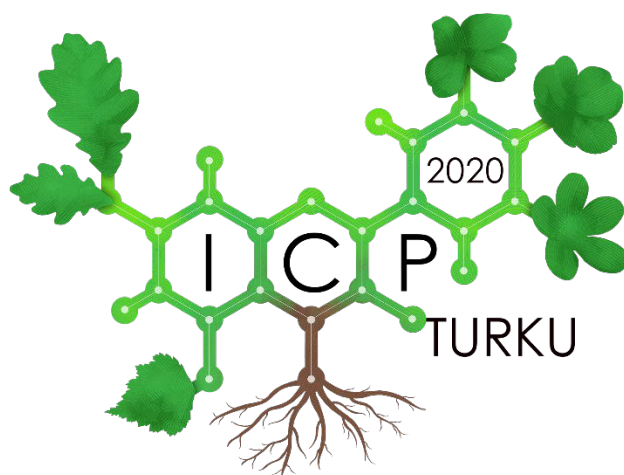




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Table of Contents

| | |
|---|----|
| Lignins and Lignification: New Developments and Emerging Concepts | 16 |
| <i>John Ralph</i> | |
| Nupharanin, a novel dehydroellagitannin from <i>Nuphar japonicum</i> | 18 |
| <i>Joanna Orejola, Manami Era, Yosuke Matsuo, Yoshinori Saito, Takashi Tanaka</i> | |
| Identification and quantification of molecular ellagitannins in Cognac eaux-de-vie by mass spectrometry method: evolution over time towards new compounds | 20 |
| <i>Mathilde Gadrat, Yoan Capello, Joël Lavergne, Catherine Emo, Stéphane Quideau, Michael Jourdes, Pierre-Louis Teissedre, Kléopatra Chira</i> | |
| New Insights of Pectin-Procyanidin Interactions: Structure/Function Relationships | 22 |
| <i>Xuwei Liu, Catherine M.G.C. Renard, Agnès Rolland-Sabaté, Carine Le Bourvellec</i> | |
| Dendrimers as color-stabilizers of anthocyanin-type dyes: how the structure and concentration of the dye modulates the interaction mechanisms | 24 |
| <i>Luis Cruz, Juan Correa, Nuno Mateus, Victor de Freitas, Maun H. Tawara, Eduardo Fernandez-Megia</i> | |
| Color expression and stability of cis and trans p-coumaric acylated cyanidin-derivatives and their UV-induced isomerization | 26 |
| <i>Yucheng Zhou, M. Monica Giusti</i> | |
| Photochemical cyclization of stilbenes isolated from Norway spruce root bark | 28 |
| <i>Riziwanguli Wufu, Harri Latva-Mäenpää, Tytti Sarjala, Pekka Saranpää, Kristiina Wähälä</i> | |
| A reaction mechanism of photo-oxidation process of catechin in relation to its bactericidal activity | 30 |
| <i>Shunichi Shishido, Rei Miyano, Takuji Nakashima, Hirotaka Matsuo, Masato Iwatsuki, Keisuke Nakamura, Taro Kanno, Hiroshi Egusa, Yoshimi Niwano</i> | |
| NMR structural determination of (+)-catechin-laccase reaction dimeric products: potential oxidation markers in grapes and wines | 32 |
| <i>Stacy Deshaies, Christine Le Guerneve, François Garcia, Laetitia Mouls, Cédric Saucier</i> | |
| On laccase-catalyzed polymerization of alkaline lignin fractions in aqueous alkaline solution | 34 |
| <i>Luyao Wang, Xiaoju Wang, Chunlin Xu</i> | |
| Orthogonal annulation strategy, enabling an efficient assembly of doubly-linked oligoflavans | 36 |
| <i>Ken Ohmori, Rikako Takeda, Vipul V. Betkekar, Keisuke Suzuki</i> | |
| Enzymatic synthesis, structures, interactions with saliva proteins and quantification in juices of a series of dehydrocaffeoylquinic acids, one of the main classes of oxidation products in apple-based beverages | 38 |
| <i>Claudia Mariana Castillo Fraire, Pascal Poupard, Sophie Guillois-Dubois, Erika Salas, Susana Soares, Elsa Brandao, Victor De Freitas, Sylvain Guyot</i> | |
| Revisiting the oxidative coupling of catechol-type flavan-3-ols: dimeric and trimeric products of (-)-epicatechin with polyphenol oxidase | 40 |
| <i>Yosuke Matsuo, Rina Kawazoe, Yoshinori Saito, Takashi Tanaka</i> | |
| A new biosynthetic intermediate of cyanidin 3-O-glucoside in black soybean seed coat | 42 |
| <i>Kumi Yoshida, Yada Teppabut, Reo Sawaguchi, Kin-ichi Oyama, Tadao Kondo</i> | |
| Hydrolyzable tannins inhibit pore-forming toxin pneumolysin | 44 |
| <i>Santeri Maatsola, Sami Kurkinen, Olli Pentikäinen, Thomas Nyholm, Juha-Pekka Salminen, Sauli Haataja</i> | |

| | |
|---|-----------|
| Synthesis and evaluation of lipophilic alkyl-polyphenols as new therapeutics toward retinal degeneration | 46 |
| <i>Espérance Moine, Nicolas Taveau, Manel Boukhallat, Maxime Vincent, Sylvie Begu, Philippe Brabet, Laurent Guillou, Thierry Durand, Joseph Vercauteren, Céline Crauste</i> | |
| The Evolution of the Color systems in Plants. A physical chemical approach | 48 |
| <i>Fernando Pina, A. Jorge Parola, Alfonso Alejo-Armijo</i> | |
| Improvement of bone health condition by oral administration of proanthocyanidin-rich grape seed extract in ovariectomized animals | 50 |
| <i>Keisuke Nakamura, Taichi Tenkumo, Alkebaier Aobulikasimu, Midori Shirato, Shunichi Shishido, Taro Kanno, Yoshimi Niwano, Keiichi Sasaki, Yoshinori Asou</i> | |
| Metabotypes of flavan-3-ol colonic metabolites after cranberry intake | 52 |
| <i>Claudia Favari, Pedro Mena, Animesh Acharjee, Saisakul Chernbumroong, Letizia Bresciani, Claudio Curti, Furio Brighenti, Christian Heiss, Ana Rodriguez-Mateos, Daniele Del Rio</i> | |
| Anthocyanin-mediated cardioprotection: an insight into molecular mechanisms | 54 |
| <i>Debora Zorzan, Francesca Cappellini, Chiara Tonelli, Katia Petroni</i> | |
| A Polyphenol From <i>Corema Album</i> L. Reduces Alpha-Synuclein Aggregation And Toxicity In Cellular And Animal Models Of Parkinson’s Disease | 56 |
| <i>Rita Rosado-Ramos, Gonçalo Poças, Mafalda Silva, Alexandre Foito, David M. Sevillano, Marcel Ottens, Derek Stewart, Regina Menezes, Markus Zweckstetter, Miguel C. Seabra, Tiago Fleming Outeiro, Pedro Domingos, Cláudia Nunes dos Santos</i> | |
| Gnetol and oxyresveratrol glucuronide metabolites: Chemical production, structural identification, metabolism by human and rat liver fractions and <i>in vitro</i> anti-inflammatory properties | 58 |
| <i>Ruth Hornedo-Ortega, Michaël Jourdes, Gregory Da Costa, Arnaud Courtois, Julien Gabaston, Pierre-Louis Teissedre, Tristan Richard, Stéphanie Krisa</i> | |
| Urolithin B inhibits IAPP aggregation: a potential strategy for Diabetes therapeutics | 60 |
| <i>Ana Raimundo, Sofia Ferreira, José Brito, Mafalda L. da Silva, Cláudia N. dos Santos, Regina Menezes</i> | |
| Unravelling the insoluble hydrolysable tannin-protein complexes | 62 |
| <i>Marica T. Engström, Valteri T.J. Virtanen, Joonas Arvola, Juha-Pekka Salminen</i> | |
| Modification of proanthocyanidins and their analysis tools for the screening of potential anthelmintic drugs of natural origin | 64 |
| <i>Iqbal Bin Imran, Marica T. Engström, Maarit Karonen, Andrew R. Williams, Juha-Pekka Salminen</i> | |
| Modulation of inflammatory responses in RAW 264.7 macrophages by purified condensed tannins and possible implication in a parasitized mouse-model | 66 |
| <i>Audrey Inge Schytz Andersen-Civil, Milla Marleena Leppä, Stig Milan Thamsborg, Juha-Pekka Salminen, Andrew Richard Williams</i> | |
| Beer effects on biochemical outcomes and gut microbiota: Alcoholic vs non-alcoholic | 68 |
| <i>Cláudia Marques, Liliana Dinis, Inês Barreiros Mota, Juliana Moraes, José B. Pereira-Leal, Joana Cardoso, Pedro Ribeiro, Helena Beato, Mafalda Resende, Christophe Espírito Santo, Ana Paula Cortez, André Moreira-Rosário, Diogo Pestana, Diana Teixeira, Ana Faria, Conceição Calhau</i> | |
| Interactions between trans-resveratrol and CpLIP2 lipase/acyltransferase: evidenced by fluorescence and <i>in silico</i> | 70 |
| <i>Thi Nga Nguyen, Eric Dubreucq, Veronique Perrier, Quang-Hung Tran, Claudine Charpentier, Clarence Charnay, Ferial Terki, Christian Jay-Allemand, Luc P. R. Bidel</i> | |
| Human metabolism of flavan-3-ols: highlights from the EU-JPI project “FOODPHYT- Food phytochemicals matter for cardiometabolic health” | 72 |
| <i>Giuseppe Di Pedè, Pedro Mena, Letizia Bresciani, Mariem Achour, Claudine Manach, Daniele Del Rio</i> | |

