ORGANISING COMMITTEE

Stefan Martens Fondazione Edmund Mach

Fulvio Mattivi University of Trento

Panagiotis Arapitsas Fondazione Edmund Mach

Urska Vrhovsek Fondazione Edmund Mach

Luisa Palmieri Fondazione Edmund Mach

Richard Blackburn University of Leeds

Floriana Marin Fondazione Edmund Mach

Michela Angeli Fondazione Edmund Mach

Gonzalo Cervantes Fondazione Edmund Mach

FONDAZIONE EDMUND MACH

UNIVERSITY OF TRENTO - Italy

C3A CENTRO AGRICOLTURA ALIMENTI AMBIENTE

10th International Workshop on Anthocyanins and Betalains - IWA&B 2019 September 9-11, 2019 San Michele all'Adige (TN), Italy

10th INTERNATIONAL WORKSHOP ON ANTHOCYANINS AND BETALAINS

09-11 September 2019
San Michele all'Adige (TN)
Italy

FONDAZIONE EDMUND MACH

UNIVERSITY OF TRENTO - Italy

C3A CENTRO AGRICOLTURA ALIMENTI AMBIENTE

Thank you to our workshop sponsors

SPEAKER

groupe polyphénols

MEDIA

biomedicines
an open access journal by MDPI

RED

Dr. Craft
Designed by Science • Made from Nature

SCIEX

ORANGE

the nature network®
PhytoLab

symrise

EXTRASYNTHESE

CHROMA
Custom-made, Healthy & Sustainable Colors

TransMIT
Gesellschaft für Technologietransfer mbH

PLANT META CHEM

SantOrsola

WINE

Vini del Trentino
CONSORZIO DI TUTELA

others

REDO
UPCYCLING

Thank you to our workshop sponsors

SPEAKER

groupe polyphénols

MEDIA

biomedicines
an open access journal by MDPI

RED

Dr. Craft
Designed by Science • Made from Nature

SCIEX

ORANGE

the nature network®
PhytoLab

symrise

EXTRASYNTHESE

CHROMA
Custom-made, Healthy & Sustainable Colors

TransMIT
Gesellschaft für Technologietransfer mbH

PLANT META CHEM

SantOrsola

WINE

Vini del Trentino
CONSORZIO DI TUTELA

others

REDO
UPCYCLING

WELCOME

The **10th International Workshop on Anthocyanins and Betalains** (IWA&B 2019) will be held in San Michele all'Adige (Italy), in region Trentino-Alto Adige/Südtirol which is located in the heart of the Italian Alps, well renowned for its excellent wine, famous fruits, and beautiful Dolomites mountains.

Anthocyanins are well known for their great diversity of colours, spanning practically the whole visible spectrum, from orange and red through to purple and blue hues. Due to their wide distribution in nature and structural diversity they have hit headlines and are linked to a range of health benefits and diverse functions in plants. Over the years, the scientific community has been focusing on these amazing molecules trying to understand their biosynthesis, properties, bioactivities and biological relevance, and possible applications. Given their relative abundance in the diet and their chemical and biological versatility, they represent a rich source of health promoting properties and only now are we truly beginning to understand how their absorption might relate to bioactivity. For the first time betalains, unique nitrogen-containing pigments found exclusively in families of the Caryophyllales order and some higher order fungi, where they replace anthocyanin pigments, are official included in the workshop topics.

Similarly, novel anthocyanin-enriching techniques are opening new opportunities for several applications in various industry sectors for manufacturing food and non-food products including their potential use as food additives, cosmetics and pharmaceuticals.

Since the year 2000, the International Workshop on Anthocyanins (IWA) has been the major bi-annual focus for cutting edge anthocyanin research dissemination. The proximity of many European countries with numerous R&D groups working on anthocyanins and betalains will surely contribute to the success of the 10th edition. IWA&B 2019 is an opportunity for academics and industrials to establish contacts with other scientists working with anthocyanins and betalains worldwide.

CONTENTS

THE VENUE

FONDAZIONE EDMUND MACH

In order to improve agriculture in what was then Tyrol, in 1874 the Austro- Hungarian Empire approved the institution of an agricultural school to be located in the Augustinian monastery of San Michele all'Adige. The school opened in the autumn with a program established by its first director, Edmund Mach. He was an efficient organizer and from the very outset fervently supported the plan to turn the institute into an innovative organization where teaching and research would together contribute to the development of agriculture in the region.

After the First World War, the Agricultural Institute of San Michele (IASMA) came under the control of the Italian government, which in turn passed it over to the authority of the Autonomous Province of Trento since 1948. In 1990 a local Law transformed IASMA into a functional agency of the Autonomous Province of Trento, integrating the land services into the pre-existing structure based on training and research. On 1st January 2008 the Institute's organization and activities were transferred to a new legal body, the Fondazione Edmund Mach (FEM), which is currently structured on the three pillars of Education and Training, Research and Innovation, and Technology Transfer.

Pioneering organisations, a young and dynamic environment, international researchers, collaborations with universities and institutions throughout the world, and the institution of high-level specialised training initiatives: all these together place FEM in a global context, which encourages the exchange of ideas and the development of innovation and produces internationally-recognised results.

Address

Fondazione Edmund Mach di San Michele all'Adige

Via E. Mach, 1 38010 S. Michele all'Adige (TN) – ITALY

Website: http://www.fmach.it

You can reach us

BY TRAIN: Direct trains to Trento run from Rome, Munich, Verona, Venice and Bologna. Mezzocorona is the nearest station (about 40 minutes by foot).All the train from Milan need change in Verona. If you are travelling within Italy by train have a look at TRENITALIA (www.trenitalia.it) to have updated information about the timetable.

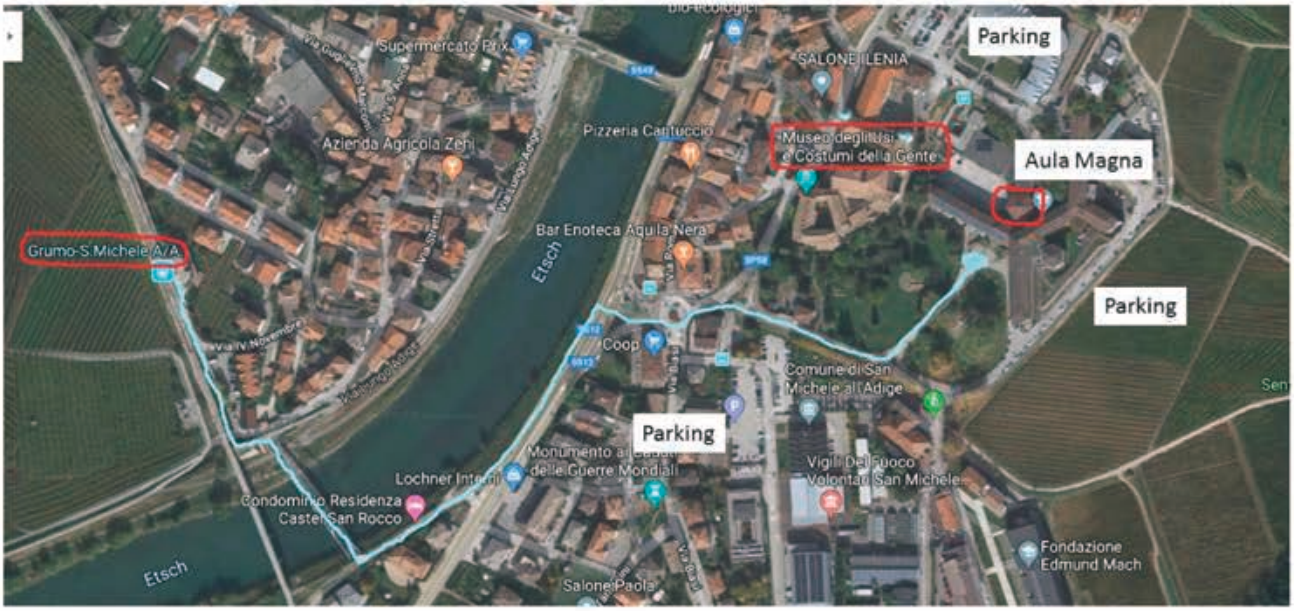
BY LOCAL TRAIN (Trentino Trasporti): Regional trains are running from Trento to San Michele all'Adige/Mezzocorona. Cost of the single way ticket is about 2,50 €. Find the timetable on https://www.trentinotrasporti.it/en/

If you choose the line "Trento-Malé" go to the small train station next to the main station in Trento. Leave at "Grumo" station. From there, take the foot pathway over a white bridge to cross the river; use the pedestrian street tunnel to cross the big street in front of S. Michele to reach the roundabout and then FEM (blue route in map).

BY BUS: Buses are running from Trento Bus Station (close to the Railway station) to San Michele all'Adige. Find detailed timetable on the Trentino Trasporti website. Note that the destination to look for is "San Michele all'Adige".

BY CAR: You can reach the venue driving on the Brennero (A22) motorway (San Michele all'Adige exit), Mezzocorona exit. The Campus is located 2 km eastward.

There are free parking areas in front of the campus (public parking) but also all over the campus available (indicated in map).



SOCIAL PROGRAMME

Sunday 8 September

Pre-registration & ice-breaking party

PALAZZO ROCCABRUNA
CAMERA DI COMMERCIO I.A.A. TRENTO

at **Palazzo Roccabruna**

Join us in Palazzo Roccabruna on Sunday evening! We will be glad to propose you a toast with typical Trentino wines, cheese, cold meats and sausages in the awful Home of Trentino products.

Monday 9 September

Social aperitif and wine tasting

The unique venue of the triangular cloister at **Trentino Folklife Museum** of San Michele all'Adige will host this event just some meters away from the conference venue. An evening accompanied by an expert sommelier from **Consorzio Vini del Trentino**. Wine tasting in the cloister with explanation of the properties of the best Trentino wines.

Tuesday 10 September

Conference dinner – Maso Franch

Located in a 19th century building amid vineyards, Maso Franch is approx. 15 to 20 min drive from San Michele all'Adige at the entrance to Val di Cembra .The restaurant features panoramic views of the Trentino countryside and with its menus, it proposes a culinary journey into the flavors and aromas of the region to discover the richness of the local wine and food heritage.

Busses will bring you directly from the venue to Maso Franch and afterwards back to San Michele and Trento.

Those who want to reach the restaurant independently from FEM have to take the SP 131 Wine Road / San Michele-Giovo. If you leave from Trento, instead, follow the directions to Lavis and then take the State Road 612 that goes up the Valle dell'Avisio. GPS coordinates 46.143325, 11.118678.

Wednesday 11 September (Optional – fee needed)

Rotaliana Wine Tour

managed by **Consorzio Vini del Trentino.**

September is also period of grape harvesting! During the tour you will have the unique opportunity to visit local vineyards and wineries: travel through the glass to see what makes the terroir of Trentino's vineyards stand apart, dive headlong into the latest wine science and, of course, taste our fantastic wines, in particular Teroldego, which is rich in anthocyanins!

Bus transfer from and back to San Michele will be organized.

SCIENTIFIC COMMITTEE

Biosynthesis and Genetics

- Stefan MARTENS, Italy
- Richard ESPLEY, New Zealand
- Kevin DAVIES, New Zealand
- Michal OREN-SHAMIR, Israel

Ecology, Function and Evolution

- Kevin GOULD, New Zealand
- Kathy SCHWINN, New Zealand
- Nicole HUGHES, USA
- Heidi HALBWIRTH, Austria

Health and Nutrition

- Mary Ann LILA, USA
- David VAUZOUR, United Kingdom
- Fulvio MATTIVI, Italy
- Sabina PASSAMONTI, Italy

Phytochemistry and Analysis

- Kumi YOSHIDA, Japan
- Urska VRHOVSEK, Italy
- Victor DE FREITAS, Portugal
- Olivier DANGLES, France

Application in Food and Industry

- Richard BLACKBURN, United Kingdom
- Panagiotis ARAPITSAS, Italy
- Cathie MARTIN, United Kingdom
- Véronique CHEYNIER, France

SPEAKERS

Marco Landi

University of Pisa (Italy) - Department of Agriculture, Food & Environment

Marco Landi is a senior scientist in plant physiology and biochemistry at University of Pisa, Italy. His research focuses on biochemical, molecular and physiological mechanisms through which Mediterranean plants accommodate environmental stress. Recent research has investigated the photoprotective role of carotenoids, anthocyanins and flavonoids sensu lato in plants subjected to climate change factors. His research group is also developing optical models to assess the occurrence of anthocyanin-metal bonding, to explore the possibility that anthocyanins might additionally function as metal chelators.

During IWA he will talk about "Fresh insights on the ecological role of foliar anthocyanins: the quest for a novel function" in the Ecology, Function and Evolution session.

Katia Petroni

University of Milano (Italy) - Dipartimento di Bioscienze

Katia Petroni is Associate Professor of Genetics in the Department of BioSciences at the University of Milan (IT), where she teaches Nutrigenomics, Genetics and Plant Biotechnology. Since the beginning of her academic life, she has studied regulatory genes controlling flavonoid and anthocyanin biosynthesis in plants. In the last 15 years, her research has focused on the development of model foods enriched in anthocyanins and their use in nutrigenomic studies to determine the protective role of anthocyanins on health.

Prof. Petroni will present a talk entitled "Cardioprotective and anti-inflammatory properties of anthocyanin-rich corn" during the Health and Nutrition session.

SPEAKERS

Elke Richling

Technische Universität Kaiserslautern (Germany) - Department of Food Chemistry and Environmental Toxicology

Elke Richling will present the talk entitled "Absorption and distribution of anthocyanin rutinosides in humans after consumption of a blackcurrant (*Ribes nigrum* L.) extract" during the Health and Nutrition session.

Nuno Mateus

University of Porto (Portugal) - Department of Chemistry and Biochemistry - Faculty of Science

Nuno Mateus graduated in Biochemistry in 1997 and obtained his PhD in Chemistry in 2002 both at the University of Porto (Portugal). He has been teaching at the University since 2002 and is currently an Associate Professor at the Department of Chemistry and Biochemistry of the Faculty of Science at the University of Porto, where he has been teaching Food Chemistry and Industrial Biochemistry (amongst other courses). His field of research concerns food chemistry and biochemistry, essentially food polyphenols and in particular red wine chemistry. He has been collaborating with local industrial companies (especially Port wine companies) and has been involved in several research projects funded essentially by the Portuguese Government. Presently, one of his main areas of research deals with the bioavailability and biological properties of food phenolics towards some cancers and age-related diseases. More recently, he started a new research line focused on the recycling of polyphenols from industrial wastes to use them in novel cosmetic formulations.

He will present a talk entitled "Exploring the colour and bioactivity of anthocyanin derivatives" in the session Application in Food and Industry.Dr. Damasio will talk about "Trends in the Use of Anthocyanins as Colour in Food Industry" in the session Application in Food and Industry.

SPEAKERS

Paolo Morazzoni

Indena S.p.A. (Italy)

Paolo Morazzoni has a degree in Biological Sciences from University of Milan. After a scientific fellowship in Biochemistry and Enzymology at Mario Negri Insitute of Pharmacology of Milan (1976-1980), he worked as senior scientist in the Pharmacological Department of Inverni Della Beffa S.p.A. (1980-1982) where then he covered the role of Head of the Metabolism and Phamacokinetic Laboratories (1983-1993) concentrating his interest on the metabolism and pharmacokinetics of pharmaceutical products of botanical origin. From 1993 to 1997 he was the Associate Scientific Director of Indena S.p.A., Milan, taking care of the development of new botanical active derivatives in different therapeutical area including cardiovascular, CNS and oncology. Since 1998 he is the Scientific Director of Indena S.p.A. with the specific role of coordinating the research and development of new botanicals, being oncology the main area of activity. Dr. Paolo Morazzoni is the author of more than 300 research articles on medical and biological issues (with particular attention to phytotherapy) and an active participants to international symposia and congresses. He is also inventor/co-inventor of more than 50 Patents.

His talk during the conference will concern "Bilberry anthocyanins:industrial development of a clinically effective standardized extract".

Maria Helena Damásio

R&D and Regulatory Advisor – Coralim Ingredients & Colours (Spain)

Maria Helena Damásio is working as R&D and Regulatory Advisor at Coralim Ingredients & Colours. Previously, she worked 20 years for the colour manufacturer Roha, where she was R&D Manager and, in the last 2 years, the company's Global Regulatory Coordinator. Her previous job was as Professor in the Faculty of Food Engineering at the University of Campinas, Brazil. She is Food Engineer and Ph.D. in Food Technology.

Dr. Damasio will talk about "Trends in the Use of Anthocyanins as Colour in Food Industry" in the session Application in Food and Industry.

SPEAKERS

Chiara Cerletti

IRCCS Istituto Neurologico Mediterraneo NEUROMED, Italy

Chiara Cerletti, Biologist, PhD in Pharmacology, Head of the Laboratory of Nutraceuticals, Department of Epidemiology and Prevention, IRCCS Neuromed (Pozzilli, IS). Long experience in experimental and human studies on biochemistry and pharmacology of blood platelet function. Contribution to the FLORA and ATHENA EU projects on the biological activities of anthocyanin from different natural sources. Specific expertise in clinical trials with drugs and nutraceuticals on the cardiovascular system. Collaboration since its start to the epidemiological study Moli-sani on genetic and environmental risk factors of chronic degenerative disease. H.I. 55, listed among the "Top Italian Women Scientists".

Her presentation will be on "Anthocyanins, polyphenols and Mediterranean diet: messages from the Moli-sani study".

Erika Salas-Muñoz

Universidad Autónoma de Chihuahua (Mexico)

PhD in Food Science. Ecole Nationales Supérieure Agronomique de Montpellier. S.N.I. nivel II. Full professor at the University of Chihuahua.

Erika will discuss about "Analysis of Anthocyanins by mass spectrometry" during the Phytochemistry and Analysis session.

SPEAKERS

Cédric Saucier

University of Montpellier (France)

Since 2014 prof. Saucier has been Director in the Oenology research and education programme at the University of Montpellier.

Richard Espley

Plant and Food Institute (New Zealand)

Dr. Espley completed a BSc in horticultural science at Reading University where he became very interested in the possibilities of biotechnology, so he joined the plant science division at Syngenta in the UK and worked on banana biotechnology, looking at ways of improving the nutritional content, in particular of pro-vitamin A. In 2002 dr. Espley moved to New Zealand to join Plant & Food Research and completed a PhD in molecular biology at The University of Auckland. In 2009, he was named one of the MacDiarmid Young Scientists of the Year for his research in apple genetics. Currently he is involved in research about plant pigments, plant gene expression, plant Biotechnology.

During IWA 2019 we will explore how to "Filling the Void – boosting the nutritional value of blueberry" with a speech to be held during the Biosynthesis and Genetics session.