| | |
|---|-----|
| Identification of plant dihydrophenanthrenes as direct activators of AMP-activated protein kinase through the allosteric drug and metabolite binding site | 74 |
| <i>Matthew Sanders</i> | |
| Ellagitannin-lipid interactions by HR-MAS-NMR spectroscopy | 76 |
| <i>Maarit Karonen, Valtteri Virtanen, Susanna Rääkkönen, Elina Puljula, Gemma Walton, Martin J. Woodward</i> | |
| In vitro bioaccessibility and protective activity of an anthocyanin-rich extract from bilberry and blackcurrant against TNF-α-induced inflammation in intestinal epithelial cells | 78 |
| <i>Antonio Speciale, Romina Bashllari, Peter J Wilde</i> | |
| Silymarin flavonolignans: news about their bioactivity, bioavailability and safety | 80 |
| <i>Kateřina Valentov, David Biedermann, Jitka Viktorov, Vladimr Křen</i> | |
| Polyphenol-bearing probes for unveiling polyphenol-proteins interactions: Synthesis and applications | 82 |
| <i>Yoan Capello, Rana Melhem, Karl Kempf, Oxana Kempf, Analle Cornu, Stphane Chaignepain, Stphane Claverol, Claire Lescoat, Alexis Groppi, Macha Nikolski, Denis Deffieux, Elisabeth Genot, Stphane Quideau</i> | |
| Boosting the bioaccessibility of dietary polyphenols by delivery as colloidal aggregate protein-polyphenol particles | 84 |
| <i>Mary Ann Lila, Jia Xiong, Mary Grace, Thiru Rathinasabapathy, Slavko Komarnytsky, Mario Ferruzzi, Colin Kay, Massimo Iorizzo</i> | |
| Comparison of different extraction techniques to determine the phenolic compound concentration in olive mill waste water | 86 |
| <i>Kelly Peeters, Ana Miklavi Višnjevec, Essakiammal Sudha Esakkimuthu, Ārtomir Tavzes, Matthew John Schwarzkopf</i> | |
| Hyperglycemia alters the polyphenol metabolome in lipoproteins: putative implications from lipoprotein’s lipid environment | 88 |
| <i>Ana Reis, Sara Rocha, Irundika Dias, Jose Luis Sanchez-Quesada, Victor Freitas</i> | |
| Decrypting bacterial polyphenol metabolism in an anoxic wetland soil | 90 |
| <i>Bridget McGivern, Malak Tfaily, Mikayla Borton, Suzanne Kosina, Rebecca Daly, Carrie Nicora, Samuel Purvine, Allison Wong, Mary Lipton, David Hoyt, Trent Northen, Ann Hagerman, Kelly Wrighton</i> | |
| Spatiotemporal Modulation of Flavonoid Metabolism in Vaccinium berries | 92 |
| <i>Catrin Guenther, Andrew Dare, Tony McGhie, Cecilia Deng, Laura Jaakola, Declan Lafferty, Blue Plunkett, Ella Grierson, Janice Turner, Nick Albert, Richard Espley</i> | |
| Two-dimensional chromatographic fingerprints of oligomeric proanthocyanidin–malvidin glycoside adducts provide new insight into the complex world of red wine chemistry | 94 |
| <i>Juuso Laitila, Juha-Pekka Salminen</i> | |
| Polyphenol targeted metabolomics to predict ros wine color | 96 |
| <i>Ccile Leborgne, Marine Lambert, Marie-Agns Ducasse, Emmanuelle Meudec, Arnaud Verbaere, Jean-Claude Boulet, Nicolas Sommerer, Gilles Masson, Jean-Roch Mouret, Vronique Cheynier</i> | |
| Identification of prenyl number, configuration, and position in (iso)flavonoids in complex plant extracts by IT-MS and HR-MS | 98 |
| <i>Sarah van Dinteren, Carla Araya-Cloutier, Wouter J.C. de Bruijn, Jean-Paul Vincken</i> | |
| An efficient strategy to boost stilbene production in <i>Vitis vinifera</i> cv. Gamay Red cell suspension | 100 |
| <i>Ru Wang, Varun Kumar, Noga Sikron-Persi, Avichai Perl, Aaron Fait, Michal Oren-Shamir</i> | |