PROGRAMME

Sunday 8 September 2019

18.00 – 20.00	**Get together & pre-registration (Palazzo Roccabruna, Trento)**

Monday 9 September 2019

8.00 – 9.00	**Registration opens**
9.00 – 9.15	**Welcome & Opening**
9.15 – 10.55	**SESSION 1: Ecology, Function and Evolution – Chair: Kevin Gould**
	PLEANARY 1: Marco Landi
	Fresh insights on the ecological role of foliar anthocyanins: the quest for a novel function
	Short 1: Kathy Schwinn
	Unlocking how red betalain pigments combat salt stress
	Short 2: Nicole Hughes
	Anthocyanin profiles of red halos surrounding *Entomosporium mespili* infections are similar to those of senescing and expanding leaves, suggesting an analogous function
	Short 3: Tanja Karl
	Betacyanins increase salt tolerance in Australasian halophytes
10.55 – 11.25	**Coffee break**
11.25 – 12.55	**SESSION 2: Application in Food and Industry - Chair: Richard Blackburn**
	KEYNOTE 1: Maria Helena Damasio
	Trends in the Use of anthocyanins as colour in food industry
	Short 4: Meryem Benohoud
	Application of anthocyanins from fruit waste in cosmetics
	Short 5: Nikitia Mexi
	Valorisation of food industry waste via a biotransformation protocol for application in consumer products
	Short 6: Ana Luísa Fernandes
	Molecular binding between anthocyanins and pectic polysaccharides: unveiling the role of pectic polysacchardies methylation degree
12.55 – 14.15	**Lunch break**

PROGRAMME continued

14.15 – 15.25	**Session 3: Phytochemistry and Analysis - Chair: Olivier Dangles**
	Keynote 2: Cedric Saucier
	Polyphenols of rosé wines: Impact on color, aroma and influence of origin
	Short 7: Kumi Yoshida
	MS analysis of hydrangea blue-complex pigment and its direct mapping in sepals of *Hydrangea macrophylla*
	Short 8: Julie-Anne Fenger
	Thermal degradation of red cabbage anthocyanins with different acylation pattern at neutral pH, and impact of metal binding
15.25 – 15.55	**Coffee break**
15.55 – 17.05	**Session 4: Biosynthesis and Genetics - Chair: Kevin Davies**
	Keynote 3: Richard Victor Espley
	Filling the void – boosting the nutritional value of blueberry
	Short 9: Henrik Brinch-Pedersen
	Dissection and engineering the anthocyanin biosynthetic pathway in orange and black carrots
	Short 10: Ralf Stracke
	Functional analysis of flavonoid biosynthesis genes in *Beta vulgaris*
17.05 – 18.00	**Poster session**
18.00 – 20.00	**WINE tasting and Aperitivo (Trentino Folklife Museum, San Michele all'Adige)**

PROGRAMME continued

Tuesday 10 September 2019

9.00 – 10.30	**SESSION 5: Health and Nutrition - Chair: Mary Ann Lila**
	KEYNOTE 4: Katia Petroni
	Cardioprotective and anti-inflammatory properties of anthocyanin-rich corn
	Short 11: Odette Marianne Shaw
	Cyanidin and ellagitanin rich BerriQi™ Boysenberry with apple juice protects against chronic lung inflammation
	Short 12: Sabina Passamonti
	Anthocyanins in colorectal cancer prevention. A systematic review of the literature in search of molecular oncotargets
	Short 13: Yang Baoru
	Impact of anthocyanins from purple potatoes on sugar metabolism and Type II Diabetes
10.30 – 11.00	**Coffee break**
11.00 – 12.10	**Session 6: Phytochemistry and Analysis - Chair: Kumi Yoshida**
	Plenary 2: Erika Salas
	Analysis of anthocyanins by mass spectrometry
	Short 14: Luis Cruz
	Impact of a water-soluble gallic acid-based dendrimer on the color-stabilizing mechanisms of anthocyanins
	Short 15: Joana Oliveira
	Stabilization of bluish pyranoanthocyanin pigments in aqueous systems using lignin nanoparticles
12.10 – 13.40	**Lunch break**
13.40 – 15.40	**Session 7: Biosynthesis and Genetics - Chair: Richard Espley**
	Short 16: Cathie Martin
	How do anthocyanins get to the vacuole?
	Short 17: Jean Chaudiere
	Oxidative transformation of flavan-3-ols by anthocyanidin synthase from *Vitis vinifera* and the role of glutathione

PROGRAMME continued

	Short 18: Sarah Moss
	Why so repressed? Evolution and mechanism for suppressed anthocyanin biosynthesis altering flower colour in snapdragon
	Short 19: Yongyan Angel Peng
	Regulation of the branch points in the anthocyanin pathway is distinct to the regulation of the core pathway
	Session 8: Health and Nutrition - Chair: Victor de Freitas
	Plenary 3: Nuno Mateus (GP Sponsored)
	Exploring the colour and bioactivity of anthocyanin derivatives
15.40 – 16.10	**Coffee break**
16.10 – 17.00	**Session 9: Anthocyanins in Mediterranean Diet - Chair: Fulvio Mattivi**
	Evening lecture: Chiara Cerletti
	Anthocyanins, polyphenols and Mediterranean diet: messages from the Moli-sani study
17.00 – 18.00	**Poster session**
19.00 – 22.30	**Conference dinner (Maso Franch, Giovo)**

PROGRAMME continued

Wednesday 11 September 2019

9.30 – 11.00	**SESSION 10: Application in Food and Industry - Chair: Nuno Mateus**
	KEYNOTE 5: Paolo Morazzoni
	Bilberry anthocyanins: industrial development of a clinically effective strandardized extract
	Short 20: Olivier Dangles
	The influence of acylation on the color and pigment stability of red cabbage anthocyanins under neutral conditions
	Short 21: Pradeep Kumar
	Deciphering the mechanism of glycosylated anthocyanin and flavonols in red mango fruits tolerance to fungal pathogens
	Short 22: Dominik Durner
	Influence of different winemaking techniques on anthocyanin fingerprints of varietal wines
11.00 – 11.30	**Coffee break**
11.30 – 12.40	**Session 11: Health and Nutrition - Chair: Sabina Passamonti**
	Keynote 6: Elke Richling
	Absorption and distribution of anthocyanin rutinosides in humans after consumption of a blackcurrant (*Ribes nigrum* L.) extract
	Short 23: Hélder Oliveira
	Recent Advances on the bioavailability of anthocyanins: a molecular approach.
	Short 24: Mary Ann Lila
	Can berry polyphenolics/anthocyanins protect the skin from environmental insult?
12.40 – 13.00	**Closing words – announcement of IWA&B 2021**
13.00 – 14.00	**Lunch boxes**
14.00 – 18.00	**Post conference wine tour**

ORAL PRESENTATION ABSTRACTS

Fresh insights on the ecological role of foliar anthocyanins: the quest for a novel function

Marco Landi

University of Pisa
marco.landi@agr.unipi.it

Marco Landi

Department of Agriculture, Food and Environment - University of Pisa, 56124 Pisa, Italy

"Dad, why are those leaves red?" None of my young son's simple questions has required so complex an answer. Most researchers accept that anthocyanins act as effective sunscreens, thereby ameliorating a plant's performance under environmental stressors. However, a considerable number of researches have failed to find any photoprotective function of foliar anthocyanins, and there is evidence that these pigments might serve additional critical functions in leaves. Here, I will present evidence for a novel function of foliar anthocyanins. When leaves of constitutively red- and green-leafed Prunus species were compared for photosynthetic assimilation rates, carbon metabolism, and photoprotective mechanisms throughout leaf ontogeny, we observed that anthocyanin biosynthesis correlated with carbon sink strength in young and senescent leaves, thus extending the leaf lifespan of purple Prunus. The dynamic interplay between anthocyanin biosynthesis, photosynthesis, sugar metabolism, and photoprotection is also of crucial importance during the autumn foliage, especially at locations characterized by high incident light and lower temperatures during the phase of chlorophyll degradation. This observation potentially offers an evolutionary explanation for the geographical distribution of red or yellow autumn leaves worldwide. A "sugar buffering" capacity, together with a photoprotective role (i.e., light abatement and the prevention of reactive oxygen species generation) offered by anthocyanins, may provide a unifying explanation for their biosynthesis in leaves, which could be collectively referred as to "photo-modulation". Perhaps this is not the right answer to my son, but these observations present the possibility of a new avenue of research into the ecophysiological functions of anthocyanin pigments.

Notes

Unlocking how red betalain pigments combat salt stress

Kathy Schwinn

Plant and Food Research
kathy.schwinn@plantandfood.co.nz

Yanfei Zhou[1], Tanja Karl[2], David H. Lewis[1], Steve Arathoon[1], Ken Ryan[2], Kevin M. Davies[1], Kevin S. Gould[2], Kathy E. Schwinn[1]

[1] The New Zealand Institute for Plant and Food Research Limited, Private Bag11-600, Palmerston North, New Zealand
[2] School of Biological Sciences, Victoria University of Wellington, PO Box 600, Wellington 6140, New Zealand

Saline soils are a growing problem and pose a threat to food production and environmental sustainability. As many crops are salt sensitive, there is interest in understanding the mechanisms for salt tolerance that occur naturally in some plants and whether that can be applied to the development of crops that are resilient to salt stress. Betalain pigments rather than anthocyanins are produced within the core Caryophyllales. Betalains (red or yellow) are formed in a pathway unrelated to that of the anthocyanins, having a tyrosine-derived central chromophore. Betalain taxa include species that survive in challenging environments such as salt marshes and sand dunes. We are studying how red betalain pigments (betacyanins) allow the native New Zealand iceplant Disphyma australe to live under high salt conditions. We are using a variety of physiological and molecular approaches. We have conducted large scale RNA-seq analyses to understand gene expression changes in response to salt and light treatments in Disphyma australe. We also have transferred betacyanin production to the anthocyanin accumulating species, Nicotiana tabacum (tobacco), in order to test whether betacyanins can improve salinity tolerance in tobacco. Our results will be presented as well as our insights into betacyanin-based salinity tolerance.

Notes

Anthocyanin profiles of red halos surrounding *Entomosporium mespili* infections are similar to those of senescing and expanding leaves, suggesting an analogous function

Nicole Hughes

High Point University
nhughes@highpoint.edu

Nicole M. Hughes[1], Sarah J. Forte[1], Harrison Seitz[1], Andrew J. Wommack[2], Mary H. Grace[3], Mary Ann Lila[1]

[1] Department of Biology, High Point University, High Point, North Carolina, USA;
[2] Department of Chemistry, High Point University, High Point, North Carolina, USA;
[3] Plants for Human Health Institute, North Carolina State University, Kannapolis, North Carolina, USA

The function of red halos commonly observed around infected spots on leaves is currently unknown. Recent studies have demonstrated that anthocyanin "fingerprints" may serve as a clue to the pigment's biochemical or physiological function. In the current study we used microscopy and HPLC-MS to characterize anthocyanins in the red halos surrounding Entomosporium mespili infection in leaves of Photinia glabra, and compared these to profiles found in red senescing and expanding leaves of the same plant; uninfected, fully-expanded green leaves were used for comparison, in addition to green tissues around the red halo of infected leaves. Anthocyanins in the red halo were the same five cyanidin-based anthocyanins found in young leaves, in similar proportions and concentrations. Senescing leaves contained four of these five anthocyanins (at ~25% concentration), while green tissues contained only very small amounts of either one (uninfected leaves) or two (infected leaves) of these anthocyanins. Cyanidin-3-galactoside comprised the greatest proportion (>75%) of the anthocyanin pool in all red tissues. Uninfected green leaves lacked this anthocyanin entirely, and only contained trace amounts of cyanidin-3-glucoside; in contrast, green areas of infected leaves contained 60:40 proportions of cyanidin-3-galactoside and cyanidin-3-glucoside. Interestingly, concentrations of cyanidin-3-glucoside were relatively constant in all leaves sampled, except in senescing leaves, where the pigment was absent. All cases of reddening corresponded with anthocyanins in palisade and spongy mesophyll tissue, reduced chlorophyll content, and reduced Fv/Fm (maximum light capture efficiency of PSII). We conclude that anthocyanins synthesized in response to E. mespili infection are likely not functioning directly in pathogen defense, and more likely play a role similar to anthocyanins in young and senescing leaves--namely light-attenuation and neutralization of reactive oxygen species.

Notes

Betacyanins increase salt tolerance in Australasian halophytes

Tanja Karl

Victoria University of Wellington
tanja.karl@vuw.ac.nz

Tanja Karl[1], Yanfei Zhou[2], Ken G.Ryan[1], Kevin M. Davies[2], Kathy E. Schwinn[2], Sergey Shabala[3] and Kevin S. Gould[1]

[1] Victoria University of Wellington, PO Box 600, Wellington, New Zealand
[2] The New Zealand Institute for Plant & Food Research, Private Bag 11 600, Palmerston North 4442, New Zealand
[3] School of Agricultural Science, University of Tasmania, Private Bag 54, Hobart, Tas 7001, Australia

Soil salinity is an increasingly important challenge to agricultural production. While most plants succumb to salt stress, some halophytes such as *Disphyma australe* thrive in saline soil. A potential reason for this could be the presence of betacyanin, a red-pigmented alkaloid found in the salt-exposed leaves of *D. australe*. Previous research has shown that the betacyanins, with their photoprotective function and their involvement in the sequestration of toxic ions, may benefit plants under salt stress.

To understand the mechanism by which betacyanins afford salt tolerance, we hypothesised that these pigments might alter the histological distribution of Na+, thereby avoiding cytotoxic effects of sodium on the photosynthetically active tissues. Using fluorescence microscopy and cryo-SEM coupled with energy dispersive X-ray spectroscopy on red and green-leafed *D. australe*, we found that in red leaves Na+ was mostly concentrated in the betacyanin-containing epidermal cells, away from the photosynthetic tissues. By contrast, in green leaves Na+ was equally distributed across tissues.

We also hypothesized that betacyanins might mitigate the cytotoxic effects of Na+ by preventing K+ leakage upon exposure to salt stress. Using Microelectrode Ion Flux Estimation (MIFE) to measure ion fluxes of mesophyll tissues from betacyanic- and green-leaves of two Disphyma species, we discovered that these halophytes not only retain K+ under salt stress, but also uptake additional K+ to maintain higher Na+/K+ ratios, thereby reducing the cytotoxic effects of Na+. This previously-undocumented discovery represents an entirely new mechanism for salinity tolerance in plants and has opened new avenues for research into this global problem.

Notes

Trends in the Use of anthocyanins as colour in food industry

Maria Helena Damasio

Coralim Ingredients & Colours
mhdamasio@coralim.com

Maria Helena Damásio

R&D and Regulatory Advisor – Coralim Ingredients & Colours

Appearance is the first thing that we notice about food and the colour is the most important factor in this attribute. Anthocyanins are commonly used as food colour in the European Union (EU) and around the world, being always considered as natural colour. They can be used to impart shades from vivid red to pink or purple, depending on the fruit or vegetable where the anthocyanin is extracted from.

Since many years, the use of natural colours has increased substantially and red artificial colours have been replaced by anthocyanins E 163. However, as the consumer perception relates the E number with "not natural", the most recent trend is to look for "clean labels" (foods without any additive, no E number).

In terms of colours, the "clean label" ingredients are the colouring foodstuffs (CF). This is a concept originated in EU that cannot be applied in USA, for example, where any ingredient used to provide colour is considered as an additive. However, the use of CFs has been extended in other countries, even without local regulations for that.

The following definition of CF can be drawn out of the EU "Guidance notes on the classification of food extracts with colouring properties" of November 2013: "a food ingredient derived from a food source processed in such a way so as not to selectively extract the pigment(s), even when used principally for the purpose of coloration of final application". From the 22 sources to be listed in the Annex III (not published yet) of this Guidance, 13 out of them are sources of anthocyanins: aronia, blackberry, blackcurrant, blueberry, black carrot, cherry, elderberry, grape, hibiscus, raspberry, red cabbage, red radish and purple sweet potato.

Notes

Application of anthocyanins from fruit waste in cosmetics

Meryem Benohoud

Keracol Ltd
m.benohoud@keracol.co.uk

Meryem Benohoud[1], Alenka Tidder[1], Richard S. Blackburn[2], Chris M. Rayner[3]

[1] Keracol Ltd, Leeds, UK;
[2] School of Design, University of Leeds, Leeds, UK;
[3] School of Chemistry, University of Leeds, Leeds, UK.

Blackcurrant, aronia and cherries are rich sources of anthocyanins, and these pigments are predominantly present in the berries' epicarp. The fruits are used in the food industry to produce various juices, jams and other preparations. Waste skins are obtained in large quantities from these products, representing a sustainable, food-grade raw material. However, anthocyanins from fruit sources have moderate stability in aqueous environments, they are also pH sensitive and overall present application limitations. Over the past years, Keracol Ltd has developed scalable processes for the extraction and purification of anthocyanins. Our studies on the extraction processes as well as the applications in formulation allow us to gain a better understanding of anthocyanin chemistry. For instance, the preservation of glycosylation in the extraction process and purification steps is of utmost importance as it enables advantageous formulation and stability. Our research efforts aim at the use of these extracts in consumer products, and in particular colour cosmetics as a natural and sustainable source of pigments and colour. We exploit biomimetic approaches for the design of our formulations that are now available under the brand Dr. Craft. Herein we will present the technology developed and used in Dr. Craft's Natural Purple Berry Brightening serum, a hair care product that neutralises yellow and brassy tones in blonde, silver and grey hair by depositing blue pigments onto the hair. Other products on the market use synthetic dyes, such as Acid Violet 43 and HC Blue No. 2, but the Dr. Craft product uses blue pigments result from delphinidin and cyanidin derivatives present in our blackcurrant extract and actually provides superior technical performance in comparison with the synthetic alternative

Notes

Valorisation of food industry waste via a biotransformation protocol for application in consumer products

Nikitia Mexia

University of Leeds
n.mexia@leeds.ac.uk

Nikitia Mexia[1,2], Meryem Benohoud[3], Christopher M. Rayner[2,3], A. John Blacker[2], Richard S. Blackburn[1,3]

[1]Sustainable Materials Research Group, School of Design, University of Leeds, LS2 9JT, UK;
[2]School of Chemistry, University of Leeds, LS2 9JT, UK;
[3]Keracol Limited, Nexus, Discovery Way, Leeds, LS2 3AA, UK

In general, anthocyanins (ANCs) lack stability in many environmental conditions, but acylated ANCs are more stable than their non-esterified counterparts. Acylation is typically the final biosynthetic step and acylated ANCs are widely distributed in nature. Large amounts of ANCs remain unexploited as waste from several food industries. This research aims to modify ANCs derived from food waste using enzyme-catalysed biotransformation reactions to produce novel compounds for application in food and cosmetics. Enzymes possess regio- and enantio-selective catalytic properties, and can be recovered and recycled; hence, they are potentially eco-friendly and sustainable. Lipases can be used with fatty acids both for ester formation and hydrolysis, depending on the substrate structure and water availability. *Candida Antarctica* lipase B immobilized on acrylic resin was employed on a model flavonoid system (the disaccharide rutin) and, after many trials under differentiated conditions where various factors were modified, an efficient and environmentally friendly acylation protocol was developed. Using *Candida Antarctica* lipase B at 55 °C over 72 hours, with silicon dioxide as dehydrating agent, led to conversion ratios of up to 50%. This optimised protocol was then applied successfully on ANC-rich food waste, specifically blackcurrant skins left over after the berries had been pressed for juice, and transferred to pilot scale. The derivatives produced were isolated, characterized and evaluated for their lipophilicity and antioxidant activity. This procedure enabled further investigation of the valorisation of ANC-rich food waste, offering a viable process leading to compounds with improved characteristics that can be formulated in novel consumer products.