| | |
|--|------------|
| Metabolomics investigation of Antioxidant Properties, Polyphenolic profile and, Anthocyanin content in Commercial, Ancient and Red-fleshed apple varieties | 102 |
| <i>Adriana Teresa Ceci, Michele Bassi, Walter Guerra, Michael Oberhuber, Peter Robatscher, Fulvio Mattivi, Pietro Franceschi</i> | |
| Phenolic compounds in agricultural residues from olive, tomato and citrus industries | 104 |
| <i>Ana Miklavčič Višnjevec, Kelly Peeters, Sudha Esakkimuthu Esakkiammal, Črtomir Tavzes, Matthew Schwarzkopf</i> | |
| Identification of AMPK activators analogues in plant extracts | 106 |
| <i>Olivier Ciclet, Ali Bakiri, Yann Ratinaud, Matthew Sanders, Martine Naranjo, Pierre-Marie Allard, Jean-Luc Wolfender, Kei Sakamoto, Denis Barron</i> | |
| Flax tissue cultures and elicitation as a strategy for bioactive compounds production | 107 |
| <i>Iride Mascheretti, Michela Alfieri, Franca Locatelli, Erica Cusano, Roberto Consonni, Gianluca Ottolina, Marina Laura, Roméo Arago Dougué Kentsop, Massimiliano Lauria, Annamaria Genga, Franco Faoro, Monica Mattana</i> | |
| Multi-method approach for extensive characterization of gallnut tannin extracts | 109 |
| <i>Aude Watrelot, H  l  ne Halle, Christine Le Guernev  , Emmanuelle Meudec, Bertrand Robillard, C  line Poncet-Legrand, V  ronique Cheynier</i> | |
| Oral cell-line based model to understand phenolic compounds astringency perception: insights from single compounds to real food matrix | 111 |
| <i>Susana Soares, Carlos Guerreiro, Elsa Brand  o, Monica Jesus, Leonor Gon  alves, Nuno Mateus, Victor de Freitas</i> | |
| Separation of Pyranoanthocyanins from Precursor Anthocyanins Using Cation-Exchange Chromatography | 113 |
| <i>Gonzalo Miyagusuku-Cruzado, Danielle M. Voss, M. Monica Giusti</i> | |
| Development of a cell-based quaternary system to unveil the effect of polysaccharides on oral astringency | 116 |
| <i>Elsa Brand  o, Carlos Guerreiro, M  nica Jesus, Nuno Mateus, Victor de Freitas, Susana Soares</i> | |
| Auronidins are a novel group of cell-wall bound red flavonoid pigments that contribute to liverwort abiotic stress tolerance | 118 |
| <i>Rubina Jibr  n, Nick Albert, Yanfei Zhou, Kathy Schwinn, Brian Jordan, John Bowman, David Brummell, Kevin Davies</i> | |
| Overexpression of dahlia chalcone reductase candidate gene in tobacco | 120 |
| <i>Kei Maruyama, Haruka Yamada, Mizuki Yokota, Ayumi Deguchi, Munetaka Hosokawa, Fumi Tatsuzawa, Motoaki Doi, Sho Ohno</i> | |
| Comparing the effect of targeting a specific-phloretin glycosyltransferase in apple by RNA silencing and CRISPR/Cas9 genome editing | 122 |
| <i>Sim  n Miranda Ch  vez, Stefano Piazza, Axel Mithoefer, Floriana Nuzzo, Alessandro Cestaro, Richard Espley, Andrew Dare, Mickael Malnoy, Stefan Martens</i> | |
| Identification of arbutin synthases in Rosaceae | 124 |
| <i>Stefan Martens, Marion Koop, Giulia Pasqualetto, Sina Stezelow, Matthias Huelsmann, Rebecca Maechtel, Wilfried Schwab, Thilo Fischer, Luisa Palmieri, Mickael Malnoy</i> | |
| Flavonoid-tannin pathway and growth of silver birch | 126 |
| <i>Paula Thitz, Tendry Randriamanana, Ann E. Hagerman, Mika L  nnenp   , Tommi Nyman, Minna Kosonen, Sadeepa Mallikarachchi, Riitta Julkunen-Tiitto</i> | |
| Creating CRISPR knockouts for two MYBs that regulate Proanthocyanidins biosynthesis in poplar | 128 |
| <i>Yalin Liu, David Ma, C.Peter Constabel</i> | |
| Interaction between root tannins and soil fungi stabilizes carbon in the soil | 130 |
| <i>Bartosz Adamczyk</i> | |

| | |
|---|------------|
| Breeding for novel flower colour in poinsettia (<i>Euphorbia pulcherrima</i>) via Genome editing and classical transgenic approaches | 132 |
| <i>Daria Nitarska, Thomas Debener, Robert Boehm, Karl Stich, Heidi Halbwirth</i> | |
| Seed-coat protective neolignans are produced by the dirigent protein AtDP1 and the laccase AtLAC5 in <i>Arabidopsis</i>..... | 134 |
| <i>Keiko Yonekura-Sakakibara, Masaomi Yamamura, Fumio Matsuda, Eiichiro Ono, Ryo Nakabayashi, Satoko Sugawara, Tetsuya Mori, Yuki Tobimatsu, Toshiaki Umezawa, Kazuki Saito</i> | |
| Towards understanding the role and regulation of condensed tannin during ectomycorrhizal symbiosis development in <i>Populus</i> roots | 136 |
| <i>Jamil Chowdhury, Jannatul Ferdous, Jenna Lihavainen, Marius Sake Imko Van Dijk, Benedicte R. Albrechtsen, C. Peter Constabel, Judith Lundberg-Felten</i> | |
| Dehydroquinase dehydratase/shikimate dehydrogenases from <i>Eucalyptus camaldulensis</i> involved in shikimate pathway, quinate metabolism, and gallate formation | 138 |
| <i>Ko Tahara, Mitsuru Nishiguchi, Evelyn Funke, Shin-Ichi Miyazawa, Takafumi Miyama, Carsten Milkowski</i> | |
| Aluminum detoxification abilities of hydrolyzable tannins identified in <i>Eucalyptus camaldulensis</i> | 140 |
| <i>Ko Tahara, Shoichi Suzuki, Mitsuru Nishiguchi, Koh Hashida, Hideyuki Ito</i> | |
| Polyphenols from pecan nut shell as multifunctional compounds for active packaging, food colorant stabilization and synthesis of silver nanoparticles | 142 |
| <i>Lucia Panzella, Federica Moccia, Rita Argenziano, Sarai Agustin-Salazar, Fabian Weber, Valeria Giosafatto, Paolo Aprea, Angela Arciello, Loredana Mariniello, Andreas Schieber, Pierfrancesco Cerruti, Alessandra Napolitano</i> | |
| Polyphenols as additives for eco-friendly and bio-inspired adhesives from soy proteins | 144 |
| <i>Rita Argenziano, Maria Laura Alfieri, Lucia Panzella, Marina DellaGreca, Alessandra Napolitano, Marco d'Ischia</i> | |
| Eco-friendly recovery of antioxidant phenolic compounds from chestnut wood fiber by optimized deep eutectic solvents (DES) extraction | 146 |
| <i>Federica Moccia, Samuele Giovando, Lucia Panzella, Alessandra Napolitano</i> | |
| Host-guest chemistry: γ-cyclodextrin interaction with pyranoanthocyanins | 148 |
| <i>Alexandra Borges, Paula Araújo, Nuno Basílio, Victor de Freitas, Joana Oliveira</i> | |
| Impact of processing technology and storage on proanthocyanidins and sensory properties of blackcurrant juices | 150 |
| <i>Oskar Laaksonen, Juha-Pekka Salminen, Leenamajja Mäkilä, Heikki Kallio, Baoru Yang</i> | |
| Investigation of protein-polyphenol conjugates in almond blanch water in food production | 152 |
| <i>Veronika Hellwig, Sabrina Görtz, Johanna Gasser</i> | |
| Oxidative coupling of chlorogenic acid with tryptophan: toward a natural product-based food dye..... | 154 |
| <i>Federica Moccia, Marina Della Greca, Maria Angeles Martin, Sonia Ramos Rivero, Lucia Panzella, Luis Goya, Alessandra Napolitano</i> | |
| Chemical / colour stability and rheological properties of cyanidin-3-glucoside in deep eutectic solvents as a gateway to design task-specific bioactive compounds^s | 156 |
| <i>Hileia Souza, Nuno Mateus, Victor de Freitas, Maria P. Gonçalves, Luis Cruz</i> | |
| Potential of industrial sweet orange waste to act as an anti-cariogenic agent | 159 |
| <i>Suvro Saha, Simon Wood, Thuy Do, Joanne Maycock, Christine Bosch</i> | |
| Anthocyanin-Polysaccharide Complexes: from nature to innovative food solutions | 161 |
| <i>Ana Fernandes, Nuno Mateus, Victor Freitas</i> | |

| | |
|--|------------|
| Preparative isolation of apple Flavan-3-ols by pH-zone-refining centrifugal partition chromatography combined with reversed-phase liquid chromatography | 163 |
| <i>Sophie Guilois-Dubois, Sylvain Guyot, Pascal Poupard</i> | |
| Inter- and Intraspecies variability of polyphenols in temperate forage species | 165 |
| <i>Supriya Verma, Juha-Pekka Salminen, Friedhelm Taube, Carsten S. Malisch</i> | |
| Functionalization of carboxylated lignin nanoparticles with amino-flavylium derivative using EDC/NHS coupling agents | 167 |
| <i>Ana Rita Pereira, Paula Araújo, Iva Fernandes, Nuno Mateus, Victor Freitas, Joana Oliveira</i> | |
| Supramolecular study of interactions between malvidin-3-O-glucoside and wine phenolic compounds. Effect on color | 169 |
| <i>Bárbara Torres-Rochera, Natércia Brás, María Teresa Escribano-Bailón, Ignacio García-Estévez</i> | |
| Biomimetic intramolecular oxidative coupling between galloyl groups of pentagalloylglucose | 171 |
| <i>Kenta Sakamoto, Takako Yamashita, Yosuke Matsuo, Yoshinori Saito, Takashi Tanaka</i> | |
| Role of ellagitannins in the synthesis of vitisin A and in the degradation of malvidin 3-O-glucoside. An approach in wine-like model systems | 173 |
| <i>Cristina Alcalde-Eon, Ignacio García-Estévez, María Teresa Escribano-Bailón</i> | |
| Investigation of pH dependance of UV-Vis spectra of gallic and ellagic acids using combined experimental and theoretical approaches..... | 175 |
| <i>Sara Štumpf, Gregor Hostnik, Jelena Tošović, Anja Petek, Urban Bren</i> | |
| Interactions of Fe(II) ion with gallic acid and vescalagin | 177 |
| <i>Gregor Hostnik, Franjo Frešer, Jelena Tošović, Sara Štumpf, Urban Bren</i> | |
| Flavan-3-ols isolated from the bark of <i>Bassia longifolia</i>..... | 179 |
| <i>Peter Bürkel, Meena Rajbhandari, Guido Jürgenliemk</i> | |
| Polyphenolic composition of cold-hardy grapes and wines..... | 181 |
| <i>Yiliang Cheng, Emily Kuelbs, Lucas Buren, Lindsey Bouska, Aude A. Watrelot</i> | |
| Kinetic and Thermodynamic characterization of 5-Hydroxy-4'-Dimethylaminoflavylium in the presence of SDS micelles | 183 |
| <i>Paula Araújo, Johan Mendoza, Fernando Pina, Ana Rita Pereira, Iva Fernandes, Victor de Freitas, Joana Oliveira</i> | |
| Investigating ultraviolet-visible energies that initiate the mechanism of cis-trans photoisomerization of acylated delphinidins and its impact on color performance..... | 185 |
| <i>Ellia H. La, M. Monica Giusti</i> | |
| High-performance countercurrent chromatography fractionation of polymethoxy flavones by off-line electrospray mass spectrometry injection profiling of <i>Citrus sinensis</i> | 187 |
| <i>Gerold Jerz, Maria Ramos-Jerz, Isabella Iuzzolino, Dennis Krygier, Recep Gök, Peter Winterhalter, Tuba Esatbeyoglu</i> | |
| Identification of oxidation markers of the reaction of grape tannins with volatile thiols commonly found in wine..... | 189 |
| <i>Lucas Suc, Peggy Rigou, Laetitia Moulis</i> | |
| Influence of red wine polysaccharides profile on the flavanol composition and precipitation | 191 |
| <i>Iglesias de Lacerda-Bezerra, Leociley Rocha Alencar Menezes, Montserrat Dueñas, Guilherme Lanzi Sasaki, Ignacio García-Estévez, María Teresa Escribano-Bailón</i> | |
| Thermal Degradation of 10-catechyl Pyranoanthocyanins Derived from Pelargonidin-, Cyanidin-, and Malvidin-3-glucosides..... | 193 |
| <i>Danielle M. Voss, Gonzalo Miyagusuku-Cruzado, M. Monica Giusti</i> | |
| Synthesis of 6-methylflavanone and its biotransformation in cultures of entomopathogenic filamentous fungi..... | 195 |
| <i>Agnieszka Krawczyk-Łebek, Monika Dymarska, Tomasz Janeczko, Edyta Kostrzewa-Susłow</i> | |

| | |
|---|------------|
| Unravelling discolouration caused by iron-flavonoid interactions: complexation, oxidation, and network formation | 197 |
| <i>Judith Bijlsma, Wouter de Bruijn, Jean-Paul Vincken</i> | |
| Distribution of lignans and lignan mono/di glucosides in freeze-fixed stem of <i>Ginkgo biloba</i> L. by cryo-TOF-SIMS/SEM..... | 199 |
| <i>Min Yu, Takuya Akita, Syunya Fujiyasu, Shunsuke Takada, Dan Aoki, Yasuyuki Matsushita, Masato Yoshida, Kazuhiko Fukushima</i> | |
| Interaction between salivary proteins and cork phenolic compounds able to migrate to wine model solutions | 201 |
| <i>Joana Azevedo, Mónica Jesus, Elsa Brandão, Susana Soares, Joana Oliveira, Paulo Lopes, Nuno Mateus, Victor Freitas</i> | |
| Formation of dehydrohexahydroxydiphenoyl esters by oxidative coupling of galloyl esters involved in ellagitannin biosynthesis..... | 203 |
| <i>Takako Yamashita, Yosuke Matsuo, Yoshinori Saito, Takashi Tanaka</i> | |
| Photochemical cyclization of stilbenes isolated from Norway spruce root bark | 205 |
| <i>Riziwanguli Wufu</i> | |
| Unveiling the iron-tannin complexes behind medieval iron gall inks..... | 207 |
| <i>Natércia Teixeira, André Neto e Silva, Paula Nabais, Nuno Mateus, Fernando Pina, Maria Rangel, Maria João Melo, Victor de Freitas</i> | |
| Production of urolithins from ellagic acid using human intestinal bacteria and activation of sirtuin-related genes by urolithins..... | 209 |
| <i>Takanori Nakajima, Hiroaki Yamamoto, Yoshinori Katakura</i> | |
| Quantification of trans-ϵ-viniferin and its glucuro-conjugated metabolites in rat plasma after oral administration | 211 |
| <i>Pauline Beaumont, Arnaud Courtois, Claude Atgé, Michael Jourdes, Axel Marchal, Chrystel Faure, Tristan Richard, Stéphanie Krisa</i> | |
| Capability of mannoproteins isolated from <i>Saccharomyces cerevisiae</i> to interact with wine polyphenolic compounds | 213 |
| <i>Diana M. Bosch-Crespo, Elvira Manjón, Ignacio García-Estévez, Montserrat Dueñas, M. Teresa Escribano-Bailón</i> | |
| Curcumin conjugates are incompletely hydrolyzed by β-glucuronidase: Detection of complex conjugates in plasma | 215 |
| <i>Paula Luis, Andrew Kunihiro, Janet Funk, Claus Schneider</i> | |
| Polyphenols from Colombian <i>Passiflora ligularis</i> Juss (granadilla) inhibit, <i>in vitro</i> and <i>in vivo</i>, inflammatory agents..... | 217 |
| <i>Juan Carlos Carmona-Hernandez, Jonathan Valdez, Bradley Bolling, Jaime Angel-Isaza, William Narvaez-Solarte, Gonzalo Taborda-Ocampo, Clara Helena Gonzalez-Correa</i> | |
| Screening novel bioactivities and bark chemistry of Finnish willows..... | 219 |
| <i>Jenni Tienaho, Dhanik Reshamwala, Tytti Sarjala, Jaana Liimatainen, Petri Kilpeläinen, Riikka Linnakoski, Anneli Viherä-Aarnio, Jarkko Hellström, Varpu Marjomäki, Tuula Jyske</i> | |
| Differences in Chemical Composition and Antioxidant Potential Between Herb and Root Ethanol Extracts of <i>Rumex alpinus</i> L. 1753. (Polygonaceae)..... | 221 |
| <i>Emilija Svirčev, Dejan Orčić, Ivana Beara, Nataša Simin, Kristina Bekvalac, Goran Anačkov, Neda Mimica-Dukić</i> | |
| <i>Bassia longifolia</i> bark extract exhibits antimicrobial activity | 223 |
| <i>Vanja Ljoljić Bilić, Peter Bürkel, Meena Rajbhandari, Ivan Kosalec, Guido Jürgenliemk</i> | |
| Uptake and anti-inflammatory properties of betalains in intestinal Caco-2 cells | 225 |
| <i>Yunqing Wang, Yap Yin Huan, Christine Boesch, Lisa Marshall</i> | |