Notes

Molecular binding between anthocyanins and pectic polysaccharides: unveiling the role of pectic polysacchardies methylation degree

Ana Fernandes

Universidade do Porto
ana.fernandes@fc.up.pt

Ana Fernandes[1], Joana Oliveira[1], Nuno Mateus[1], Jean-Paul Vincken[2], Victor de Freitas[1]

[1] REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências da Universidade do Porto, Portugal
[2] Wageningen University, Laboratory of Food Chemistry, Bornse Weilanden 9, 6708 WG Wageningen, The Netherlands

Anthocyanins-pectic polysaccharides interactions have been shown to be important for the colour stabilization mechanisms of anthocyanins. In this work, isothermal titration calorimetry (ITC), NMR and UV-Visible spectrophotometry were used to study the interactions between a representative food anthocyanin and four pectic polysaccharides differing in substitution pattern, at pH 3.5. The main goal of this work was to understand the role of pectic polysaccharides fine structure (methylation/amidation degree and pattern of esterification) for anthocyanins-pectic polysaccharides binding affinity.

For that, low methylesterified (HG 30%), amidated (AHG 30%) and high methylesterified (HG-B and HG-R 70%) pectic polysaccharides were selected to study their binding affinity with cyanidin-3-O-glucoside. The binding constant (Ka) and associated thermodynamic binding parameters determined showed a weak non-covalent interaction between cyanidin-3-O-glucoside and the selected pectic polysaccharides. Equilibrium binding constants determined for the interaction with the different homogalacturonans fractions were in the range 104 to 102 M-1 (expressed per galacturonic acid equivalents) and in the order: HG 30% > AHG 30% > HG-B 70% > HG-R 70%. These results were correlated with anthocyanins colour impact, resulting on a higher red colour intensity of a cyanidin-3-O-glucoside model solution due to HG 30% fortification probably due to the establishment of electrostatic interactions between anthocyanin flavylium cations and free pectic carboxyl groups.

The presented results are expected to promote the application of natural pectic polysaccharides as anthocyanins colour modulators.

Notes

Polyphenols of rosé wines: Impact on color, aroma and influence of origin

Cédric Saucier

University Montpellier, INRA
cedric.saucier@umontpellier.fr

Cédric Saucier[1], Mélodie Gil[1], Christelle Reynes[2], Fabian Avila[3], Philippe Louazil[4], Guillaume Cazals[5], Véronique Cheynier[1], Christine Enjalbal[5], Nerea Iturmendi[4], Leonardo Santo[3], Robert Sabatier[5], Virginie Moine[4]

[1] SPO, Univ Montpellier, INRA, Montpellier SupAgro, Montpellier, France.
[2] Univ Montpellier, IGF, CNRS INSERM, Montpellier, France
[3] Laboratory of Asymmetric Synthesis, Institute of Chemistry and Natural Resources, Universidad de Talca, Talca, Chile.
[4] Biolaffort, 126 Quai de la Souys, 33100 Bordeaux, France.
[5] Univ Montpellier, IBMM, Montpellier, France

Rosé wine is becoming more and more popular in France with sales rising from 11 % to 32 % within the last 20 years. A tendency is to have rosé wines with a lighter pink color with an aroma close to white wines with a higher varietal thiol content. The use of different grape varieties, the soil where they grow, the viticulture and the winemaking technology used will have impact on the polyphenol, color and aroma content of these wines. A very common practice during rosé winemaking is the use of Polyvinylpolypyrrolidone (PVPP) to adjust its color an polyphenol content. We have investigated the polyphenol adsorption by PVPP by comparing their polyphenol content compared to controls (biological triplicates) by UPLC-ESI-MS/MS. Specific adsorption affinity for some polyphenols like flavanols and anthocyanins were measured. Coumaroylated anthocyanins showed strong adsorption affinity compared to the other anthocyanins. Molecular modelling calculations were used to further understand this specific binding affinity. Last but not least, we showed that the thiol aroma content of rosé wine wines can be increased up to 200 % by using PVPP compared to the controls.

In another study the influence of the origin of rosé wine was investigated by semi-targeted polyphenomics. 60 commercial wines from the Bordeaux, Languedoc and Provence regions were discriminated by a few key polyphenolic markers. This was achieved by using two independent wines and datasets and a specicific data analysis algorithm.

Notes

MS analysis of hydrangea blue-complex pigment and its direct mapping in sepals of *Hydrangea macrophylla*

Kumi Yoshida

Nagoya University
yoshidak@i.nagoya-u.ac.jp

Takaaki Ito[1], Dan Aoki[2], Kin-ichi Oyama[3], Kazuhiko Fukushima[2], Tadao Kondo[4], Kumi Yoshida[4]

[1] Graduate School of Information Science, Nagoya University, Chikusa, Nagoya 464-8601, Japan
[2] Graduate School of Bioagricultural Sciences, Nagoya University, Chikusa, Nagoya 464-8601, Japan
[3] Research Center for Materials Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan
4 Graduate School of Informatics, Nagoya University, Chikusa, Nagoya 464-8601, Japan

The blue sepal color of *Hydrangea macrophylla* is due to a metal complex anthocyanin composed of 3-O-glucosyldelphinidin (1) and an Al3+ with the copigments 5-O-caffeoylquinic acid (2) and/or 5-O-p-coumaroylquinic acid (3). These are essential for blue color development, but the complex is unstable and only exists in aqueous solution, thus, the chemical structure was not clarified. We could obtain the same blue solution as that of sepals by mixing with 1, 2 or 3 and Al3+ in buffered solution of pH 4. To determine the composition of the complex, we tried measurement of the solution by electrospray-ionization mass spectrometry (ESI-TOF MS). When hydrangea blue-complex was re-constructed in a low-concentration of buffer (2.0 mM), we succeeded to detect the molecular ion peak of the hydrangea blue-complex at m/z = 843 (positive detection) and m/z = 841 (negative detection), indicating that the blue solution has a ratio of 1:1:1 for the complex. By using 3, the observed mass number was m/z = 827 (positive detection) and the ratio of 1, 3 and Al3+ was also 1:1:1.

Next, to map the distribution of hydrangea blue-complex in sepal tissues, we carried out cryo-time-of-flight secondary ion mass spectrometry analysis (cryo-TOF-SIMS). The analysis of the reproduced hydrangea blue-complex with negative mode-detection gave a molecular ion at m/z = 841, which was consistent with the results of ESI-TOF MS. We could detect the same molecular ion peak at m/z = 841 in the freeze-fixed blue sepal-tissue. In sepals, the blue cells were located in the second layer and the mapping of the ion at m/z = 841 overlapped with the same area of the blue cells. In colorless epidermal cells, atomic ion of Al3+ was hardly detected and only potassium adduct ion of 5-O-caffeoyl and/or 3-O-acylquinic acid were found. This is the first evidence that aluminum and hydrangea blue-complex exist in blue sepal cells and are involved in blue coloration.

Notes

Thermal degradation of red cabbage anthocyanins with different acylation pattern at neutral pH, and impact of metal binding

Julie-Anne Fenger

Avignon University, INRA
julie-anne.fenger@univ-avignon.fr

Julie-Anne Fenger[1], Rebecca J. Robbins[2], Tom M. Collins[2], Olivier Dangles[1]

[1] Avignon University, INRA, UMR408, 84000 Avignon, France;
[2] Mars Wrigley Confectionary, 800 High St., Hackettstown, NJ 07840, USA

Red cabbage contains cyanidin glycosides acylated by hydroxycinnamic acid (HCA) residues that can express vibrant blue colors at pH 8 or at lower pH after addition of Fe2+ ions. Although the color is relatively stable at room temperature, even moderate heating (50°C) triggers irreversible processes leading to a myriad of derivatives, most being colorless.

After acidification, product analysis was carried out by UHPLC-DAD-MS with ion trap and Q-ToF analyzers. Fe2+ ions were mostly deleterious to the non- and mono-acylated anthocyanins. By contrast, the stability of diacylated anthocyanins was not impacted by Fe2+. In the latter case, tight Fe2+-pigment binding (confirmed by independent experiments) is proposed as a stabilization mechanism.

From the structural analysis of the main products, a scenario for the degradation of anthocyanins at neutral pH is proposed: a) autoxidation of the electron-rich anionic base with concomitant H2O2 production, b) addition of H2O2 to the cyanidin nucleus (possibly, also to the trans-chalcone) followed by Bayer-Villiger rearrangements and subsequent cleavage of the C1'-C2 and C2-C3 bonds, leading to products with C6 to C9 skeletons, some possibly evolving by oxidative dimerization. In the process, the acylated glycosyl group at C3-OH is generally released. Interestingly, HCA residues acylating secondary OH groups appeared prone to migration within the glycosyl moiety, not only in the anthocyanins but also in the cleavage products.

In conclusion, double acylation and/or tight iron binding favor the expression of stable blue colors under neutral conditions. However, protecting anthocyanins against autoxidation is also very important. To obtain stable blue colors for food or cosmetic products, acylated anthocyanins are prime candidates but strategies should be carefully designed to also include pH control, optimal metal binding and addition of antioxidants / H2O2 scavengers.

Notes

Filling the void – boosting the nutritional value of blueberry

Richard Espley

Plant & Food Research
richard.espley@plantandfood.co.nz

Blue J. Plunkett[1], Declan Lafferty[2,3], Andrew P. Dare[1], Nick W. Albert[2], Catrin Guenther[1], Tony McGhie[2], Janice L. Turner[4], Lisa Jones[5], Ella Grierson[2], Kathy E. Schwinn[2], Kevin M. Davies[2], Andrew C. Allan[1,3], Laura Jaakola[6], Richard V. Espley[1]

[1] The New Zealand Institute of Plant & Food Research (PFR), Auckland, New Zealand
[2] PFR, Palmerston North, New Zealand
[3] School of Biological Sciences, University of Auckland, Auckland, New Zealand
[4] PFR, Motueka, New Zealand
[5] PFR, Ferndale, WA, USA
[6] Climate Laboratory, Department of Arctic and Marine Biology, University of Tromsø, Tromsø, Norway

Fresh fruit and vegetables as part of the daily diet are one way to increase 'health-span': a longer healthy life. With advances in understanding the genomics and genetics of crop plants, there is an opportunity to enhance key consumer traits such as appearance, flavour, health, storage, and convenience. The best-accepted compounds in fruits and vegetables that affect health are vitamin C, vitamin A, and flavonoids, including anthocyanins. These anthocyanins provide many of the reds, blues and purples of fruit and often provide the 'wow factor' for consumers. The added benefit of these colour compounds is that they are either measurably healthy or considered by the consumer as healthy. Hence, anthocyanin concentration is a key target for fruit improvement. In many fresh plant products the health benefits, such as anthocyanins, are mainly located in the skin. Fruit skin makes up less than 10% of a fruit, leaving a large volume of flesh ready for 'improvement'.

As an exemplar for this, we have started using Vaccinium species to create a novel, anthocyanin rich berry. Blueberry has deeply pigmented skin but colourless flesh, while its close relative, bilberry, has both deeply pigmented skin and flesh, and contains much higher concentrations of anthocyanins. We are using hybridisation techniques of blueberry and bilberry in an attempt to produce hybrid berries with coloured flesh to deliver hybrid fruit with up to five times the phytochemical content of normal blueberry, and create an attractive new fruit for consumers. Transcriptomics and metabolomics approaches will create knowledge on why different fruit tissues amass different concentrations of anthocyanin. This will inform alternative strategies to create new fruit types with elevated anthocyanin across a range of crops.

Notes

Dissection and engineering the anthocyanin biosynthetic pathway in orange and black carrots

Henrik Brinch-Pedersen

Aarhus University
hbp@mbg.au.dk

Shrikant Sharma[1], Giuseppe Dionisio[1], Miyako Kodama[2], Inger B Holme[1] and Henrik Brinch-Pedersen[1]

[1] Aarhus University, Department of Molecular Biology and Genetics, Research Center Flakkebjerg, Forsoegsvej 1, DK-4200 Slagelse, Denmark;
[2] Centre for GeoGenetics, Section for Evolutionary Genomics, Natural History Museum of Denmark, University of Copenhagen

The anthocyanin biosynthetic pathway in black carrot has been studied. The study aimed to analyze global transcription levels, to mine MYB, bHLH and WD40 genes that may function as positive or negative regulators in the carrot anthocyanin biosynthetic pathway and to evaluate candidate genes in carrots by genetic engineering and new breeding techniques. RNA was isolated from differently colored calli, as well as tissue samples from taproots of various cultivars across the course of development. RNA-Seq data were obtained, aligned to the recently published carrot genome, and gene expression levels of colored and non-colored tissue and callus samples were compared. MYB, bHLH and WD40 genes that are consistently down or upregulated in a purple color-specific manner within the various cultivars and different time points sampled were identified. Candidate genes were introduced in orange carrots by genetic engineering or knocked out in purple carrots by CRISPR/cas, respectively. Simultaneous transgenic expression of specific black carrot transcription in the orange carrot cultivar 'Danvers 126' lead to consistent upregulation of anthocyanin related biosynthetic genes and significant accumulation of anthocyanins in leaves, stems and taproots. The anthocyanin profile of the transformants were significantly different from the profile in the reference black carrot Deep Purple.

Notes

Functional analysis of flavonoid biosynthesis genes in *Beta vulgaris*

Ralf Stracke

Bielefeld University
ralf.stracke@uni-bielefeld.de

Boas Pucker, Daniela Holtgräwe, Ralf Stracke, Hanna M. Schilbert, Kali Swichtenberg, Sebastian Blunk, Bernd Weisshaar

Genetics and Genomics of Plants, CeBiTec, Bielefeld University, 33615 Bielefeld, Germany

Beta vulgaris (sugar beet) belongs to the flowering plant order Caryophyllales, which is well known for a complex pigment evolution. Anthocyanins and betalains are considered mutually exclusive pigments thus different taxonomic lineages of the Caryophyllales are expected to produce either only anthocyanins or betalains. Although *B. vulgaris* produces betalains, the genes of the anthocyanin biosynthesis pathway are present in the genome. We set out to identify the genetic cause for the lack of anthocyanins in *B. vulgaris*.

Genes of the flavonoid biosynthesis, which provide anthocyanin precursors, were characterized through phylogenetic analysis, gene expression analysis, and complementation of *Arabidopsis thaliana* mutant lines. The binding and activation of candidate promoters by potential regulators of this pathway was studied through transient transfection of *A. thaliana* protoplasts.

Functionality of several enzyme-encoding genes was demonstrated. Although there are several gene copies for each step in the pathway, functionality was observed for only one or a few copies. Also a gene was identified encoding a functional bHLH-type anthocyan regulator. Since committed encoded proteins of the anthocyanin biosynthesis appear functional, we speculate that the production of anthocyanins in sugar beet is possible, but hindered by reduced transcript abundances. Future research might identify betalain and anthocyanins production within the same species.

Notes

Cardioprotective and anti-inflammatory properties of anthocyanin-rich corn

Katia Petroni

Università degli Studi di Milano
katia.petroni@unimi.it

Katia Petroni

Department of Biosciences, Università degli Studi di Milano, 20133 Milan, Italy

The importance of a healthy diet in preventing chronic diseases is supported by epidemiological studies, which indicate that consumption of polyphenol-rich foods reduces the risk of cardiovascular disease, cancer and neurodegenerative diseases. Among polyphenols, anthocyanins are renowned as red, purple and violet pigments that, in addition to a strong in vitro antioxidant capacity, may regulate cellular signaling pathways involved in antioxidant and anti-inflammatory response. Using isogenic anthocyanin-rich and anthocyanin-free corn in rat models, we have previously demonstrated that dietary anthocyanins (ACNs) reduced myocardial injury upon ischemia/reperfusion and increased cardiac glutathione levels and omega-3 levels in blood, suggesting that dietary ACNs modulate cardiac antioxidant defences and the conversion of plant □-linolenic acid into omega-3 fatty acids. Cardioprotection was also effective against cardiotoxicity induced by the chemotherapeutic drug doxorubicin (Dox). In animals fed dietary ACNs from purple corn treated with Dox, medium-term survival was improved and Dox-induced cardiac histopathological alterations were prevented compared to animals fed ACN-free diet form yellow corn. Cardiomyocytes viability was preserved, without affecting the antitumoral activity of Dox. Finally, we showed that the administration of ACN-rich purple corn extract has a protective effect on the development of orofacial allodynia in an in vivo model of inflammatory trigeminal (TG) pain, reduces TG macrophage infiltration and microglial activation both in vivo and in vitro. The protective effect of purple corn is comparable to the anti-inflammatory effects of acetyl salicylic acid, which nevertheless does not modify microglia activation. Therefore, a possible application of ACN-rich dietary supplements as co-adjuvant to pharmacological treatments or as a preventive strategy against TG pain, aimed at reducing drugs dosage and side effects may be proposed.

Notes

Cyanidin and ellagitanin rich BerriQi™ boysenberry with apple juice protects against chronic lung inflammation

Odette Shaw

Plant & Food Research
odette.shaw@plantandfood.co.nz

Odette M. Shaw[1], Greg M. Sawyer[1], Janine Cooney[2], Duncan Hedderley[3], Roger D. Hurst[1]

[1] Food & Wellness Group, Food Innovation, The New Zealand Institute for Plant & Food Research. Palmerston North 4474 New Zealand
[2] Physiological Chemistry, Biological Chemistry & Bioactives Group Food Innovation, The New Zealand Institute for Plant & Food Research. Hamilton 3214 New Zealand
[3] Data Science, The New Zealand Institute for Plant & Food Research. Palmerston North 4474 New Zealand

There is epidemiological evidence that diet influences immune system function and studies show increased consumption of fruits and vegetables improves lung function and reduces airways inflammation. We have previously shown that anthocyanin containing fruits protect against allergic airways inflammation.
Our objective was to determine if the combination of Boysenberry (predominantly containing anthocyanin cyanidin glycosides and ellagitanins) and apple (phenolic acids, flavonols and chalcones) protected against chronic allergic airways inflammation.
We used a 10 week chronic ovalbumin model of allergic airways inflammation. After 5 weeks of ovalbumin challenges we started treatment by feeding mice with a low dose BerriQi™ Boysenberry with apple juice (2.5kg/kg) for the remaining 5 weeks of ovalbumin challenges. We then quantified the immune response to ovalbumin.
Using LCMS we identified cyanidin glycosides, ellagitanins, and chlorogenic acid as the major components in BerriQi™ Boysenberry with apple juice as well as other minor components. Repeated ovalbumin challenges resulted in increased immune cell into the lung, including eosinophils, monocytes and antigen presenting cells. Consumption of the BerriQi™ Boysenberry with apple juice significantly ($P<0.05$) reduced the total number of infiltrating immune cells into the lung. AB-PAS staining showed that BerriQi™ Boysenberry with apple juice also reduced the amount of mucous production in the lung. We observed histological improvements in collagen deposition, and significant ($P<0.05$) correlations ($r= 0.93$) between cytokines associated with wound healing and collagen content following Boysenberry with apple juice consumption.
We have found that consumption of BerriQi™ Boysenberry with apple juice that is rich in cyanidin, ellagitannins and other polyphenols reversed inflammatory lung tissue damage and helped ameliorate the immune response to chronic allergen exposure.