| | |
|--|------------|
| Health-promoting effects of lingonberry (<i>Vaccinium vitis-idaea</i> L.) in obesity: impact on lipid and glucose metabolism and low-grade inflammation..... | 227 |
| <i>Riitta Ryyti, Mari Hämäläinen, Antti Pemmari, Rainer Peltola, Eeva Moilanen</i> | |
| Effect of the presence of mannoproteins on the interaction between flavanols, salivary proteins and oral epithelial cells | 229 |
| <i>Alba M. Ramos-Pineda, Ignacio García-Estévez, M. Teresa Escribano-Bailón</i> | |
| Protective effect of Manuka honey against inflammation and its related diseases | 231 |
| <i>Massimiliano Gasparrini, Tamara Yuliett Forbes-Hernandez, Danila Cianciosi, Francesca Giampieri</i> | |
| Chestnut (<i>Castanea sativa</i> Mill.) shells: A promising source of polyphenols as valuable compounds for cosmetic industry..... | 233 |
| <i>Diana Pinto, Elsa Vieira, Andreia F. Peixoto, Vitor Freitas, Paulo Costa, Cristina Delerue-Matos, Francisca Rodrigues</i> | |
| <i>In vitro</i> and <i>in vivo</i> bioassay-guided fractionation of olive mill wastewaters for effective biocontrol of <i>Verticillium dahliae</i> in tomato plants and <i>Phytophthora capsici</i> in pepper plants..... | 235 |
| <i>Alba Gutiérrez Docio, Clara Lago, Dani Marchena, Inmaculada Larena, Marta Lois, Raquel Núñez, Javier Veloso, José Díaz, Esperanza Mollá, Marin Prodanov</i> | |
| Agrifood waste as a source to obtain natural bioactive compounds | 237 |
| <i>Andrea Palos-Hernández, M. Yolanda Gutiérrez Fernández, José Escuadra Burrieza, José Luis Pérez Iglesias, Ana M. González-Paramás</i> | |
| Comparison of <i>In vitro</i> assays to determine inhibition of α-amylase enzyme activity of anthocyanins..... | 239 |
| <i>Sadia Zulfiqar, Lisa Marshall, Christine Boesch</i> | |
| Polyphenols in six less cultivated fruit and berry species cultivated in Estonia | 241 |
| <i>Reelika Rätsep, Liina Arus, Hedi Kaldmäe, Alar Aluvee</i> | |
| Microwave-assisted extraction of kiwiberry leaves for cosmetic purposes: Phenolic composition and bioactivity screening | 243 |
| <i>Ana Margarida Silva, Diana Pinto, Iva Fernandes, Vitor de Freitas, Paulo Costa, Cristina Delerue-Matos, Francisca Rodrigues</i> | |
| Biological and physicochemical properties of <i>Solanum tuberosum</i> L. var. Vitelotte anthocyanins rich extract and its impact on membrane, albumin and cancer cells..... | 245 |
| <i>Paulina Strugała-Danak, Anna Urbaniak, Alicja Z. Kucharska, Maciej Ugorski, Janina Gabrielska</i> | |
| <i>In vitro</i> antibacterial activity against <i>Helicobacter pylori</i> of oligomeric and highly polymerised procyanidin-rich fractions from grape seed extract | 247 |
| <i>Alba Gutiérrez Docio, Esperanza Guerrero, Jose Manuel Silván, Teresa Alarcón, Marin Prodanov, Adolfo J. Martinez-Rodriguez</i> | |
| Bio-accessibility of bioactive compounds in blueberry smoothie enriched with pea protein: an <i>in-vitro</i> gastrointestinal digestion..... | 249 |
| <i>Latifeh Ahmadi, Michael Rogers, Kailah Sprowl</i> | |
| RoB oncogene increased synthesis of phenolic compounds and bioactivity of <i>Dionaea muscipula</i> J. Ellis..... | 251 |
| <i>Wojciech Makowski, Aleksandra Królicka, Barbara Tokarz, Halina Ekiert, Agnieszka Szopa, Rafał Banasiuk, Krzysztof Tokarz</i> | |
| Protein Precipitation Capacity of Chemically Well-Defined Proanthocyanidin Oligomers and Polymers..... | 253 |
| <i>Mimosa Sillanpää, Marica T. Engström, Juha-Pekka Salminen</i> | |
| Sugarcane polyphenols as non-antibiotic growth promoters in animal feeds | 255 |
| <i>Matthew Flavel, Barry Kitchen, Xin Yang, Stefanie Prendergast, Roya Afshari, Mia Bettio</i> | |