Notes

Anthocyanins in colorectal cancer prevention. A systematic review of the literature in search of molecular oncotargets

Sabina Passamonti

University of Trieste
spassamonti@units.it

Nevenka Medic, Federica Tramer, Sabina Passamonti

Dept. Of Life Sciences, University of Trieste, Via Weiss 2, 34128 Trieste, Italy

Background

Colorectal cancer (CRC) is the malignant process that surges in the terminal part of gastrointestinal tract when adenomatous polyps convert to neoplastic cells able to infiltrate the submucosa. CRC is still one of the leading causes of cancer death worldwide. Defining the precise role of the diet and its particular molecular moieties in CRC prevention is of constant scientific interest years behind. Anthocyanins (AC) have been reported to have some role in counteracting CRC carcinogenesis. Nonetheless, evidence coming out the pre-clinical, clinical and epidemiological studies is still controversial. This review is addressing the need to better comprehend the causes of missing data and discrepancies in investigations on the role of dietary AC in modulating CRC carcinogenesis.

Methods

We have analyzed the scientific literature, available in PubMed database, according to PRISMA statement methodology for systematic reviews.

Results

From the pool of 82 identified publications, we selected 19 articles reporting experimental or observational data on the effect of AC enriched diets in CRC prevention in humans or murine species. Furthermore, we selected 10 articles reporting about molecular mechanisms of action of pure AC in CRC experimental models.

Conclusions

The major outcome of this review is that AC shows essentially no effect in human studies, whereas AC-enriched diets proved to be effective in experimental murine models of CRC. In cell culture tests, AC showed to interfere with cell signaling pathways related to cell growth and differentiation, apoptosis, oxygen stress and inflammation response. Further molecular characterizations are required to include AC is the panel of disease-modifying agents.

Notes

Impact of anthocyanins from purple potatoes on sugar metabolism and Type II Diabetes

Baoru Yang

University of Turku
baoru.yang@utu.fi

Baoru Yang[1], Johanna Jokioja[1], Kang Chen[1], Matti Viitanen[2], Tuomo Sainio[3], Yumei Zhang[4], Kaisa Linderborg[1]

[1] Food Chemistry and Food Development, Department of Biochemistry, University of Turku, Itäinen Pitkäkatu 4c, FIN-20014 Turku, Finland
[2] Department of Geriatrics, University of Turku, Turku City Hospital, Turku, Finland
[3] School of Engineering Science, Lappeenranta University of Technology, Skinnarilankatu 34, FIN-53850 Lappeenranta, Finland
[4] Department of Nutrition and Food Hygiene, School of Public Health, Peking University, Beijing 100191, China

Anthocyanins have a wide range of physiological effects in supporting health and reducing risk of chronic health problems. The average intake of anthocyanins is low due to limited availability of anthocyanin-rich food in the diet. Blue and purple potatoes are rich sources of acylated anthocyanins, which represent important alternative sources of dietary anthocyanins. However, there has been limited studies on the physiological effects of acylated anthocyanins. The current research aimed to investigate the potential of anthocyanins of purple potatoes for reducing the risk of metabolic syndrome and type II diabetes.

Two clinical studies were performed to investigate purple potatoes and anthocyanin extract from purple potatoes on postprandial glycaemic response in healthy male human subjects after a high carbohydrate meal. In the first clinical trial, a meal of mashed purple potato (*Solanum tuberosum*) 'Synkeä Sakari' induced significantly lower postprandial response in glycaemia and insulinemia than did the meal of yellow potato. The postprandial glycaemic response was also lower than that induced by the meal containing equal amount of potato starch and bilberries (*Vaccinium myrtillus*). In the second clinical trial, inclusion of purple potato anthocyanin extract in the meal of yellow potatoes reduced the postprandial glucose and insulin levels in plasma compared to the meal of yellow potatoes alone, confirming positive impact of anthocyanins.

The metabolic impacts of bilberry anthocyanins and acylated anthocyanins from potatoes were compared in a six-week feeding trial with obese diabetic Zucker rats. Non-targeted NMR metabolomics was used to study the metabolomics profiles of fasting plasma after 8-week intervention with the anthocyanin extracts. While both anthocyanin extracts showed positive impact on sugar and lipid metabolism of the diabetic rats, the two extracts differed clearly in the effecting mechanism, targeting on different metabolic pathways.

Notes

Analysis of anthocyanins by mass spectrometry

Erika Salas

Universidad Autónoma de Chihuahua
esalas@uach.mx

Erika Salas

Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua, Circuito Universitario s/n, Campus 2, C.P. 31125, Chihuahua, México

Anthocyanins are considered the principal phenolic pigments responsible for the color diversity observed in the plant world and, also, they are one of the largest groups of water-soluble pigments. Anthocyanins are natural pigments and non-toxic. These phenolic pigments develop a wide range of different colors. Fruits, vegetables, plants and flowers are rich in anthocyanins.

Mass spectrometry (MS) is a very important tool for research, and its analytical power is relevant for structural studies on anthocyanin composition. Mass spectrometry involves the measurement of the mass (m) of a compound as a function of charge (z), m/z.

Different types of mass spectrometer sources and mass analyzers are available and can be combined to provide a large range of analytical possibilities. Electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI), were introduced for the analysis of different polyphenols. ESI yields multiply charged ions (z =1,2,3..), while MALDI yields mostly singly charged ions (z =1). To facilitate the interpretation, additional information can be obtained by multiple step mass spectrometry (MS/MS or MSn) experiments.

HPLC coupled with diode array and mass spectrometric detection (HPLC-DAD-MS) provides an efficient method of rapid identification of anthocyanins in a mixture. The combination offers the possibility of taking advantage of chromatography as a separation method and MS as an identification tool. With regard to the MS analysis of anthocyanins, ESI in the positive mode has been widely used for the analysis of anthocyanins in food and beverages.

This work will summarize the recent advances in anthocyanin analysis by mass spectrometry while comparing different sources of ionization, different types of matrix (in MALDI analysis), and the addition of proteins in order to enhance ionization.

Notes

Impact of a water-soluble gallic acid-based dendrimer on the color-stabilizing mechanisms of anthocyanins

Luis Cruz

Universidade do Porto
luis.cruz@fc.up.pt

Luís Cruz[1], Nuno Basílio[3], Johan Mendoza[3], Nuno Mateus[1], Victor de Freitas[1], Maun H. Tawara[2], Juan Correa[2], Eduardo Fernandez-Megia[2]

[1] REQUIMTE/LAQV, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, s/n, 4169-007 Porto, Portugal.
[2] Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS) and Departamento de Química Orgánica, Universidade de Santiago de Compostela, Jenaro de la Fuente s/n, 15782 Santiago de Compostela, Spain.
[3] LAQV, REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal.

The interaction of two anthocyanins with a water-soluble polyanionic dendrimer was studied through UV-Vis, stopped-flow and NMR spectroscopy. Cy3glc revealed a stronger interaction than mv3glc at pH 1 according to their apparent association constants. A higher color increased was also obtained for cy3glc at pH 3.5 as a result of this stronger interaction. A high-frequency chemical shift of the cy3glc aromatic protons suggest the formation of ionic pairs. The interaction parameters (K~700 M-1, n~295) indicated the binding of approximately two anthocyanin molecules by each sulfate group. The equilibrium and rate constants of cy3glc in the presence of dendrimer showed an increased stability of the flavylium cation and a higher protection of this species from hydration (pK'a and pKh increased almost one pH unit). The tuning and color stabilization of anthocyanins using this dendrimer envisage novel applications as colorimetric sensors for food packaging.

Notes

Stabilization of bluish pyranoanthocyanin pigments in aqueous systems using lignin nanoparticles

Joana Oliveira

Universidade do Porto
jsoliveira@fc.up.pt

Paula Araújo[1], Ana Costa[2,3], Iva Fernandes[1], Nuno Mateus[1], Victor de Freitas[1], Bruno Sarmento[2,3,4], Joana Oliveira[1]

[1] REQUIMTE – Laboratório Associado para a Química Verde, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal,
[2] Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal,
[3] Instituto de Engenharia Biomédica (INEB), Universidade do Porto, Rua Alfredo Allen 208, 4200-135 Porto, Portugal,
[4] CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde & Instituto Universitário de Ciências da Saúde, Rua Central de Gandra 1317, 4585-116 Gandra, Portugal.

Lignin nanoparticles (LNP) loaded with pyranoanthocyanin dimers (PD) and portisins (P) were produced using nanoprecipitation and dialysis techniques. The results obtained for LNP and P-LNP showed particles presenting sizes around 200 nm with a very narrow size distribution (PDI ≤ 0.2) using both methods. For PD-LNP, the nanoprecipitation method yielded particles with high particle sizes (Z-average ≈ 800 nm) and PDI ≈ 0.45 when compared to the dialysis method that produced particles with an average size around 200 nm and a PDI below 0.2. Concerning the average zeta potential, values around – 40 mV were obtained for both methods. TEM, SEM and STEM images showed that the particles present a spherical and compact shape and that these characteristics were maintained for 28 days at room temperature. High encapsulation efficiencies of 86±2 and 85±4 were observed for PD and P, respectively. LNP stability was followed at pH ≥ 5 for at least 7 days and the LNP encapsulated systems (PD-LNP and P-LNP) showed a good colour stability towards pH change after 8 days of incubation, when compared with the pure bluish pigments.

Notes

How do anthocyanins get to the vacuole?

Cathie Martin

John Innes Centre
cathie.martin@jic.ac.uk

Cathie Martin, Elaine Barclay, Kim Findlay and Ingo Appelhagen

John Innes Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom

Anthocyanins are synthesised in the cytoplasm but need to be stored under acidic conditions in the vacuole, to gain stability and to exhibit their colours. It has been a matter of debate how anthocyanins are transported to the vacuole with two contrasting models having been proposed: 1) anthocyanin transporters in the membrane of the vacuole (the tonoplast) transport anthocyanins to the vacuole or 2) vesicle-mediated transport that is independent of tonoplast transporters transports anthocyanins to the vacuole. We hypothesized that, in fact, these two pathways are non-exclusive and that the same transport proteins that localise to the tonoplast also load vesicles with anthocyanins, while trafficking through the endomembrane system, from the site of their biosynthesis (the ER) to their final destination (the vacuole). We tested these ideas using tobacco transiently and stably producing high levels of anthocyanins and results from these investigations, including assessment of the role of autophagy in anthocyanin transport will be reported.

Notes

Oxidative transformation of flavan-3-ols by anthocyanidin synthase from *Vitis vinifera* and the role of glutathione

Jean Chaudiere

University of Bordeaux
jean.chaudiere@u-bordeaux.fr

Jean Chaudière[1], Jia-Rong Zhang[1], Claudine Trossat-Magnin[2]

[1]CBMN (UMR 5248), University of Bordeaux, 33600 Pessac, France;
[2]ISVV (UMR 1287), University of Bordeaux, 33140 Villenave d'Ornon, France

Recombinant ANS from *Vitis vinifera* (VvANS) has been expressed in E. coli, and purified by nickel affinity chromatography. Ascorbate behaves as an essential cofactor of the enzyme.

Leucocyanidin, dihydroflavonols (dihydroquercetin, dihydrokampferol and dihydromyricetin) and flavan-3-ols (catechin, gallocatechin and afzelechin) are recognized as substrates by VvANS, and mass spectrometry studies (MS and MS/MS) lead to the conclusion that the first catalytic step of ANS is a C3-hydroxylation that systematically produces a 3,3-gem-diol.

Dihydroflavonols are oxidized into flavonols, and 3,4-cis-leucocyanidin (natural stereoisomer) is only transformed into quercetin.

Significant amounts of anthocyanidins are produced only from flavan-3-ols, and we have monitored the oxidative transformation of (+)-catechin into intermediates and products in real time by mass spectrometry.

A catechin dimer as well as a covalent adduct ascorbate-cyanidin are produced in the absence of the coenzyme glutathione (GSH). Physiological concentrations of GSH considerably modify the transformation pattern of (+)-catechin and (+)-gallocatechin. In the presence of (+)-catechin and GSH, we observe only two major products, cyanidin and a cyanidin-glutathione thioether, with much higher production yields than in the absence of GSH. In the presence of (+)-gallocatechin, a similar delphinidin-glutathione thioether adduct is observed. The anthocyanidin-glutathione adducts are most likely C4-thioethers which decompose into anthocyanidin and GSH.

Our results suggest that anthocyanidins could be produced in vivo from a flavan-3ol substrate via a glutathione thioether intermediate.

Notes

Why so repressed? Evolution and mechanism for suppressed anthocyanin biosynthesis altering flower colour in snapdragon

Sarah Moss

Plant and Food Research
sarah.moss@plantandfood.co.nz

Sarah M.A. Moss[1], Kathy E. Schwinn[1], Kevin M. Davies[1], Andrew Hudson[2], Cathie Martin[3], Yanfei Zhou[1], Shin-Mei Yeh[4], Nick W. Albert[1]

[1] The New Zealand Institute for Plant and Food Research Limited, Private Bag 11-600, Palmerston North, New Zealand;
[2] Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh, Scotland, United Kingdom;
[3] John Innes Centre, Norwich Research Park, Colney, Norwich, United Kingdom;
[4] The New Zealand Institute for Plant and Food Research Limited, Private Bag 92169 Auckland, Palmerston North, New Zealand

Snapdragons (*Antirrhinum spp.*) are native to the Mediterranean region and exhibit a range of colour patterns in their flowers. The colour and patterns occur because of the accumulation of red (anthocyanin) and yellow (aurone) pigments. Pollinators recognise colours and patterns, and pollinator preference limits gene-flow between populations. Anthocyanin pigmentation is regulated by a transcription factor complex consisting of R2R3-MYB, bHLH and WD-Repeat proteins (MBW complex), which activate the expression of the biosynthetic genes, resulting in anthocyanin accumulation. We characterised ELUTA (EL), a gene that suppresses anthocyanin accumulation in snapdragon flowers, leading to flowers that have white lobes and corolla tubes and a magenta 'bulls-eye' on the pollinators' landing platform. This phenotype is distributed throughout the *Antirrhinum* genus, and is proposed to maintain species separation by inhibiting unfavourable hybrid colour morphs. A candidate MYB repressor gene was identified using RNAseq and was confirmed as EL by transposon-tagging. Functional characterisation was completed using particle-bombardments in snapdragon, transient analysis in Nicotiana benthamiana leaves, yeast 2-hybrid analysis and over-expression in Nicotiana tabacum. Our results suggest that EL sequesters the bHLH Delila, resulting in the reduced anthocyanin accumulation in flowers. EL alleles were investigated across the *Antirrhinum* genus to investigate how these alleles have contributed to flower colour and patterning, and whether they have contributed to speciation within the genus. This expands the understanding of mechanisms that control floral colour and patterning in plants.

Notes

Regulation of the branch points in the anthocyanin pathway is distinct to the regulation of the core pathway

Yongyan Angel Peng

University of Auckland
yongyan.peng@plantandfood.co.nz

Yongyan A. Peng[1], Richard V. Espley[1,2], Andrew C. Allan[1,2]

[1] School of Biological Science, The University of Auckland.
[2] The New Zealand Institute for Plant and Food Research Limited, Mt Albert, Auckland

Kiwifruit belongs to the genus *Actinidia*, which has over 70 species with a great diversity of traits. Some kiwifruit accumulate only cyanidin-based anthocyanins around the centre of the core in the flesh, whereas other species also accumulate delphinidin-based anthocyanins and have purple coloured flesh and skin. The objective of this study was to understand the regulation of anthocyanins in kiwifruit, with a focus on delphinidin biosynthesis and identification of transcription factors (TF) involved in the branch point of anthocyanin biosynthesis. The intense pigmentation throughout the skin and flesh of purple kiwifruit is due to the accumulation of both cyanidin- and delphinidin-based anthocyanins. The kiwifruit TF, MYB110, is an anthocyanin activator and is associated with the expression of the biosynthetic genes chalcone synthase (CHS) and flavonoid 3′-glycosyltransferase (F3GT) in the core anthocyanin pathway. However, MYB110 was unable to activate the promoters of flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′, 5′-hydroxylase (F3′5′H), which are the key branch points of the anthocyanin pathway responsible for the biosynthesis of cyanidin and delphinidin, respectively. These results suggest that the core pathway and the branch points are differentially regulated and MYB110 may not be the sole regulator. Transcriptomic analysis of purple kiwifruit identified two candidate TFs, a MYB and a WRKY, which were differentially expressed between the purple and the non-anthocyanin accumulating kiwifruit. Results from promoter activation assays indicated that these two TFs are the activators of the cyanidin and delphinidin branch points. A better understanding of the regulation of the branch points and the core pathway will add to knowledge of anthocyanin regulation in plants and lead to the development of fruit with enhanced dietary properties.

Notes

Exploring the colour and bioactivity of anthocyanin derivatives

Nuno Mateus

University of Porto
nbmateus@fc.up.pt

Nuno Mateus, Iva Fernandes, Luís Cruz, Joana Oliveira, Victor de Freitas

REQUIMTE/LAQV, Department of Chemistry and Biochemistry, Faculty of Sciences, University of Porto, Porto, Portugal

There have been several evidences regarding the positive association of dietary anthocyanin intake with healthy biological effects displayed in vivo. Some of their metabolized forms occurring in vivo after ingestion have been detected, and their benefits for human health are being discovered.

Red wine has always been a source of inspiration for anthocyanin chemical transformations yielding new classes of pigments with a wide array of colours from orange to bluish hues. Many of these pigments share similar antioxidant and/or bioactivities than their anthocyanin precursor. This has led the research on dietary anthocyanins to go beyond the nutritional standpoint to the development of research towards novel technological applications of anthocyanins in different industrial fields.

In the cosmetic industry, there has been a growing market demand for the incorporation of plant-derived ingredients in new products. Besides their appealing colours, anthocyanins have been shown to display many antioxidant and bioactive properties such as free radical scavenging, metal-chelating, antimicrobial, wound healing and chemopreventive activities. On the other hand, the ability to prevent oxidative damages has led to the incorporation of natural bioactives in lotions and facial creams to prevent skin diseases and premature ageing, therefore the biological activities of anthocyanins make them novel potential compounds for cosmetic formulations. However, anthocyanin and anthocyanin-derived pigments present a low solubility in lipophilic media, which compromises their effective application. Therefore, strategies have been conducted to overcome or partially solve this issue. Other approaches may involve the use of lignin nanoparticles for the encapsulation of some derivatives such as pyranoanthocyanin dimers. In addition, the physical-chemical features of some anthocyanin derivatives make them interesting targets for putative therapeutic applications such as photodynamic therapy (PDT).

Notes

Anthocyanins, polyphenols and Mediterranean diet: messages from the Moli-sani study

Chiara Cerletti

IRCCS Neuromed
chiara.cerletti@moli-sani.org

Chiara Cerletti, on behalf of the ATHENA Project and Moli-sani study Investigators

Department of Epidemiology and Prevention, IRCCS Neuromed, Pozzilli (IS)

Adherence to a Mediterranean diet has been shown to be beneficial to human health. Fruit and vegetables represent some of the main components of the Mediterranean diet and their role has been increasingly considered to prevent or reduce the risk of chronic degenerative diseases.

Both the FLORA and ATHENA EU funded projects investigated a possible beneficial effect of dietary anthocyanins: human studies were designed to verify whether anthocyanin supplementation would counteract the inflammatory response to stress conditions, namely a fatty meal in healthy subjects, or the skin toxicity consequent to a radiotherapy treatment in women with breast cancer.

The Moli-sani study is a prospective cohort study that recruited 24,325 men and women aged ≥35 years from the general population of the Molise Region, to investigate genetic and environmental risk/protection factors for cardio- cerebrovascular disease and cancer. Among the environmental and lifestyle factors, emphasis was given to dietary habits and to the degree of adhesion to Mediterranean diet.

Four major classes of polyphenols (phenolic acids, flavonoids - including anthocyanins -, stilbenes and lignans) were calculated to assess their total dietary content and the polyphenol antioxidant content (PAC) score (–28, 28) constructed by an appropriate algorithm. A composite score (INFLA-score) of low grade inflammation based on plasmatic (C-reactive protein) and cellular (leukocyte and platelet counts and granulocyte : lymphocyte ratio) biomarkers has been proposed and validated. This score was associated negatively with the polyphenols intake and directly with total mortality risk. The Moli-sani study was the first one to address the relationship between the traditional Mediterranean diet and platelet and leukocyte (WBC) counts as emerging cellular biomarkers of low grade inflammation.

A cross-sectional analysis of the overall polyphenol dietary content was associated with a more favourable pulmonary function, partially mediated by WBC in men.

Finally, all-cause mortality risk was reduced by a polyphenol-rich diet consumed by the Moli-sani (median follow up of 8.3 y), an effect possibly mediated by anti-inflammatory mechanisms.

Notes

Bilberry anthocyanins: industrial development of a clinically effective strandardized extract

Paolo Morazzoni

INDENA SPA
scientificadvisor.pm@indena.com

Paolo Morazzoni

Indena S.p.A., Milan, Italy

Bilberry (*Vaccinum myrtillus L.*) is extremely difficult to grow and cannot be cultivated. The berries are collected from wild plants and processed after being frozen to avoid degradation of the relevant compounds, i.e. anthocyanins (0.3-05% on fresh weight) , the reddish coloured flavonoidic pigments. Anthocyanins are antioxidant, cytoprotective, antimicrobial, chemo-preventive agents and are also indicated to have high potentiality in the management of age-related neurodegeneration, weight control and prevention of chronic-degenerative diseases. For preparing a GAP- and GMP-grade standardized extract (Mirtoselect®) a long list of sequential steps have been standardized including: correct identification of the botanical species (also through DNA-barcoding techniques), controlled freezing-drying cycle, industrial extractive (water-ethanol) procedure combined with resin absorption process of purification and adequate drying process. Analytical controls with both specific/quantitative (HPLC,...) and qualitative/semi-quantitative (NMR, FT-IR,..) techniques in order to guarantee the batch-to-batch consistency of composition have been performed. Mirtoselect® has been standardized to contain 36% anthocyanins along with additional polyphenols such as polymeric (9.8%), dimeric (0.4%) and monomeric (1.5%) proanthocyanidins, monoglycosidic flavonols (2%), flavonols and phenolic acids (4%) and small amounts (0.3%) of anthocyanidins. The clinical development of Mirtoselect® has been mostly focused in ophthalmology (retinic adaptability, diabetic retinopathy, macular degeneration, dry eye, eye fatigue) and in peripheral circulation disorders (venous insufficiency, haemorroids, chronic dysmenorrhea, intra-and post-operatory bleeding).

Notes

The influence of acylation on the color and pigment stability of red cabbage anthocyanins under neutral conditions

Olivier Dangles

Avignon University, INRA
olivier.dangles@univ-avignon.fr

Julie-Anne Fenger[1], Rebecca J. Robbins[2], Tom M. Collins[2], Olivier Dangles[1]

[1] Avignon University, INRA, UMR408, 84000 Avignon, France,
[2] Mars Wrigley Confectionary, 800 High St., Hackettstown, NJ 07840, USA

Acyl groups derived from hydroxycinnamic acids (HCAs) are known to stabilize the color of anthocyanins owing to pi-stacking interactions (intramolecular copigmentation, self-association) involving the anthocyanidin nucleus and the HCA residues. This work investigated the factors governing pigment and color stability at neutral pH for a set of acylated anthocyanins (one monoacylated, two diacylated + the nonacylated control) from red cabbage, which exhibit blue colors under such conditions. Color stability was evaluated from direct spectral monitoring at pH 7 or 8, 50°C. Pigment stability was determined after subsequent acidification of the samples to pH 1 and complete conversion of the accumulated trans-chalcone to the flavylium ion. Experiments were repeated after addition of Fe2+ (0.6 equiv.) or antioxidants (caffeic acid, N-acetylcysteine) and under low O2 atmosphere.

For optimal data interpretation, the time dependence of the mole fractions of colored, hydrated and degraded forms was quantitatively analyzed. This kinetic analysis showed that HCA residues are much more efficient at providing protection against water addition (reversible color loss) than against the complex combination of hydrolytic/autoxidative pathways leading to degradation (irreversible color loss). Moreover, the colorless forms appear much less sensitive to degradation than the electron-rich colored forms (particularly, the anionic base).

Finally, the comparative study also showed that the anthocyanidin nucleus of the acylated pigments (especially, the diacylated ones) is efficiently protected against the nucleophilic addition of hydrogen peroxide (mostly irreversible) and bisulfite (reversible), two processes possibly involved in bleaching in food products.

Notes

Deciphering the mechanism of glycosylated anthocyanin and flavonols in red mango fruits tolerance to fungal pathogens

Pradeep Kumar

Volcani Center
pradeepkumar2k@gmail.com

Pradeep Kumar.S, Oleg Feygenberg, Dalia Maurer, Noam Alkan

Department of Postharvest Science, Volcani Center, Agricultural Research Organization, Rishon LeZion, 7505101, Israel.

Mango (*Mangifera indica L.*) fruit that was exposed to sunlight at the orchard accumulate anthocyanin and flavonols and develop red skin color. Those red mango fruits are more resistant to the pathogenic fungus Colletotrichum gloeosporioides. Some flavonoids metabolites are known to have antifungal activity. The objective of this study was to identify the different anthocyanin and flavonols in 'Shelly' mango peel and evaluate their antifungal activity against *C. gloeosporioides*. Organic extraction of red fruit peel showed increased inhibition of conidia germination and hyphal growth in comparison to extract from green mango peel. HPLC analysis of non-hydrolyzed samples showed that the major anthocyanin peaks were cyanidin-3-galactoside and 7-O-methylcyanidin-3-O-β-D-galactopyranoside, and the major flavonol peaks were glucoside derivatives of quercetin and kaempferol. Thus, all the identified flavonoids were glycosylated. Transcriptome analysis of host-pathogen interaction of mango and C. gloeosporioides showed an increase of large sets of β-glucosidase genes related to fungal pathogenicity and host defense response during decay development. When the peel extract from both red and green fruit was treated with β-glucosidase it inhibited much more the C. gloeosporioides hyphal growth and conidia germination. Additionally, aglycones of quercetin (0.3 mM) and cyanidin (0.2 mM) inhibited significantly more the C. gloeosporioides hyphal growth and conidia germination in comparison to glycosylated quercetin and cyanidin. Similarly, β-glucosidase treatments on the peel extract released a large amount of volatiles with, three unique compounds 'methyl salicylate, benzyl alcohol, phenyl acetaldehyde' which further inhibited the fungal growth. These indicate that glycosylated flavonoids play an important role in red fruit defense mechanism towards fungal pathogens, which control postharvest decay.

Notes

Influence of different winemaking techniques on anthocyanin fingerprints of varietal wines

Dominik Durner

Weincampus Neustadt
dominik.durner@dlr.rlp.de

Dominik G. Durner[1,2], Patrick Nickolaus[1], Ulrich Fischer[1]

[1] Institute for Viticulture and Enology, DLR Rheinpfalz, 67435 Neustadt
[2] University of Applied Science Kaiserlautern, Weincampus Neustadt, 67435 Neustadt

Many studies have reported about the differences in the relative proportions of the 3-O-glycosides of cyanidin, delphinidin, malvidin, peonidin, and petunidin in different grape varieties. Acetylated and coumaroylated forms have been examined for the purpose of varietal typification. The objective of the presented work was to examine the influence of grape and wine processing on the anthocyanin fingerprints of varietal wines. Pinot noir, Cabernet Sauvignon and Trollinger (Vernatsch) were de-stemmed, crushed and processed at original and decreased pH (addition of 4 g/L tartatric acid). Fermentations were conducted at 28°C for 10 days in pilot-scale fermenters. Cap management was either carried out by punch downs, air push, pump over or in a rotating fermenter. Pinot noir and Trollinger were additionally processed by thermovinification allowing the separated view of anthocyanin extractability and reactivity. HPLC-DAD monitoring of anthocyanins revealed that the relative proportions of anthocyanins changed during fermentation for all investigated cap management techniques. Threefold substituted anthocyanins were found to be increasingly dominating during the winemaking process. A lower pH shifted the anthocyanin proportions in favor of acetylated and coumaroylated forms. Although the use of the air push fermenter and the pump-over treatment yielded lowest overall anthocyanin content, these techniques supported delphinidin-3-O-glucoside, malvidin-3-O-glucoside, and petunidin-3-O-glucoside contents. It was found that heat extraction – as compared to fermentation on skins – yielded higher concentrations in twofold substituted anthocyanins in the juice. However, cyanidin-3-O-glucoside and peonidin-3-O-glucoside showed disproportionately high losses during the fermentation of the heat-extracted juices. It is speculated that cyanidin-3-O-glucoside and peonidin-3-O-glucoside are (more) susceptible for enzymatic cleavage of the glycosidic bond resulting in insolubility.

Notes

Absorption and distribution of anthocyanin rutinosides in humans after consumption of a blackcurrant (*Ribes nigrum* L.) extract

Elke Richling

Technische Universitaet Kaiserslautern
richling@chemie.uni-kl.de

Elke Richling[1], Teresa Roehrig[1,2], Verena Kirsch[1], Dorothea Schipp[3], Jens Galan[4]

[1] Division of Food Chemistry and Toxicology, Department of Chemistry, Technische Universitaet Kaiserslautern, Erwin-Schroedinger-Strasse 52, D-67663 Kaiserslautern, Germany
[2]Helmholtz Institute for Pharmaceutical Research Saarland, 66123 Saarbrücken, Germany
[3] Pirnaer Strasse 1, 01824 Rosenthal-Bielatal, Germany
[4] Hochgewanne 19, 67269 Gruenstadt, Germany

The anthocyanin spectra of blackcurrant (*Ribes nigrum L.*) is dominated by anthocyanin rutinosides, namely delphinidin-3-O-rutinoside and cyanidin-3-O-rutinoside. Data on their absorption and distribution in the human body are rather limited. We have performed a human pilot study with volunteers consuming a blackcurrant extract. In urine and plasma samples rutinosides and their degradation products (gallic acid and protocatechuic acid) were measured. Overall recoveries were low, but significant quantities of rutinosides and their respective degradation products circulated in the plasma and were excreted via urine. This study provided insight into the body distribution and excretion of this special class of anthocyanins.

Notes

Recent Advances on the bioavailability of anthocyanins: a molecular approach

Hélder Oliveira

University of Porto
helder.oliveira@fc.up.pt

Hélder Oliveira, Iva Fernandes, Victor de Freitas, Nuno Mateus

REQUIMTE/LAQV, Chemistry and Biochemistry Department, Faculty of Sciences, University of Porto, Portugal

Although the consumption of anthocyanins may easily reach 200 mg/day, their bioavailability has been reported to be amongst the lowest of polyphenols. These pigments have unique physical-chemical properties that affect their behaviour in vivo, and so, their bioavailability can be assessed from a simple nutritional view. Due to their rapid appearance in plasma, the absorption on anthocyanins is likely to occur not only at the intestine, but also at the gastric level. Nevertheless, the information on this matter remains very scarce.

This study reports recent advances to further elucidate the mechanism of absorption of anthocyanins at the gastric level, the role of the anthocyanin structure and the role of food matrices at the gastrointestinal level, using state-of-the-art techniques.

Transepithelial transport assays analysis showed that structurally complex anthocyanins have a gastric and intestinal absorption comparable to the common monoglucoside anthocyanins. The presence of food matrix such as glucose and proteins affected the overall transport efficiency, but protected anthocyanins during the digestion processes. Using gold nanoparticles functionalized against GLUT-1 and GLUT-3, coupled with the transepithelial transport assays, it was shown that glucose transporters are indeed crucial for anthocyanin gastric absorption. Also, for the first time, anthocyanin inner fluorescence was used to track them in an intracellular approach. The results showed anthocyanins concentrated in specific cell regions.

Altogether, these findings bring important insights on the comprehension of anthocyanins bioavailability.

Notes

Can berry polyphenolics/anthocyanins protect the skin from environmental insult?

Mary Ann Lila

North Carolina State University
mlila@ncsu.edu

Mary Ann Lila, Erika Pambianchi, Alessandra Pecorelli, Mary Grace, Francesca Ferrara, Brittany Woodby, Jean-Philippe Therrien and Giuseppe Valacchi

Plants for Human Health Institute, North Carolina State University, North Carolina Research Campus, Kannapolis, NC 28081 USA

Skin, our largest organ, is the primary target exposed to environmental insults such as pollutants (ozone and particulates). Although the skin acts as a shield against these insults, prolonged or repetitive exposure can lead to reactive oxygen species (ROS) formation and inflammatory status, which provoke skin aging, and underlie adverse skin pathologies. Native American and Alaska Native cultures, as well as tribal nations across Australasia and Africa, traditionally used berry compotes for healing wounds and treating skin abrasions. Zoopharmacognosy chronicals the use of berries by wild animals to help heal wounds incurred in combat. Can berry phytoactive compounds similarly protect human skin today from modern vulnerabilities? The inflammasome, one of the cardinal characters in the skin's inflammatory response, is a multiprotein complex that serves an important pro-inflammatory role in the innate immune system and is responsible for activation of the inflammatory process in skin by promoting the maturation of cytokines. We examined the ozone-induced inflammasome activation in human skin using human skin explants (2D keratinocyte cultures), 3D reconstituted human epidermis models, and human skin biopsies. Ozone exposure clearly provoked the oligomerization of proteins (Caspase1, ASC and NLRP1) to activate the inflammasome multiprotein complex, as determined by immunofluorescence, gene & protein expression, and cytokine release. Pretreatment with anthocyanin-rich berry extracts (blackberry, blackcurrant, and black raspberry) was able to quench and suppress inflammasome activation. Topical pretreatment with anthocyanin-rich blueberry extracts in all three skin models (2D, 3D and biopsies) prevented key inflammasome proteins from oligomerizing, resulting in suppression of cytokine release and attenuation of inflammatory responses. Topical treatment with berry phytoactives dramatically accelerated wound repair and reduced skin inflammation.

Notes

POSTER PRESENTATION ABSTRACTS

P1

Improving naringenin scaffold for fermentative production of anthocyanins in engineered yeast

Camilla Valdesturli

Fondazione Edmund Mach
camilla.valdesturli@fmach.it

Camilla Valdesturli[1], Tobias Weil[1], Luisa Palmieri[1], Michael Naesby[2], Michael Eichenberger[2], Stefan Martens[1]

[1] Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, San Michele all'Adige, (TN), Italy;
[2] Evolva AG, Duggingerstrasse 23, 4153 Reinach, Switzerland

Anthocyanins (ACNs) represent an important class of pigments, which confers specific colors to flowers, fruits and vegetables. The relative abundance of ACNs in the human diet and their potency against a range of chronic diseases have made them the subject of intense research as they have an enormous potential as natural food and cosmetic colorants or health-promoting supplements in functional foods. However, both the limited range of commercially available ACNs and the great expense of pure preparations led to numerous studies that used crude extracts of plants which are neither standardized for its content nor for the amounts of each metabolite they contain. Biotechnological approaches, such as engineering of yeast as cell factories, showed high potential to overcome this bottleneck.

Especially, the baker's yeast *Saccharomyces cerevisiae* was recently shown to be a promising and suitable candidate for heterologous de novo synthesis of ACNs. Inspired by nature the whole plant derived pathway starting from aromatic amino acids was introduced. Several modifications of the entire host metabolism already improved the titer of the end product. However, for an industrial application further significant improvements are needed.

The naringenin (NAR) scaffold, starting from primary pathway and composed of the phenylpropanoid pathway steps, paves the way for ACNs production. One purpose of this work was to compare the gene expression profiles of different NAR producing yeast strains in order to identify changes in the transcriptome due to the new pathway. Therefore, candidate genes obtained by microarray analysis of gene expression were confirmed by quantitative real-time PCR and subsequently correlated with the metabolite profile of the respective strains to draw an overall-picture of the underlying process. The gained knowledge will help to identify key factors and bottlenecks in NAR production and thus, improve the overall titer required for further synthesis towards ACNs.

Notes

P2

Anthocyanins on demand

Constantinos Patinios

Wageningen University
constantinos.patinios@n-chroma.com

Rumyana Karlova[1], Mark Levisson[1], Constantinos Patinios[3], Nikolay Outchkourov[2] and Jules Beekwilder[2]

[1]Wageningen University, Laboratory of Plant Physiology, Wageningen, The Netherlands
[2]Wageningen Plant Research, Wageningen, The Netherlands
[3]Wageningen University, Laboratory of Microbiology, Wageningen, The Netherlands

Anthocyanins are plant secondary metabolites that have received significant attention due to their natural colour properties (orange, red, purple and blue), their health promoting antioxidant functions and their biological potency. Depending on the pH and their chemical modifications, anthocyanins can change colour from red to purple and blue. Production of secondary metabolites in plants requires tight spatiotemporal regulation of various developmental processes ensuring that biosynthetic enzymes, transporters and storage capacity are present at the correct time and place. Understanding these metabolic pathways requires a system for coordinated induction of these components in a tissue- and development independent manner. Here, we established such a system for studying the program of anthocyanin-biosynthesis in tomato. Based on this knowledge we have developed a yeast-based production platform for anthocyanins and these data may provide new insights for improved microbial production of anthocyanins.