| | |
|---|------------|
| The bioactive & bioavailability properties of polyphenol- rich extract from sugarcane (<i>Saccharum officinarum</i>) | 257 |
| <i>Barry Kitchen, Matthew Flavel, Roya Afshari, Mia Bettio, Stefanie Prendegast, Xin Yang</i> | |
| Hydrophobicity and logP of hydrolysable tannins | 259 |
| <i>Valtteri Virtanen, Maarit Karonen</i> | |
| UPLC-PDA-Q/TOF-MS profiling of phenolic compounds and anti-neurodegenerative potential of <i>Hippophaë rhamnoides</i> L. berries | 261 |
| <i>Karolina Tkacz, Aneta Wojdyło, Igor Turkiewicz, Paulina Nowicka</i> | |
| Phenolic profile and biological activities of <i>Chaenomeles</i> microencapsulated powders | 263 |
| <i>Igor Turkiewicz, Aneta Wojdyło, Karolina Tkacz, Anna Michalska-Ciechanowska, Paulina Nowicka</i> | |
| Profiling of polyphenols by LC-MS-ESI-QTOF, characteristics of nutritional compounds and in vitro effect on α-amylase, α-glucosidase, lipase activities of <i>Prunus avium</i> and <i>P. cerasus</i> leaves and fruits | 265 |
| <i>Aneta Wojdyło, Paulina Nowicka, Karolina Tkacz, Igor Piotr Turkiewicz</i> | |
| Prospecting for bioactives with group-specific and molecular networking MS/MS approaches | 267 |
| <i>Ilari Kuukkanen, Marianna Manninen, Erika Alander, Niko Luntamo, Minttu Matturi, Thao Nguyen, Essi Suominen, Matias Kari, Juha-Pekka Salminen</i> | |
| Effect of climate change on the polyphenolic composition of the main varieties of grape from La Rioja (Spain) and new oenological strategies to correct these effects on the quality of red wine | 269 |
| <i>Carlos Asensio-Regalado, Andrea Sasía-Arriba, Aimar Ayelen Poliero, Rosa Mara Alonso-Salces, Blanca Gallo Hermosa, Luis ngel Berrueta Simal</i> | |
| Characterization of Finnish apple ciders by means of polyphenol profiles..... | 271 |
| <i>Wenjia HE, Oskar Laaksonen, Ye Tian, Maarit Heinonen, Baoru Yang</i> | |
| Epicuticular polyphenols as potential chemotaxonomic markers for common Finnish tree species..... | 273 |
| <i>Marianna Manninen, Maarit Karonen, Juha-Pekka Salminen</i> | |
| Biotransformation of 5-O-caffeoylquinic acid by gut bacteria: an interesting oxidative pathway..... | 275 |
| <i>Gentiana Balaj, Zohreh Tamana-Shacoori, Aurelie Sauvager, Solenn Ferron, Isabelle Rouaud, Latifa Bousarghin, Sandrine David-Le Gall, Sylvain Guyot, Dashnor Nebija, Sophie Tomasi, Marie-Laurence Abasq</i> | |
| Cranberry proanthocyanidins enhance chemotherapy-induced esophageal adenocarcinoma cell death | 277 |
| <i>Yun Zhang, Katherine Weh, Connor Howard, Kiran Lagisetty, Dyke McEwen, Jules Lin, Rishindra Reddy, Andrew Chang, David Beer, Amy Howell, Laura Kresty</i> | |
| Metabolomic approach of Arbosana olive (<i>Olea europaea</i> L.) leaves dried by different technologies to identify polyphenols related to antioxidant capacity | 279 |
| <i>Ma Elena Daz, Juan Marin, Miguel Gaston, Itxaso Filgueira, Mara-Jose Saz-Abajo</i> | |
| Cranberry proanthocyanidins mitigate bile-induced injury in primary normal esophageal cell lines isolated from patients with esophageal adenocarcinoma | 281 |
| <i>Katherine Weh, Danielle Turgeon, Joel Rubenstein, Amy Howell, Laura Kresty</i> | |
| Untargeted metabolomic LC-MS fingerprinting of apple cultivars for the identification of biomarkers related to resistance to rosy apple aphid | 283 |
| <i>Andrea Sasa-Arriba, Rosa Mara Alonso-Salces, Carlos Asensio-Regalado, Aimar Ayelen Poliero, Beatriz Abad-Garca, Enrique Dapena, Blanca Gallo, Luis ngel Berrueta</i> | |
| Effect of temperature and developmental stage on the content of anthocyanins and phenolic acids in potato cultivars..... | 285 |
| <i>Liz Gutierrez Quequezana, Anssi Vuorinen, Heikki Kallio, Baoru Yang</i> | |

| | |
|---|------------|
| Diarylheptanoids – strong antioxidants in alder bark growing in Latvia: chemical profiling, isolation and their application potential..... | 287 |
| <i>Liga Lauberte, Galina Telysheva, Jevgenija Ponomarenko, Alexander Arshanitsa, Anna Andersone, Sarmite Janceva, Jelena Krasilnikova</i> | |
| Extraction and identification of polyphenols from spruce bark using HPLC-DAD-ESI-MS/MS | 289 |
| <i>Esakkiammal Sudha Esakkimuthu, Ana Miklavčič Višnjevec, Petra Jenuš, Aleksander Učakar, Črtomir Tavzes, David DeVallance, Andreja Kutnar, Kelly Peeters</i> | |
| Comparing aryltetralin lignans production by adventitious roots from three <i>Linum</i> species | 291 |
| <i>Michela Alfieri, Iride Mascheretti, Gianluca Ottolina, Roberto Consonni, Roméo Arago Dougué Kentsop, Franca Locatelli, Monica Mattana</i> | |
| Are Dirigent like Domains from Bacteria Belonging to the DIR protein Family? | 293 |
| <i>Merlin Bardin, Pierre Rousseolt Pailley, Thierry Tron, Viviane Robert</i> | |
| Exploring extractable and non-extractable polyphenols in banana flower and banana pseudo-stem. Effect of harvest year | 300 |
| <i>Sara Ramírez-Bolaños, Jara Pérez-Jiménez, Sara Díaz, Lidia Robaina</i> | |
| Snailase is a powerful tool for the enzymatic hydrolysis of flavonoids | 302 |
| <i>Christoph Kornpointner, Jakob Scheibelreiter, Heidi Halbwirth</i> | |
| Isolation and purification of betalains from red beetroot (<i>Beta vulgaris</i> L.) using automated flash chromatography | 304 |
| <i>Ganwarige Sumali N Fernando, Natalia Sergeeva, Lisa Marshall, Christine Boesch</i> | |
| Salicis cortex: influences of sex and harvest season on polyphenolic content in four <i>Salix</i> species..... | 306 |
| <i>Thomas Olaf Gruber, Jörg Heilmann, Gregor Aas, Guido Jürgenliemk</i> | |
| Procyanidin variation in leaves and stems of wild and cultivated <i>Vaccinium</i> species..... | 309 |
| <i>Oana-Crina Bujor, Mona Elena Popa</i> | |
| Influence of choice of solvents and extraction techniques on the recovery of phenolic phytochemicals linked to the antioxidant and enzyme inhibition potential of <i>Clerodendrum glandulosum</i> Lindl..... | 311 |
| <i>Prashanta Kumar Deb, Amrita Chatterjee, Rajdeep Saha, Biswatrish Sarkar</i> | |
| Exudate flavonoid diversification of <i>Primula auricula</i> L. populations in an ecological context..... | 313 |
| <i>Clara Priemer, Danka Bukvicki, Karin Valant-Vetschera</i> | |
| A transfer to a new host plant and a change in a polyphenol content can affect the metabolism of <i>Lymantria mathura</i> larvae | 315 |
| <i>Suvi Vanhakylä, Martin Volf, Juha-Pekka Salminen</i> | |
| Chemical composition and biosynthesis of poplar bud resin in <i>Populus trichocarpa</i> and <i>Populus balsamifera</i>..... | 317 |
| <i>Eerik-Mikael Piirtola, C. Peter Constabel</i> | |
| Leaf proanthocyanidins act as in planta antioxidants and protect poplar trees against the effects of oxidative stress from drought and UV-B | 319 |
| <i>Peter Constabel, Geraldine Gourlay</i> | |
| Combined effects of ozone stress with drought or salt stress on selected parameters of the antioxidant machinery in city trees | 321 |
| <i>Michael Kurta, Silvija Marinovic, Anne-Charlott Fitzky, Jürgen Greiner, Hans Sandén, Heidi Halbwirth</i> | |
| Altered polyphenol metabolism associated with cut carrot blackening | 323 |
| <i>Katie Schulz, Robert D. Hancock, Barbara Karpinska, Susan R. Verrall, Paul J. Knox, Christine H. Foyer</i> | |