Notes

P3

Anthocyanin accumulation and biosynthesis mechanism in purple-head Chinese cabbage

Lugang Zhang

Northwest A&F University
lugangzh@163.com

Qiong He[1,2], Junqing Wu[1], Yihua Xue[1], Zhanfeng Zhang[3], Lugang Zhang[1]

[1] State Key Laboratory of Crop Stress Biology for Arid Areas, College of Horticulture, Northwest A&F University, 3 Taicheng Road, Yangling 712100, Shaanxi, People's Republic of China
[2] College of Life Sciences, Northwest A&F University, Yangling, People's Republic of China
[3] College of Plant Protection, Northwest A&F University, Yangling, People's Republic of China

It was known that anthocyanins has high free radical scavenging abilities in healthcare. Thus, vegetables rich in anthocyanin are becoming a research hot spot in world. Chinese cabbage is a significant dietary vegetable for its edible heading leaves in Asia countries. a new purple head Chinese cabbage(11S91) has been successfully bred from a intersubspecies of a common Chinese cabbage(94S19) and a flowering purple Chinese cabbage(95T2-5) in Northwest A&F University, China. In present. The tissue distribution, the anthocyanin components and the key gene of purple trait in 11S91 was observed and analyzed, the results showed that the purple pigment mainly resided in three cell layers under the upper epidermis and two cell layers under the lower epidermis respectively. The purple pigments in purple-head Chinese cabbage were anthocyanins. thirty-two anthocyanins were separated and identified in purple head of 11S91, 70% of them were glycosylated and acylated cyanidins including four major anthocyanins. Combining the results of the gene expression and map-based cloning, A novel dominant gene BrMYB2 was identified as kay gene controlling purple head. Sequence alignment indicate that both CDS and gDNA of BrMYB2 of 11S91 and 95T2-5 were unanimous, whereas two SNPs existed in cMYB211S91 and cMYB294S19, a large deletion appeared in the first intron of gBrMYB211S91. Genetic transformation of Wild-type Arabidopsis showed that both gBrMYB211S91 and cBrMYB2 can lead to purple phenotype in transgenic Arabidopsis except for gBrMYB294S19. These paper firstly illustrated that BrMYB2 was a new gene controlling anthocyanins accumulation in purple-head Chinese cabbage. The large deletion in the first intron of gBrMYB2 was the reason to activate the anthocyanin biosynthesis in purple-head Chinese cabbage. These evidences not only supplied a new understanding on the anthocyanin biosynthesis in Brassica vegetables, but also laid a basis for new purple-head Chinese cabbage breeding.

Notes

P4

A tool box for elucidation and reconstitution of anthocyanin biosynthesis pathways by transient expression in *Nicotiana banthamiana*

Sylvestre Marillonnet

Leibniz Institute of Plant Biochemistry
smarillo@ipb-halle.de

Ramona Grützner, Kristin Köning, Claudia Horn, Thomas Vogt and Sylvestre Marillonnet

Department of Cell and Metabolic Biology, Leibniz Institute of Plant Biochemistry, Weinberg 3, 06120 Halle, Germany

Anthocyanins are responsible for the red, purple and blue colors in the flowers of many plants. Anthocyanins are made from phenylalanine by a series of enzymatic reactions that take place in the cytosol and vacuole of plant cells. The first nine or ten reactions lead to biosynthesis of the basic anthocyanins pelargonidin-, cyanidin- and delphinidin-3-glucoside, which display the colors orange/red, red/purple and purple/blue, respectively. These basic anthocyanins are then modified by a series of enzymatic reactions that include glycosylation, acylation and methylation, which affect the color and/or stability of the final anthocyanins. The nature and substrate specificity of the enzymes involved in these reactions vary considerably among plant species, providing the large diversity of anthocyanins found in nature. While the biosynthetic pathways that lead to the formation of the basic anthocyanins are quite well understood, less is known of the genes and enzymes that are involved in the biosynthesis of the more complex anthocyanins that are present in plant tissues. To facilitate the analysis of putative anthocyanin biosynthetic genes, we have developed a tool box to allow transient expression of complete anthocyanin biosynthetic pathways in leaves of *Nicotiana benthamiana* plants. As an example, we produced in *Nicotiana benthamiana* leaves some of the complex anthocyanins that are normally made in *Arabidopsis*.

Notes

P5

Regulation of pigmentation patterning in *Antirrhinum*

Nick Albert

Plant & Food Research
nick.albert@plantandfood.co.nz

Nick W. Albert[1], Kathy E. Schwinn[1], Sarah M.A. Moss[1], Eugenio Butelli[2], Kevin M. Davies[1] and Cathie Martin[2]

[1] The New Zealand Institute for Plant & Food Research Limited, Private Bag 11-600, Palmerston North, New Zealand.
[2] John Innes Centre, Norwich Research Park, Colney, Norwich UK

Colour and pigmentation patterning are common in animal-pollinated flowers. The formation of complex patterns can provide high contrast visual cues to attract pollinators, acting as 'nectar guides', directing them towards their reward and ensuring pollination. In *Antirrhinum*, flowers are pigmented with magenta anthocyanins as full colour, venation, bi-colour or 'bulls-eye' patterns. These colour patterns are genetically controlled by the interaction between the anthocyanin regulatory loci: Rosea, Venosa, Delila, Incolorata and Eluta. Anthocyanin pigmentation is regulated by a transcription factor complex consisting of R2R3-MYB, bHLH and WDR transcription factors, which activate the anthocyanin biosynthesis genes. Rosea and Venosa encodes R2R3-MYB proteins, while Delila corresponds to an R-clade bHLH transcription factor. Other components of the conserved MBW gene regulation network have yet to be identified in *Antirrhinum*. We report on the identification of key components of the anthocyanin regulation machinery: an AN1-clade bHLH and a WDR protein. The genetic interaction between the regulatory loci, mechanisms for controlling anthocyanin biosynthesis, and establishment of pigmentation patterning will be discussed.

Notes

P6

Several B-box proteins coordinately mediate light-induced anthocyanin in red pear fruits

Ruiyan Tao

Zhejiang University
ywteng@zju.edu.cn

Ruiyan Tao, Songling Bai, Lei Yin, Junbei Ni, Yunjing Ma, Yuanwen Teng

Department of Horticulture, Zhejiang University, Hangzhou 310058, Zhejiang, PR China

The red coloration of pear (*Pyrus pyrifolia*) results from anthocyanin accumulation in the fruit peel. Light is indispensable for anthocyanin biosynthesis in red pear, but detailed characterization of the regulatory mechanism is needed. In the present work, we found that PpHY5, the essential transcription factor mediating light signal transduction, lacked the trans-activity although it bound to the promoter of PpMYB10. To unravel the regulatory model of PpHY5 on the anthocyanin biosynthesis, we screened the Yeast two-hybrid cDNA library using PpHY5 as bait protein. Finally, we identified three PpHY5 associated BBX proteins, PpBBX16, PpBBX18 and PpBBX21. Overexpression of PpBBX16 and PpBBX18 resulted in the highly accumulation of anthocyanin in *Arabidopsis*. Further analysis showed that although both PpBBX16/PpBBX18 could not directly bind to the promoter of PpMYB10, the complex of PpBBX16/PpBBX18-PpHY5 strongly trans-activated PpMYB10 promoter in tobacco. Furthermore, overexpressing of these two BBX proteins also resulted in high anthocyanin accumulation in transgenic pear calli under light condition. However, although both BBX proteins shared a similar regulatory model, RNA-seq analysis using the transgenic calli revealed significant transcriptome differences between these two overexpression lines. In addition, we also found that PpBBX21 repress the anthocyanin biosynthesis in both Arabidopsis and pear calli. Further analysis showed that PpBBX21 physically interacts with PpHY5 and PpBBX18 and such interactions hamper the formation of the active transcription activator complex of PpHY5/PpBBX18. Finally, these BBX proteins were transient expressed or silenced in pear fruit and the results confirmed that these proteins were involved in the anthocyanin biosynthesis. These results showed that PpBBX proteins associated with PpHY5 and fine-tuned the anthocyanin biosynthesis via transcriptionally regulate PpMYB10.

Notes

P7

ABA and R2R3 MYB transcription factors control anthocyanin biosynthesis in bilberry (*Vaccinium myrtillus* L.) fruit

Katja Karppinen

University of Oulu
katja.karppinen@oulu.fi

Katja Karppinen[1,2], Pinja Tegelberg[1], Nelli Mikkola[1], Hely Häggman[1], Laura Jaakola[2,3]

[1] Department of Ecology and Genetics, University of Oulu, Oulu, Finland;
[2] Climate laboratory Holt, Department of Arctic and Marine Biology, UiT the Arctic University of Norway, Tromsø, Norway;
[3] NIBIO, Norwegian Institute of Bioeconomy Research, Ås, Norway

Bilberry (*Vaccinium myrtillus L.*) is an economically important wild berry species growing in boreal forests of Northern Europe. Bilberry fruits accumulate anthocyanins both in peel and pulp during the berry ripening leading to high content and rich profile of anthocyanins in mature blue fruits. The fruit ripening associated anthocyanin biosynthesis is under developmental and hormonal regulation and in many non-climacteric fruits plant hormone abscisic acid (ABA) has a key regulatory role. The transcription of anthocyanin biosynthetic genes is directly controlled by an array of R2R3 MYB transcription factors as a part of MBW regulatory complex. In our study, we have investigated the hormonal and transcriptional regulation of ripening-related anthocyanin biosynthesis in bilberry fruit.

Our results show that exogenous application of ABA on unripe bilberry fruits accelerates anthocyanin biosynthesis and accumulation as well as up-regulates the expression of many ripening-related genes. In our study, we have also characterized 18 different flavonoid biosynthesis related R2R3 MYB transcription factors from bilberry. Some of the genes associate with anthocyanin biosynthesis based on their expression pattern and functional analyses. The expression of VmMYBA1 and VmMYB2 is induced at the onset of fruit ripening, elevated by exogenous ABA treatment and markedly down-regulated in white berry mutants lacking anthocyanins. Thus, our results confirm the role of ABA as an important regulator in bilberry fruit anthocyanin biosynthesis and provide insight into the complex regulation of flavonoid biosynthesis by R2R3 MYB transcription factors.

Notes

P8

Metabolic engineering of anthocyanin biosynthesis in chrysanthemum for developing blue flower color

Naonobu Noda

National Agriculture and Food Research Organization
naonobun@affrc.go.jp

Naonobu Noda[1], Satoshi Yoshioka[1], Sanae Kishimoto[1], Masayoshi Nakayama[1], Mitsuru Douzono[1], Yukihisa Katsumoto[2], Yoshikazu Tanaka[2], Ryutaro Aida[1]

[1] Institute of Vegetable and Floriculture Science, National Agriculture and Food Research Organization, 2-1 Fujimoto, Tsukuba, Ibaraki 305-0852, Japan;
[2] Research Institute, Suntory Global Innovation Center Limited, Suntory World Research Center, 8-1-1, Seika-cho, Soraku-gun, Kyoto 619-0284, Japan

Chrysanthemum cultivars have red and yellow flowers due to accumulation of cyanidin-based anthocyanins and carotenoid, respectively. However, there are no chrysanthemum cultivars with blue or violet flowers because they lack the flavonoid 3',5'-hydroxylase gene (F3'5'H), which is essential for the bluish delphinidin-based anthocyanin biosynthesis. In this study, we tried to generate violet and blue-colored chrysanthemums through genetic engineering. Our results showed that overexpression of the F3'5'H derived from Canterbury bells (CamF3'5'H), which is under control of the chrysanthemum F3H promoter, changed the flower color from pink to violet. Co-expression of the UDP-glucose:anthocyanin 3',5'-O-glucosyltransferase gene derived from butterfly pea (CtA3'5'GT) with CamF3'5'H allowed blue color to develop in the ray florets of chrysanthemums. Next, we screened copigments with roles in blue coloration by interacting with the newly synthesized 3',5'-diglucosylated delphinidin 3-malonylglucoside called ternatin C5 and performed experiments to reconstruct the blue color in vitro. Our findings showed that blue-colored chrysanthemums could be developed by intermolecular copigmentation of ternatin C5 and flavone 7-malonylglucosides. Copigmentation was also observed with delphinidin 3-malonylglucoside and cyanidin 3-malonylglucoside, which are major pigments of violet transgenic chrysanthemums and the original pink chrysanthemums, respectively. These results suggest that chrysanthemums are colored by copigmentation with anthocyanins and flavone glycosides. As observed in case of 7-malonylglucosyl-flavones, other flavones and flavonol glycosides also contribute to the bluish coloration of ternatin C5 in vitro.

Notes

P9

Anthocyanins and dihydroflavonol 4-reductase substrate specificity in *Gerbera hybrida*

Teemu H. Teeri

University of Helsinki
teemu.teeri@helsinki.fi

Saku Mattila, Lorenzo Mollo, Hany Bashandy and Teemu H. Teeri

Viikki Plant Science Centre, Department of Agricultural Sciences, University of Helsinki, 00014 UH, Finland

Gerbera (*Gerbera hybrida*) is an ornamental model plant of the Asteraceae family, with many cultivars with different inflorescence colors and patterns. The flavonoid metabolites in gerbera consist of flavones, flavonols and anthocyanins. Of anthocyanins, pelargonidin and cyanidin 3-O-glucosides are the most abundant, and cultivars can be grouped into anthocyanin free, pelargonidin-, cyanidin- and mixed types. Chemical blocking of the flavonoid B-ring hydroxylation at position 3' with the inhibitor tetcyclasis shifts the anthocyanin composition towards pelargonidin in mixed types but blocks anthocyanin biosynthesis altogether in cyanidin types. This rouse our interest in substrate specificity of the gerbera dihydroflavonol 4-reductase enzyme DFR, often responsible for the choice of the anthocyanin pathway branch kept active. We characterized three allelic forms of the enzyme, GDFR1-1 and GDFR1-2 expressed in pelargonidin type cultivars and GDFR1-3 expressed in a cyanidin type cultivar that reacts to tetcyclasis by turning acyanic. The DFR encoding sequences were expressed from pEAQ-HT vectors in agroinfiltrated Nicotiana benthamiana leaves, which yielded highly active protein extracts. Our expectations were that GDFR1-1 and 1-2 might not show substrate preference, but that GDFR1-3 would prefer not to reduce dihydrokaempferol, the precursor of pelargonidin. Instead, we found that GDFR1-3 does not differentiate between the three substrates leading to pelargonidin, cyanidin and delphinidin (dihydrokaempferol, dihydroquercetin and dihydromyricetin, respectively) but both GDFR1-1 and 1-2 show a strong preference for dihydrokaempferol. Accordingly, something apart from DFR substrate specificity prohibits pelargonidin formation in cyanidin type cultivars. Now, we are analyzing the petal transcriptomes of gerbera cultivars of different anthocyanin types.

Notes

P10

Role of light quality in regulation of anthocyanin biosynthesis in wild bilberry and woodland strawberry

Amos Samkumar

Arctic University of Norway
amos.s.premkumar@uit.no

Amos Samkumar[1], Florence Omowumi Omole[1], Katja Karppinen[1], Inger Martinussen[2], Laura Jaakola[1,2]

[1] Climate laboratory / Holt, Department of Arctic and marine Biology, UiT The Arctic University of Norway, Tromsø, Norway
[2] Norwegian Institute of Bioeconomy Research (NIBIO), Tromsø, Norway

Wild berries are abundant with health-beneficial bioactive compounds such as flavonoids, carotenoids, vitamins and polyphenolic compounds that accumulate in both skin and flesh of the fruit throughout the ripening process in some species. Interestingly, berries from northern latitudes are found to contain more phenolic compounds compared to southern clones. This genetic adaptation is most likely favored by environmental conditions such as extended day length, cool temperature and light spectral qualities. Anthocyanins, one of the most conspicuous classes of flavonoids are important plant pigments responsible for the red, purple and blue colors in berries with diverse biological functions. The regulation of anthocyanin biosynthesis in response to various environmental cues is less explored in wild berries. The present study is focused on gaining knowledge on this regulatory process with response to different light spectral conditions in two non-climacteric wild berry species, bilberry (*Vaccinium myrtillus*) and woodland strawberry (*Fragaria vesca*). Controlled experiments have been carried out in phytotrons with a local Norwegian clone of bilberry and three different strawberry clones (northern latitudes of Finland, Italy and Norway), which were subjected to continuous exposure of different light wavelengths from early to late ripening stages. Samples of leaves and berries were collected during berry ripening for the qRT-PCR analysis of the expression of flavonoid pathway genes and regulatory genes such as R2R3-MYB transcription factors. Our results show that red and blue light wavelengths are up-regulating anthocyanin biosynthesis and accumulation in bilberries but varying response pattern was detected among the three northern strawberry clones. Our results will bring better understanding on the light-mediated flavonoid biosynthesis in berries and can be applied in future breeding programs.

Notes

P11

Expression of anthocyanin biosynthesis-related genes as a function of time of day in corollas of lisianthus (*Eustoma grandiflorum*)

Daiki Mizuta

Nihon University
mizuta.daiki@nihon-u.ac.jp

Daiki Mizuta, Ryusei Tadokoro, Yoshiyuki Muramatsu, and Satoshi Kubota

College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa, Kanagawa, 252-0880, Japan

Lisianthus is one of the main commercial cut flower species in Japan. Many lisianthus cultivars in various flower colors, such as purple, red, pink, white, and yellow, have currently been bred. During a recent intensely hot summer season, however, high temperatures in cultivated greenhouses were responsible for dulling the colors of lisianthus flowers, with the resulting poor quality lowering their market value. In the present study, we investigated the expression trend of genes related to anthocyanin biosynthesis at different times of the day to obtain basic molecular information regarding the mechanism of coloration in lisianthus corollas during anthesis.

Pigmented petals were collected after flowering from two lisianthus cultivars, pink-flowered 'Granas Pink' and purple-flowered 'Korezo Blue'. Nine samples per cultivar were obtained every 3 h beginning at midnight on 31 August 2018. Using real-time PCR, we evaluated the transcript levels of five anthocyanin biosynthetic genes, namely, PAL, C4H, CHI, DFR, and ANS.

Our expression analysis based on time of day revealed that DFR and ANS, which function in the downstream portion of the anthocyanin biosynthetic pathway, were highly expressed in post-anthesis petals of both cultivars at all sampling times compared with the expressions of the upstream genes PAL, C4H, and CHI. In pink-flowered 'Granas Pink', the maximum transcript levels of each gene were observed at 9:00 or 12:00; in purple-flowered 'Korezo Blue', however, gene expressions were highest at 12:00 or 15:00. In contrast, none of the five genes were strongly expressed in lisianthus leaves. These results indicate that expression levels always differed between upstream and downstream genes of the anthocyanin biosynthetic pathway, with especially high levels observed in DFR and ANS, which function in the downstream portion of the pathway. Furthermore, the maximum expression levels of these five genes were detected during daylight hours regardless of cultivar.