| | |
|--|------------|
| Phenolic compounds profile of <i>Dionaea muscipula</i> J. Ellis leaves and traps after UV-A treatment | 325 |
| <i>Karolina Miernicka, Rafał Banasiuk, Wojciech Makowski, Barbara Tokarz, Aleksandra Królicka, Krzysztof Tokarz</i> | |
| The different substrate specificities of the <i>Zea mays</i> dihydroflavonol 4-reductase paralogs A1 and A1* are determined by few amino acids | 327 |
| <i>Christian Haselmair-Gosch, Silviya Marinovic, Christian Molitor, Daria Nitarska, Lukas Eidenberger, Emmanuelle Bignon, Serge Antonczak, Heidi Halbwirth</i> | |
| Synthesis of flavonol-bearing probes and proteomic analysis of Asteraceae petals via affinity-based protein profiling | 329 |
| <i>Karl Kempf, Oxana Kempf, Yoan Capello, Christian Molitor, Rana Melhem, Stéphane Chaignepain, Stéphane Claverol, Elisabeth Genot, Claire Lescoat, Alexis Groppi, Macha Nikolski, Heidrun Halbwirth, Stéphane Quideau, Denis Deffieux</i> | |
| Experimental warming induces species-specific changes in phenolic chemistry of boreal tree seedlings | 331 |
| <i>Virpi Virjamo, Katri Nissinen, Riitta Julkunen-Tiitto, Heli Peltola</i> | |
| Iron solubilization in mangrove sediments associated with leaf-derived polyphenols and benthic animals | 333 |
| <i>Ko Hinokidani, Yasuhiro Nakanishi</i> | |
| Insect and fungus specialists on aspen leaves have opposite relationships to condensed tannins | 335 |
| <i>Benedicte R. Albrechtsen</i> | |
| Membrane assisted solid-liquid extraction for the recovery of polyphenolic fractions from grape pomace | 337 |
| <i>Laura Alicia Orozco-Flores, Erika Salas, Beatriz Adriana Rocha-Gutiérrez, María del Rosario Peralta-Pérez, Guillermo González-Sánchez, María de Lourdes Ballinas-Casarrubias</i> | |
| Exploring the colour and bioactivity of anthocyanin related structures towards skin healthcare – bridging food and therapeutics | 339 |
| <i>Patrícia Correia, Hélder Oliveira, Paula Araújo, Ana Rita Pereira, Patrícia Coelho, Lucinda Bessa, Paula Gameiro, Victor de Freitas, Nuno Mateus, Joana Oliveira, Iva Fernandes</i> | |
| Valorisation of food wastes to obtain polyphenolic rich extracts and extract fractions | 341 |
| <i>Linards Klavins, Ruta Muceniece, Una Riekstina, Maris Klavins</i> | |
| Can plant polyphenol inspired surface modifications improve tissue integration of titanium implants? | 343 |
| <i>Florian Weber, Alejandro Barrantes, Hanna Tainen</i> | |
| Polymerization possibilities of polyphenols from the flavonoid group (Funding: National Science Centre, Poland, grant No. 2018/31/N/ST8/02565) | 345 |
| <i>Malgorzata Latos-Brozio, Anna Masek</i> | |
| Extraction of grape polyphenols during maceration and by organic solvents in relation to vineyard relief | 347 |
| <i>Alenka Mihelčič, Paolo Sivilotti, Vrščaj Borut, Lisjak Klemen, Vanzo Andreja</i> | |
| Optimization of alcohol extraction of polyphenols from distillery stillage | 349 |
| <i>Wioleta Mikucka, Magdalena Zielińska</i> | |
| Capability of yeast mannoproteins to modify phenolic compound-salivary protein aggregation | 351 |
| <i>Elvira Manjón, Alberto Recio-Torrado, Alba M. Ramos-Pineda, Ignacio García-Estévez, M. Teresa Escribano-Bailón</i> | |

| | |
|---|------------|
| Effect of mannoproteins obtained from different oenological yeast on pigment and color stability of red wine | 353 |
| <i>María Oyón-Ardoiz, Elvira Manjón, M.Teresa Escribano-Bailón, Ignacio García-Estévez</i> | |
| Comparing the lignin degrading abilities of lower and higher termites | 355 |
| <i>Hongjie Li, Xue Kang, Mengyi Yang, Boris Kassenev, Xuguo Zhou, Hongwei Shan, Shiyong Liang, Xiaojie Zhang, Yu Liu, Cameron Currie, John Ralph, Daniel Yelle</i> | |
| Anti-aging effects of constituents in Wine or Wine compression residue | 357 |
| <i>Akiyoshi Sawabe, Ayato Tanaka, Ryuji Takeda</i> | |
| Surfactant-mediated green extraction of polyphenols from red grape pomace | 359 |
| <i>Darija Sazdanić, Veljko Krstonošić, Mira Mikulić, Jelena Cvejić, Milica Atanacković Krstonošić</i> | |
| Polyphenols and urban mining. A green alternative for the recovery of valuable metals from scrap printed circuit boards | 361 |
| <i>Gemma Reguero-Padilla, María F. Alexandre-Franco, Carmen Fernández-González, Marta Adame-Pereira, Agustina Guiberteau-Cabanillas, Eduardo Manuel Cuerda-Correa</i> | |
| Polyphenols-mediated green synthesis of nZVI for the removal of dyes from water | 363 |
| <i>M Cristina Rodríguez Rasero, Carmen Fernández González, María F Alexandre Franco, Agustina Guiberteau Cabanillas, Eduardo M Cuerda Correa</i> | |
| Impact of green tea extraction in ternary deep eutectic solvent on chitosan-based films properties for food applications | 365 |
| <i>Tiago Filipe P. Alves, Natércia Teixeira, Jorge Vieira, António A. Vicente, Victor de Freitas, Hiléia K. S. Souza</i> | |
| The “Groupe Polyphénols” (International) today: beyond the hopes of its founders, 50 years ago! | 367 |
| <i>Joseph Vercauteren</i> | |
| The Lignans – A Family of Biologically Active Polyphenolic Secondary Metabolites | 369 |
| <i>Jean-Philip Lumb</i> | |
| Total synthesis of hybrid type polyphenols and confirmation of its absolute configuration | 371 |
| <i>Toshiyuki Kan</i> | |
| The potential of low molecular weight (poly)phenol metabolites for attenuating neuroinflammation and treatment of neurodegenerative diseases | 373 |
| <i>Daniela Marques, Rafael Carecho, Diogo Carregosa, Cláudia Nunes dos Santos</i> | |
| Relevance of dietary flavonoids on the mitigation of metabolic disorders | 375 |
| <i>Patricia Oteiza</i> | |
| Applications of MS-based metabolomics to investigate the biomarkers of the co-metabolic processing of apple polyphenols | 377 |
| <i>Fulvio Mattivi, Maria M. Ulaszewska</i> | |
| Deciphering complex natural mixtures through metabolome mining of mass spectrometry data: the plant specialized metabolome as a case study | 379 |
| <i>Justin J.J, van der Hooft, Madeleine Ernst, Daniel Papenberg, Kyo Bin Kang, Iris F. Kappers, Marnix H. Medema, Pieter C. Dorrestein, Simon Rogers</i> | |
| Analysis of Proanthocyanidins in Food Ingredients by the 4-Dimethylaminocinnamaldehyde Reaction | 381 |
| <i>Jess Reed</i> | |
| Why should non-extractable polyphenols be systematically included in polyphenol analysis? | 383 |
| <i>Jara Pérez Jiménez</i> | |
| Colour bio-factories: production of anthocyanins in plant cell cultures | 385 |
| <i>Cathie Martin, Ingo Appelhagen</i> | |

| | |
|--|------------|
| The puzzle of displaying orange: Substrate specificity of dihydroflavonol 4-reductase | 387 |
| <i>Teemu H. Teeri, Dalia Sultana, Saku Mattila, Jere Vainio, Lingping Zhu</i> | |
| Advances in biobased thermosetting polymers | 389 |
| <i>Hélène Fulcrand, Laurent Rouméas, Guillaume Billerach, Chahinez Aouf, Eric Dubreucq</i> | |
| Advanced Polyphenol-Based Materials via Supramolecular Assembly | 391 |
| <i>Frank Caruso</i> | |
| Author index..... | 392 |

P3.12

Extraction and identification of polyphenols from spruce bark using HPLC-DAD-ESI-MS/MS

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MAIN CONCLUSION

The results indicated that Fe₃O₄-CA exhibits better efficiency than bare Fe₃O₄ and 1h of shaking was optimal (4h shaking degraded the original polyphenol composition, and 15 min was not efficient). MNPs show the potential in extracting viable polyphenol from bark (hot water extraction) followed by different modified Fe₃O₄ separation, and possibly continuous (cycling) processes.

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INTRODUCTION

Spruce is a widely used raw material in the pulp and paper industry where it contributes significantly to considerable amounts of biomass waste generated. As with most woody biomass used for pulp and paper, the removed bark from spruce ends up being a low-value by-product. In spruce, the bark constitutes 10-15% total weight of tree stems, is detrimental in the pulping process, as it has to be removed before processing. Once removed, the bark is generally used as an energy source. Extraction of commercially viable compounds from the bark before burning is an interesting value-added option. Bark is heterogenous both morphologically and chemically, as it contains cellulose, hemicelluloses, pectins, lignin and various extractives in different proportions. Spruce bark is a renewable source of biologically active compounds. More than 60 antioxidant compounds can be isolated from spruce bark including piceid astringin, quercetin and resveratrol [1]. These polyphenolic compounds showed anti-inflammatory, anti-microbial, anti-tumor, antioxidants and antiaging properties, and therefore, are potential ingredients for cosmetics, food and pharmaceuticals.

The present study describes the extraction of polyphenols from spruce bark using hot water extraction followed by the separation of polyphenols from the debarking water using magnetic beads through adsorption and desorption methodology. In the experimental section, the efficiency of magnetic iron oxide nanoparticles (MNPs - bare Fe₃O₄, modified Fe₃O₄), concentration and shaking time were tested.

MATERIALS & METHODS

Magnetic nanoparticles synthesis: MNPs were synthesized by coprecipitation from an aqueous solution of Fe²⁺ and Fe³⁺ salts in a two-step process. First, the pH was raised to 3 and after 30 min to pH 11 by using ammonia solution (25%). The synthesized particles were washed several times with water, collected by magnet and re-dispersed in water in a form of water-based suspension with a concentration of 10 g/L (bare Fe₃O₄). Additionally, citric acid (CA) was adsorbed on a bare Fe₃O₄ to modify their surface charge (Fe₃O₄-CA).

Bark polyphenol extraction: 500mg of samples (38 to 850 μm) were taken in 20mL of water and ultrasonicated at 80°C for 30min followed by filtration. Different concentrations (0.5, 2.5 g/L) of Fe₃O₄ and Fe₃O₄-CA were added into the filtrate at different shaking times (15min, 1h and 4h). Then, MNPs were collected by a magnet and debarking water was then separated and analyzed; 5mL of methanol was added to the collected MNPs shaken and the separated methanol was analyzed.

RESULTS & DISCUSSION

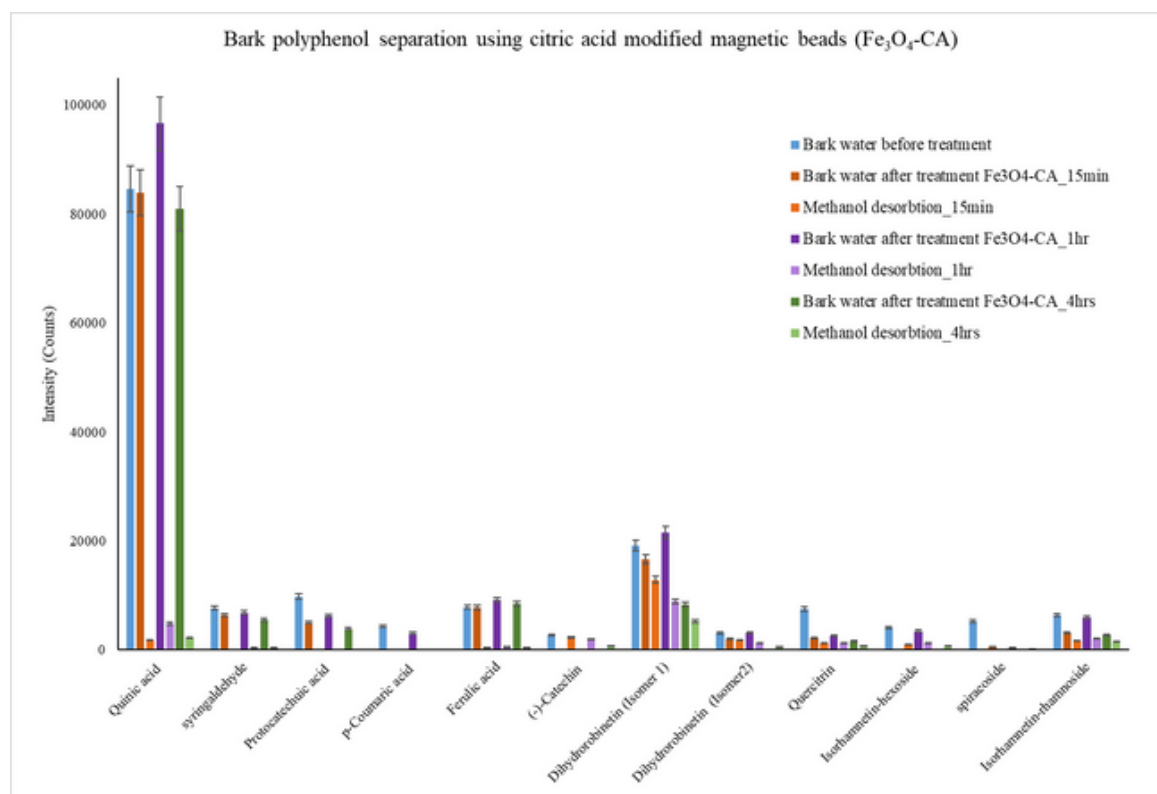


Figure 1. Bark polyphenol separation using citric acid modified magnetic beads ($\text{Fe}_3\text{O}_4\text{-CA}$).

Identification and determination of phenolic compounds in the methanol fraction after desorption from the Fe_3O_4 and $\text{Fe}_3\text{O}_4\text{-CA}$ were analyzed using LCMS-QTOF MS/MS. Based on the fragmentation pattern reported in the literature [2], quinic acid, syringaldehyde, p-coumaric acid, ferulic acid, dihydrorobinetin, isorhamnetin-hexoside, and spiraeoside were identified with Fe_3O_4 .

The efficiency of Fe_3O_4 and $\text{Fe}_3\text{O}_4\text{-CA}$ with different shaking time (15min, 1h, and 4h) was tested and the results for $\text{Fe}_3\text{O}_4\text{-CA}$ (2.5 g/L) is presented in Fig.1, where individual polyphenol concentrations are shown for bark water before and after $\text{Fe}_3\text{O}_4\text{-CA}$ treatment, and in methanol desorption samples. The results demonstrated that 15min shaking time is not sufficient to separate the polyphenols from the debarking water, whereas 4 h of shaking time degraded the polyphenols, as exhibited by the lower MS intensity counts. On the other hand, 1 h shaking time showed a better result compared to either 15 min or 4 h. Further experiments were also carried out with different $\text{Fe}_3\text{O}_4\text{-CA}$ concentrations (0.5 and 2.5g/L) and the results indicated that an increase in $\text{Fe}_3\text{O}_4\text{-CA}$ concentration enhances the extraction yield of polyphenols.

Therefore, the efficiency of bare Fe_3O_4 and $\text{Fe}_3\text{O}_4\text{-CA}$ for polyphenols separation was compared at the higher concentration (2.5 g/L & 1 h). $\text{Fe}_3\text{O}_4\text{-CA}$ extract contains additional polyphenols such as catechin, quercitrin, and isorhamnetin-rhamnoside. No such compounds can be found with bare Fe_3O_4 extraction. However, comparing the methanol desorption results with the debarking water before and after magnetic nanoparticles (Fe_3O_4 and $\text{Fe}_3\text{O}_4\text{-CA}$), it is evident that only a small quantity of the polyphenols was removed. Therefore, the follow-up investigation will focus on either increasing the concentration of $\text{Fe}_3\text{O}_4\text{-CA}$, different modification methods for Fe_3O_4 , or the possibility of several MNP extraction cycles.

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