Notes

P12

Genome editing approach for silencing FLS in *Petunia* × *hybrida* cultivar Sophistica Blackberry

Daria Nitarska

Technische Universität Wien
daria.nitarska@tuwien.ac.at

Daria Nitarska[1], Ina Menz[2], Thomas Debener[2], Heidi Halbwirth[1]

[1] Institute of Chemical Engineering, Technische Universität Wien, Vienna, Austria
[2] Institute of Plant Genetic, Leipniz Universität Hannover, Hannover, Germany

The flavonoid pathway plays a main role in pigment formation in petunia flowers. *Petunia* × *hybrida* Sophistica Blackberry (cv. Blackberry) is a black cultivar with alluring flowers. It has been shown that obtaining such dark colors flavonols, with anthocyanins, play a major role and serve as co-pigments. Flavonols form stable complexes with anthocyanins, leading to more intense color. Flavonol synthase (FLS), an enzyme that converts dihydroflavonols to flavonols, is pivotal to flavonol synthesis. Dihydroflavonols also serve as substrate for dihydroflavonol 4-reductase (DFR), one of the key enzymes in the anthocyanin biosynthesis pathway. Enzyme balance is a deciding factor in color formation. To evaluate the impact of flavonols on pigment formation in cv. Blackberry, the FLS gene was silenced using CRISPR/Cas9. Transgenic plants were created by Agrobacterium mediated transformation. Regenerated shoots were screened by PCR for nptII and Cas9 genes. In total 12 transgenic lines were obtained. Mutation success was confirmed by cloning and sequencing of the FLS fragment. Also, very low levels of FLS gene expression was detected in comparison to wild types. After flower development there was, however, no discernable change in the flower color in transgenic lines. Obtained results suggest that silencing of the FLS gene was successful, but the impact of this gene on flower color in this species is slight. Lack of visible change in flower color could also be explained by a higher availability of the substrate for DFR, which can result in higher production of anthocyanins. Further analysis of pigment composition in transgenic flowers is needed to confirm this. The research performed represents one of the first successful attempts at genome editing on petunia. The fact that it was performed on non-standard, commercial line, adds commercial relevance to this work.

Notes

P13

Order of glucosylation reactions in the biosynthesis of anthocyanins in red cabbage

Nobuhiro Sasaki

Toyo University
sasaki076@toyo.jp

Nobuhiro Sasaki, Ayaka Ogino, Natsumi Okawa

Toyo University, Department of Food and Nutritional Sciences, Faculty of Food and Nutritional Sciences, Japan

Red cabbage (*Brassica oleracea* var. capitata f. rubra) is commonly used in food as a natural colorant as it contains a highly stable pigment. The major anthocyanins of red cabbage are cyanidin 3-sophoroside-5-glucoside (CytriG) and mono- or di acylated CytriG [1,2]. The thermal and pH stability of these anthocyanins have been evaluated, and the acyl moieties attached to the anthocyanin molecules have been indicated to contribute to their stability [3]. However, the biosynthetic pathway for this polyacylated anthocyanin remains unclear. To elucidate the order of the three glucosylation reactions for cyanidin triglucoside, we assessed glucoslyltrasnferase activity using anthocyanins and UDP-glucose. Crude protein prepared from red cabbage leaf was added to the reaction mixture; the reaction products were then analyzed using HPLC. A product peak was detected on the HPLC chromatogram for the GT reaction with cyanidin 3-glucoside (Cy3G) as the acceptor; the retention time did not correspond to that of Cy3,5-diglucoside, while the m/z was observed at 611 corresponding to that of cyanidin diglucoside. Furthermore, enzymatic reactions with this new product elicited a new product peak with an m/z = 773, corresponding to that of CytriG. No peaks were detected when Cy3,5dG was used as the acceptor. These data imply that the glucosylation of CytriG in red cabbage occurs in the order Cy3→Cy3-sophoroside→Cy3triG.

To isolate the candidate cDNA encoding these anthocyanin glucosyltransferases, we performed RNA-seq analysis using mRNA extracted from red or green cabbage. Approximately 110,000 contigs were obtained via de novo assembly from short reads using the Trinity algorithm. A BLAST search yielded one and 3 contigs homologous to Cy3GGT and anthocyanin 5-GT, respectively.

[1] Tanchev, S.S., Timberlake, C.F. (1969) Phytochem. 8: 1825–1827.

[2] Idaka, E., et al. (1987) Chem. Lett. 6: 1213–1216.

[3] Ahmadiani, N., et al. (2016) Food Chem. 197: 900–906.

Notes

P14

Identification and characterization of MYB-bHLH-WD40 regulatory complexes controlling anthocyanin biosynthesis in flowers of *Freesia hybrida*

Xiang Gao

Northeast Normal University
gaoxiang424@163.com

Yueqing Li[1], Xiaotong Shan[1], Ruifang Gao[1], Song Yang[1], Li Wang[1], Xiang Gao[1,2]

[1] Key Laboratory of Molecular Epigenetics of MOE and Institute of Genetics & Cytology, Northeast Normal University, Changchun, China
[2] National Demonstration Center for Experimental Biology Education, Northeast Normal University, Changchun, China

The anthocyanin-promoting MBW complex has been well characterized in plants. Presently, a whelming proportion of MBWs has been well elucidated in core dicots, whereas few were studied in monocots, which are important to decipher their functional diversities among angiosperms during evolution. *Freesia hybrida*, a monocotyledonous genus of herbaceous perennial flowering plants in the family Iridaceae, is grown universally as a cut flower plant with a wide range of flower colors. A strategy combining transcriptomic data, expression profiles, gain-of-function experiments and transient protoplast transfection assays was undertaken to identify anthocyanin regulators. Consequently, FhPAP1, FhTT8L and FhTTG1, orthologs of AtPAP1, AtTT8 and AtTTG1, respectively, and two negative MYB regulators (FhMYB4 and FhCPC) were characterized. FhPAP1, FhTT8L and FhTTG1 were found to interact and activate anthocyanin biosynthesis. Moreover, evidences showed that R2R3-MYB FhMYB4 with strong repression capacity functioned as part of the MBW complex by interacting with bHLH proteins and changed the positive MBW complex into negative one. Comparatively, R3-MYB FhCPC functioned as negative regulators by competing bHLH regulators with anthocyanin-promoting MYBs, which titrated the formation of anthocyanin-promoting MBW complex. The precise regulatory cascade and feedback regulation in Freesia anthocyanin biosynthesis were much similar like that in core eudicot plants. However, divergent regulatory characteristics were also concluded. For example, FhPAP1 shared the same target genes but displayed higher transactivation capacity than its homologous genes in *Arabidopsis* and tobacco. Together, it can be deduced that the regulatory mechanisms between monocots and dicots were conserved in many aspects irrespective of evolutionary divergences, which benefited for the understanding of the regulatory mechanism accounting for flower pigmentation in monocots.

Notes

P15

The conserved and divergent roles of R2R3-MYB regulator FhPAP1 from *Freesia hybrida* in flower anthocyanin biosynthesis

Yueqing Li

Northeast Normal University
liyq339@126.com

Yueqing Li[1], Xiaotong Shan[1], Linna Tong[1], Chao Wei[1], Keyu Lu[1], Shuying Li[1], Shadrack Kimani[1,3], Shucai Wang[1], Li Wang[1], Xiang Gao[1,2]

[1] Key Laboratory of Molecular Epigenetics of MOE and Institute of Genetics & Cytology, Northeast Normal University, Changchun, China
[2] National Demonstration Center for Experimental Biology Education, Northeast Normal University, Changchun, China
[3] Karatina University, P.O Box 1957-10101, Karatina, Kenya

Anthocyanin biosynthesis is known to be controlled by MYB-bHLH-WD40 (MBW) complexes that modulating the expression of anthocyanin biosynthetic genes. The MYB regulators involved in anthocyanin biosynthesis arose early during plant evolution, thus may function divergently in monocots and dicots. Although the anthocyanin-promoting R2R3-MYB regulators in dicots has been comprehensively explored, little consensus has been reached about the discrepencies or the conservations among MYB regulators of different sources. Transcriptome analysis, gene expression profiles, gain-of-function experiments and transient protoplast transfection assays were herein integrated to functionally characterize the monocot *Freesia hybrida* anthocyanin MYB regulator gene FhPAP1 which showed synchronous correlations with anthocyanin biosynthetic genes. FhPAP1 was able to activate anthocyanin biosynthetic genes as well as TT8-clade genes FhTT8L, AtTT8 and NtAN1 when overexpressed in Freesia, Arabidopsis and tobacco. Consistently, FhPAP1 could interact with FhTT8L and FhTTG1 to form the conserved MBW complex which was indispensable for the floral anthocyanin biosynthesis. Prominently, FhPAP1 shared the same target genes but displayed higher transactivation capacity than its homologous genes in Arabidopsis and tobacco. These results suggested conserved characters or mechanisms among the MBW complex from monocot and core eudicot plants during evolution, whereas MYB regulators might be functionally divergent according to their transactivation capacities.

Notes

P16

Improvement of anthocyanins content in red table grapes by viticulture treatments for saving irrigation water supply

Pasquale Crupi

CREA-VE
pasquale.crupi@crea.gov.it

Pasquale Crupi and Antonio Coletta

CREA-VE - Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria – Viticoltura ed Enologia, Via Casamassima 148 - 70010 Turi (BA) – Italy.

Table grapes need of substantial water supply for achieving commercial requirements. Viticulture practices such as girdling (G) and gibberellic acid (GA) application, as well as water supply, can improve table grape quality. The study aimed to assess the counterbalance effect of these viticulture practices on the anthocyanins profile (determined by HPLC-DAD analyses) in case of a significant and unusual irrigation water reduction (40%) applied to a table grape variety. Water stress seemed to have a greater impact on anthocyanin composition; indeed, even though a significant increase in grape total anthocyanins (AT) was observed in full water supply (FWS) treatment, the higher concentrations of malvidin-3O-glucoside and peonidin-3O-glucoside were synthesized in the berries under water deficit (RWS). Conversely, the non-methoxylated anthocyanins, cyanidin-3O-glucoside and delphinidin-3O-glucoside were more abundant in the non stressed grapes. These results let to suppose that the different concentrations of anthocyanins might be due to the modification of their biosynthetic pathway; indeed, considering the ratios between methoxylated and non-methoxylated anthocyanins in the two irrigation treatments, it can be concluded that water deficit stimolated the anthocyanin methoxylation favoring the enzymatic conversion of hydroxylated anthocyanins into their more stable methoxylated ones. A significant decrease of AT, with the exception of delphinidin-3-glucoside, was observed in grapes from vine treated with G and GA, too. Moreover, an evident ordinal interaction between water management and viticulture treatments was observed; in particular, it is very interesting the Mv-3-g and Pn-3-g contents that was revealed in the RWS grapes under GA condition, suggesting how this viticulture treatment was able to get more stable color, thus improving the quality of grapes but maintaining a relative high yield and berry weight even in RWS conditions.

Notes

P17

A gene-specific approach illustrates the anthocyanins tissue-specificity on citrus species and related genera

Chiara Catalano

CREA Research Centre for Olive, Citrus and Tree Fruit
chiara1995catalano@hotmail.it

Chiara Catalano[1,2], Fabrizio Salonia[1,2], Angelo Ciacciulli[1], Maria P. Russo[1], Lara Poles[1], Paola Caruso[1], Gaetano Distefano[2], Marco Caruso[1], Giuseppe Russo[1], Concetta Licciardello[1]

[1] CREA Research Centre for Olive, Citrus and Tree Fruit, Corso Savoia 190 - 95024 Acireale (CT), Italy
[2] Department of Agriculture, Food and Environment, University of Catania, Via Valdisavoia 5 - 95123 Catania, Italy

Anthocyanin pigmentation characterizes young leaves and flower buds of many citrus genotypes having citron (*Citrus medica*) as parent. In addition, some blood oranges (*C. sinensis*), namely Moro, Tarocco and Sanguinello, accumulate anthocyanins in flesh and rind, even though they phylogenetically derive from complex and interspecific crosses between ancient mandarin (*C. reticulata*) and pummelo (*C. maxima*). The present work consists in the transcriptional analysis of PAL, early and late biosynthetic genes (respectively EBGs - CHS, CHI, F3H - and LBGs - DFR, ANS, UFGT), GST and regulatory (Ruby and Noemi) genes responsible of anthocyanin pigmentation on eight different tissues (flesh/juice, rind, young leaves, flower bud, petals, stamens, stylus, stigma) of twenty-eight accessions belonging to fourteen species (C. *medica, C. sinensis, C. limon, C. limonia, C. aurantifolia, C, latifolia, C. meyeri, C. latipes, C. celebica, C. hystrix, Microcitrus. australasica, M. paniculata, Severina distica, S. buxifolia*). Pigmentation is not only limited to the genus Citrus, but it is also diffused among citrus relatives. The CREA citrus germplasm collection (Catania, Italy) represents a precious genetic resource for investigating on the patterns of anthocyanin pigmentation of several tissues from citrus and related genera, never studied before. qRT-PCR showed that: (1) there is no consistent correlation between EBG and anthocyanin content in all samples; (2) otherwise a positive correlation was observed for LBG and GST; (3) Ruby and Noemi, respectively a Myb and a bHLH-type (part of the MBW complex), control the anthocyanins accumulation in flesh/juice, rind, stamen and stigma (exclusively for Ruby). This is the first time in which a gene specific picture was given on pigmented flower tissues, as stamen, stylus and stigma, of Citrus and related genera, which data could contribute to elucidate the complex origin of citrus and the effect of external factors on anthocyanin accumulation.

Notes

P18

Genetic control and regulation of anthocyanins in raspberry fruits

Luisa Palmieri

Fondazione Edmund Mach
luisa.palmieri@fmach.it

Muhammad Zubair Rafique, Luisa Palmieri and Stefan Martens

Research and Innovation Center, Fondazione Edmund Mach – IASMA, via E. Mach 1, 38010 San Michele all'Adige, Italy

Pigmentation is an important indicator of fruit quality trait that is usually controlled by complex genetic architecture. In raspberries, anthocyanins and carotenoids are responsible for imparting colour to the fruits, while red fruits are mainly considered due to the varying contents of anthocyanins. Raspberries also contain different concentrations of phenolic metabolites, however, a block of certain class was not observed except anthocyanins.

The present study provides new insights in the mechanisms of biosynthesis of pigments in raspberry fruits. Transcriptomic analysis of flavonoid pathway genes revealed the reduced Ans transcripts in "Anne (yellow)" as compared to "Tulameen (red)" which provided the basis at gene level for anthocyanin-less fruits. The in-silico search of an available Rubus "Heritage" genome draft with Ans of *Fragaria x ananassa* as template enabled the assembly of a putative ans gene of Rubus. Further, molecular cloning of Ans gene revealed a 5 bp mutation leading to a truncated protein in "Anne", which lacks conserved region for substrate, cosubstrate and iron binding sites. The complementation of 'KO' line of *Arabidopsis* via *Ans* gene from "Tulameen" elaborates the functional role of this gene in the anthocyanin biosynthesis pathway in raspberry. Molecular analysis of *Ans* gene was extended to several available red, yellow and orange fruiting varieties. The analysis indicated various Rubus *Ans* mutation types (RAMT-1 to -6) in the *Ans* gene which produce short/truncated proteins in many yellow and orange fruiting varieties. Therefore, we suggest that these raspberry genotypes also have originated from a mutation event in the *Ans* gene.

Present study led us to conclude that the yellow and orange fruit character of cultivar of most of the raspberry varieties originated from different mutation events in the *Ans* gene. Carotenoids seem to be responsible for the yellow and orange colour and they might are just masked by anthocyanins in red varieties.

Notes

P19

Anthocyanin and flavonoids role in mango fruit resistance to fungal pathogens and to chilling

Pradeep Kumar

Volcani Center
noamal@volcani.agri.gov.il

Pradeep S Kumar, Velu Sivankalyani, Ortal Galsarker, Oleg Feygenberg, Dalia Maurer, Noam Alkan

Department of Postharvest Science of Fresh Produce, Volcani Center, Agricultural Research Organization, Rishon LeZion, 7505101, Israel.

Fruits of red mango cultivars that accumulate anthocyanin are more resistant to both biotic (anthracnose) and abiotic (chilling) stress. To validate that anthocyanin correlates to biotic and abiotic resistance, red and green 'Shelly' mango fruit from the exterior and interior of tree canopy were evaluated. Red mango fruits from the exterior of tree canopy accumulated more anthocyanin, flavonoids and antioxidant, while the ripening parameters of both red and green mango fruit were similar. In response to storage at suboptimal temperature, the green fruit responded with ROS, lipid peroxidation and developed significantly more chilling injury symptoms than red fruit. Furthermore, red fruit had a more diverse stem end microbiome and had less postharvest decay. Red fruits were also more resistant to *C. gloeosporioides* inoculation both at the red and green side of the red fruit, which suggest the involvement of induced resistance. This induced resistance was further evaluated by transcriptome analysis, which showed that phenylpropanoid, anthocyanin and flavonoid biosynthesis pathways were upregulated. Interestingly, the resistance of red mango fruit include both induced resistance and direct antifungal activity. The direct antifungal activity was evaluated by organic extraction of red fruit peel that showed increased inhibition of conidia germination and hyphal growth in comparison to extract of green fruit. During the characterization of flavonoids and anthocyanin's, we found that un-glycosylated flavonoids from mango was more active against pathogenic fungi. To summaries, red mango fruit that accumulate high amount of anthocyanin showed increased resistance to chilling and fungal pathogens by direct antifungal activity and by activation of induced resistance. Using pruning and preharvest application of phytohormones, we were able to induce red color in fruit peel and fruit resistance to pathogens and chilling in several mango cultivars.

Notes

P20

Identification of anthocyanins in medicinal and aromatic plants: new phenotypes or plant physiological response?

Stefan Martens

Fondazione Edmund Mach
stefan.martens@fmach.it

Eirini Sarrou[1], Paschalina Chatzopoulou[1], Stefan Martens[2]

[1] Institute of Plant Breeding and Genetic Resources-Hellenic Agricultural Organization DEMETER, Thermi 57001, Thessaloniki, Greece;
[2] Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, San Michele all'Adige, (TN), Italy

Medicinal and aromatic plants (MAPs) species have been in the focus of research due to a wide class of bioactive substances they contain. Besides aromatic constituents, terpenes (di- or triterpenes) and polyphenols, the identification of anthocyanins in MAPs received limited attention so far. *Sideritis scardica*, commonly known as mountain tea and Greek oregano (*Origanum vulgare spp. hirtum*) are two of the main species that are widely cultivated in Greece. Concerning *S. scardica*, some genotypes expressing dark red apical bracts on inflorescences, a trait that has been undesired from producers and the local market as it is believed to be attributed to damaged and/or degraded tissue. Greek oregano plants express apical red leaves during the autumn of first developmental year, while the tendency of this phenomenon becomes limited during the rest years of plants life-circle. To investigate the above experimental cases two independent evaluations have been set up, in order to qualitatively and quantitatively identify the anthocyanins within the target plants. For *Sideritis*, the bracts were separated from inflorescences and divided in three parts; apical, intermediate and basal. For oregano, apical leaves were collected in autumn during first and second year of development, including a sampling also from summer. All tissues were extracted with 80% methanol and subjected to targeted LC-MS/MS. The analysis revealed cyanidin-3-O-galactoside (Cy3gal) as the most predominant anthocyanin in both species. In Sideritis bracts the concentration of Cy3gal varied between 19.8-216.3 mg 100g-1 dry weight (DW) from basal to apical part, while peonidin-3-O-galactoside was also detected in relatively lower amounts of 2.4-6.8 mg 100g-1 DW. In red oregano leaves, the content of Cy3gal was 121.9 mg 100g-1 DW and the related glucoside was identified as well in 5.3 mg 100g-1 DW. No anthocyanins were identified in the leaves of oregano collected during second developmental year.

Notes

P21

Influence of anthocyanin accumulation on carbohydrate content in *Prunus cerasifera* leaves: is this a strategy to limit the feedback regulation of photosynthesis?

Ermes Lo Piccolo

University of Pisa
ermes.lopiccolo@gmail.com

Ermes Lo Piccolo, Marco Landi, Rossano Massai, Damiano Remorini, Lucia Guidi

Department of Agriculture, Food and Environment, University of Pisa, 56124 Pisa, Italy

Photosynthesis is finely tuned by CO2 availability as well as sink/source balance. The feedback regulation of photosynthesis is a complex process, which depends on the cooperation of multiple signals, including sugars. In this experiment, shoot girdling was operated in 3-year-old green- and red-leafed Prunus cerasifera (GLP and RLP, respectively), to study the possible influence of anthocyanins in limiting the accumulation of sugars promoted by girdling. Leaf gas exchange, carbohydrate and anthocyanin content were monitored on a daily basis during the three days after girdling. On the first day at 12:00, net photosynthesis (A390) and stomatal conductance were only reduced (29 and 33 %, respectively) in girdled GLP which also showed higher soluble sugar concentrations than controls. Girdled RLP showed the first reduction of A390 only at 18:00 with no significant differences in sucrose, glucose and fructose concentration, although sorbitol was strongly accumulated. The increase in anthocyanin concentration only detected in girdled RLP at the second and third day might have contributed in lowering the hexoses accumulation that, together to the constitutive ability of RLP to produce more sorbitol than starch, influenced the circadian leaf starch metabolism and also attenuated the feedback downregulation of photosynthesis. The effectiveness of non-photochemical quenching, namely pNPQ, also revealed the ability of anthocyanins to photoprotect photosystem II (PSII) from supernumerary photons reaching the chloroplast, whose performances were compromised by girdling. Overall, a sugar-buffering role exerted by anthocyanins might have positively influenced the feedback regulation of photosynthesis and, on another hand, anthocyanin accumulation also improved the PSII photoprotection from excitation energy excess.

Notes

P22

Untargeted metabolomics strategy based on LC-MS-Orbitrap for discovering new polyphenol metabolites in humans after acute ingestion of *Vaccinium myrtillus* berry supplement

Lapo Renai

University of Florence
maria.ulaszewska@fmach.it

Lapo Renai[1], Claudia Ancillotti[1], Marynka Ulaszewskab[2], Fulvio Mattivib[2,3], Massimo Del Bubbaa[1]

[1] Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Florence, Italy.
[2] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via Mach 1, 38010 San Michele all'Adige, Trento, Italy.
[3] Center for Agriculture Food and Environment, University of Trento, San Michele all'Adige, Italy

In this work, liquid chromatography coupled with an electrospray ionization hybrid linear ion trap quadrupole/Orbitrap mass spectrometry, has been used to accurately identify polyphenol metabolites in human serum and urine after acute ingestion of a Vaccinium myrtillus berry supplement. The supplement was obtained by cryo-milling of bilberries, which were freeze-dried within one week after their harvesting, so as to maintain the berry native composition. Thirty-six derivatives of benzoic acids, hydroxyhippuric acids, cinnamic acids, phenylpropionic acids, phenylvaleric acids, phenylpentenoic acids and abscisic acid, together with two berry-native anthocyanins, one flavonol metabolite and two catechol derivatives, were putatively identified in the investigated biofluids. The annotated compounds included thirteen metabolites, among glucuronides and sulphates of phenylvaleric and phenylpentenoic acids, which have been identified for the first time in human biofluids after ingestion of V. myrtillus berries. It should be emphasized that the presence of phenylvaleric and phenylpentenoic acid derivatives is in agreement with their origin from fruit native flavanol monomers and oligomers, which are widely distributed in Vaccinium berries, but usually overlooked in metabolomics studies regarding bilberry. The identification of these compounds confirmed the key-role of untargeted metabolomics approach in the discovery of new metabolites which could result biologically active

Notes

P23

Anthocyanins and their cardioprotective role against doxorubicin

Debora Zorzan

Università degli Studi di Milano
debora.zorzan@unimi.it

Debora Zorzan, Francesca Cappellini, Chiara Tonelli and Katia Petroni

Department of Biosciences, Università degli Studi di Milano, 20133 Milan, Italy

Doxorubicin is one of the most effective chemotherapeutic drugs, but its clinical use is severely limited by its dose-dependent side effects. Among these, cardiotoxicity is the most feared one, since it can lead to cardiomyopathy, heart failure and eventually to death. Its onset may be delayed up to 10 years after the end of the chemotherapeutic treatment, placing paediatric cancer patients among the most at risk. The finding of new molecules which can counteract doxorubicin side effects is extremely important to guarantee a better life quality to cancer patients. Anthocyanins have recently gained interest as cardioprotective agents, thanks to their anti-oxidant and anti-inflammatory properties. We previously demonstrated that dietary anthocyanins from purple corn are able to counteract the doxorubicin-induced cardiotoxicity in mice, by ameliorating mid-term survival and preventing the cardiac histopathological alterations associated to doxorubicin treatment. However, the molecular mechanisms involved are not elucidated yet. In this study, we aim at understanding which pathways lead to the anthocyanin-mediated cardioprotection. Our analyses were performed in vitro using the murine atrial cardiomyocyte HL-1 cell line and a previously characterized purple corn extract (RED). Cells were treated with doxorubicin and/or RED extract and gene and protein expression, together with the activity and the interactions of specific factors involved in the AMPK-SIRT1-p53 pathway were analysed. Doxorubicin affects AMPK activity and, as a consequence, p53 acetylation and subcellular localization, thus increasing cardiomyocyte apoptosis. Overall, our data suggest that RED extract has a prominent role in promoting cell survival through the AMPK-SIRT1-p53 axis.

Notes

P24

Elderberry anthocyanins: a promising resource to support mitochondria-targeted therapeutic and nutritional approaches

Dina Neves

Universidade do Porto
dinaneves4@gmail.com

Dina Neves, Patrícia Valentão, João Bernardo, Paula B. Andrade, Romeu A. Videira

REQUIMTE/LAQV, Laboratório de Farmacognosia, Departamento de Química, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, Nº 228, 4050-313, Porto, Portugal

It is well-known that mitochondria dysfunction plays a key role in the onset and progression of several degenerative brain diseases. However, effective strategies to overcome the mitochondrial dysfunction and restore the interlinked cell redox balance remain a challenge. In the present work the potential of elderberry anthocyanins (*Sambucus nigra L.)* to support mitochondria-targeted therapeutic and nutritional approaches is characterized. Elderberries are rich in anthocyanins, which allow to obtain an anthocyanin-enriched extract dominated by cyanidin-3-O-sambubioside, cyanidin-3-O-glucoside and their 5-O-glucosides. This anthocyanin-enriched extract exhibits one-electron reversible redox behaviour (Epa=+0.079 V, Epc=+0.018 V), in the range of redox potentials where the mitochondrial redox chain operates, that emerge from its anthocyanin content. Studies with rat brain mitochondria revealed that elderberry anthocyanins i) have affinity for the lipid bilayer of mitochondrial membranes; ii) do not affect the respiration of mitochondria isolated from healthy animals; iii) are able to overcome the mitochondrial dysfunction promoted by rotenone (complex I inhibitor) creating an alternative pathway for electron delivery in the inner-mitochondrial membrane. Additionally, this anthocyanin-enriched extract, in the concentration range up to 50 µg/mL, protects neuron-like SH-SY5Y cells from rotenone-induced cytotoxicity, modulating the cell redox state and decreasing the reactive oxygen species generation. In the absence of toxic stimuli, anthocyanin-enriched extract promotes significant improvement of the functionality of mitochondrial respiratory complexes and of antioxidant enzyme system of the neuronal cells. Therefore, elderberry anthocyanins emerge as a promising tool to support the development of new functional foods and/or therapeutic approaches to fight against degenerative brain diseases connected with mitochondrial dysfunctions.

Notes

P25

Nutritional properties of betalains

Christine Boesch

University of Leeds
c.bosch@leeds.ac.uk

Christine Boesch

School of Food Science and Nutrition, University of Leeds, LS2 9JT, Leeds, UK

Betalains are a group of water-soluble pigments responsible for the red-violet and yellow colouring in plants of the order *Caryophyllales* such as beetroot, amaranth and cactus species. Besides their high tinctorial strength, betalains have demonstrated potential to inhibit inflammatory processes and scavenge free radicals, thereby potentially attenuating features of metabolic disease and aging. The intake of beetroot, a major source of betalains, has indeed been linked to improved health outcome, such as lowering of blood pressure, and there is a high interest in the development of functionalized beetroot products. However, there is limited evidence on betalain bioavailability and biochemical and molecular mechanisms that would explain associated health benefits. Current work in our group considers gastrointestinal effects of betalains, including carboanydrase enzyme inhibitory actions, betalain availability and molecular effects. Our results demonstrate in vitro and in vivo anti-diabetic properties following beetroot juice consumption, however, it is not clear whether the effects are due to betalains, nitrate or other components in the beet. Further research is underway to clarify the role of individual bioactives from beetroot in order to evaluate the relevance of betalains for human nutrition and health.

Notes

P26

In vitro inhibition of α-amylase and α-glucosidase by *Hibiscus sabdariffa*

Lisa Marshall

University of Leeds
L.J.Marshall@leeds.ac.uk

Sadia Zulfiqar, Lisa Marshall, and Christine Boesch

School of Food Science and Nutrition, Nutritional Sciences and Epidemiology, University of Leeds, LS2 9JT, Leeds, UK.

Hibiscus sabdariffa (HS), also known as red tea or Roselle, is a rich source of bioactive compounds and with a unique anthocyanin profile. Hibiscus intake has, besides lowering of blood pressure and blood cholesterol, been associated with the prevention of type 2 diabetes. The reduction of postprandial glycaemia is recognized as effective approach to control the risk of diabetes and inhibition of carbohydrate digesting enzymes α-amylase and α-glucosidase has been shown for a range of polyphenol compounds. However, there is limited evidence available regarding HS anthocyanins on mechanisms that contribute to anti-diabetic properties.

The aim of our project was to investigate the inhibitory effect of hibiscus and its main anthocyanins (delphinidin- and cyanidin-sambubiosides) on the activity of α-amylase and α-glucosidase enzymes. The total phenolic (Folin assay) and anthocyanin contents (pH differential method) as well as anthocyanin composition (HPLC) were analysed in extracts of hibiscus. The activity of α-glucosidase (S. cerevisae) was determined via absorbance assay using p-nitrophenyl-α-D-glucopyranoside as a substrate. Acarbose, a synthetic inhibitor of α-glucosidase was used as a positive control. Activity of α-amylase was determined using DNS method with human salivary enzyme and potato starch as a substrate.

The results demonstrate that HS preparations inhibit α-glucosidase activity in a dose-dependent manner with an IC50 of 10 mg/ml extract (equivalent to 55.7 mg/ml anthocyanins). The pure anthocyanin compounds do not indicate inhibition up to a concentration of 100 μM. Neither hibiscus nor individual anthocyanins are able to inhibit α-amylase, however, the assay was impacted by colour interference. Although in vitro experiments clearly indicate the potential of Hibiscus sabdariffa to reduce α-glucosidase activity, the enzyme inhibitory effect is not due to the major anthocyanins but to other ingredients of hibiscus which requires further investigation.

Notes

P27

Constituents in wine or wine compression residue alleviates aging of skin

Akiyoshi Sawabe

Kindai University
sawabe@nara.kindai.ac.jp

Akiyoshi Sawabe[1], Ryuji Takeda[2], Akira Iida[1]

[1] Department of Applied Biological Chemistry, Faculty of Agriculture, Kindai University, 3327-204 Nakamachi, Nara 631-8505, Japan;
[2] Faculty of Health and Welfare, Kansai University of Welfare Sciences, Kashiwara, Osaka 582-0026

Japan is seeing a rapid shift toward an aging society and a surge in the number of lifestyle disease's due to the westernization of eating habits. In the elderly or lifestyle disease's, in vivo glycation occurs, causing various diseases. Therefore, we focused on anti-glycation in this study and searched for an anti-glycation substance that can be utilized as health food and cosmetics. As a means to evaluate anti-glycation, we conducted the AGE (advanced glycation end product) inhibition test. Remarkable inhibitory active substance in wine or wine compression residue was separated by gel filtration and silica gel column chromatography. As for the ethyl-bridged anthocyanins-(epi)catechin dimers and its glycosides which are substances peculiar to wine, high inhibitory activity was shown. In conclusion, it might be used in general as food additives or cosmetics because remarkable AGEs inhibitory activity was found from the wine or wine compression residue.

Notes

P28

A systematic review and meta-analysis investigating the effects of a bilberry and blackcurrant anthocyanin extract (MEDOX®) on the lipid profile: Preliminary results

Laura Headley

Evonik Nutrition & Care GmbH
laura.headley@evonik.com

Laura Headley, Christopher Studte

Evonik Nutrition & Care GmbH, 64293 Darmstadt, Germany

Anthocyanins have been associated with improvements in health for many years, however one clear direction of research appears to be around cardiovascular disease (CVD). A bilberry and blackcurrant anthocyanin extract (known commercially as MEDOX®) has been investigated across a number of different clinical trials, yet with no definitive review quantifying a tangible health benefit.

Thus, the primary objective of the research was to report a systematic review and meta-analysis of randomised controlled trials that have been conducted using MEDOX®, in order to assess any influence on markers of cardiovascular disease risk, namely the lipid profile.

All known studies (n=17) supported by the manufacturers of MEDOX® were collected. PubMed (which included MEDLINE, www.ncbi.nlm.nih.gov/pubmed), the Cochrane Central Register of Controlled Trials (www.cochranelibrary.com/central), the International Clinical Trials Registry Platform (ICTRP - http://apps.who.int/trialsearch/) and the International Standard Randomized Controlled Trials Number Register (www.isrctn.org) were searched for any unpublished trials, the searches covered from January 1950 to January 2019.

The results indicated that MEDOX® significantly reduces LDL cholesterol (weighted mean difference (WMD) -12.36 (95% CI: -15.91, -8.82), P<0.00001) and ApoB (WMD 6.40mg/dL (95% CI: -11.38, -1.43), P=0.01). In addition, there were significant increases in HDL cholesterol (WMD 5.67mg/dL (95% CI: 4.01, 7.34), P<0.00001) and ApoA1 (WMD 4.89mg/dL (95% CI: 0.12, 9.67), P=0.04).

In conclusion, preliminary results appear to show that MEDOX® has a consistent and positive influence on the lipid profile of those individuals at increased risk of cardiovascular disease risk. Further research would be beneficial to understand the influence in healthy people and to also extend into health areas such as cognitive health, where CVD risk factors have been shown to also influence outcomes.

Notes

P29

Lingonberry juice decreases mean arterial pressure via cyclooxygenases in hypertensive rat models

Anne S Kivimäki

University of Helsinki
anne.kivimaki@helsinki.fi

Anne S. Kivimäki[1], Aino E. Siltari[1,2], Sanna H. Lehtonen[3], Riitta A. Korpela[1], Heikki I Vapaatalo[1]

[1] Medical Nutrition Physiology, Faculty of Medicine, Pharmacology, University of Helsinki, Helsinki, Finland
[2] Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland
[3] Department of Pathology and Research Program for Clinical and Molecular Metabolism, Faculty of Medicine, University of Helsinki, Helsinki, Finland

Lingonberry juice has been shown to decrease blood pressure, improve vascular function and ameliorate low-grade inflammation caused by hypertension in spontaneously hypertensive rats. These effects are most likely due to phenolic compounds of the lingonberry. Lingonberry is rich in anthocyanins, such as cyanidin-3-galactoside, cyanidin-3-glucoside and cyaniding-3-arabinocide. We investigated the effects of ingested lingonberry juice on mean arterial pressure (MAP) of spontaneously hypertensive rats (SHR) and salt-loaded Wistar Kyoto rats (WKY). To define the molecular mechanisms involved, we analyzed the expression levels of proteins involved in prostanoid synthesis and blood pressure control in the kidneys of SHR and WKY rats. Rats were given cold-compressed lingonberry juice ad libitum for eight weeks. As a result, MAP was calculated from systolic and diastolic blood pressure values measured by the tail-cuff method. Immunohistochemical staining of angiotensin-converting enzyme 1 (ACE1), cyclo-oxygenase 1 (COX1), cyclo-oxygenase 2 (COX2) and 8-hydroksy-2-deoxyguanosine (8-OHdg) from the kidney sections were done. MAP was decreased by lingonberry juice in hypertensive rats ($p \leq 0.01$). COX2 protein expression in kidney Macula Densa was significantly increased in lingonberry groups. In high-salt WKY group, COX2 protein expression was almost completely inhibited, but lingonberry significantly ameliorated the effect ($p \leq 0.01$). Lingonberry increased the Macula Densa COX2 protein expression also in hypertensive rats. COX1 was elevated by lingonberry, when the two experimental models were combined. ACE1 and 8-OHdg showed no significant difference. In conclusion, lingonberry decreases MAP of hypertensive rats. Lingonberry increases protein expressions of COX1 in the kidney cortex and COX2 in the Macula Densa. The blood pressure lowering mechanisms of the lingonberry juice is at least partially related to prostanoid synthesis and cyclooxygenase pathway.

Notes

P30

Bilberry (*Vaccinium myrtillus*) extract dosage and pharmacokinetics

Marie Kosehira

Omnica Co., Ltd.
kosehira@omnica.co.jp

Marie Kosehira

Omnica Co., Ltd. 112-0002 Tokyo, Japan

Introduction

Bilberry (*Vaccinium Myrtillus* : VM)extract has 15 anthocyanins which are composed of 5 anthocyanidin skeletons, each occurring as 3-O-glycoside with three varieties of mono sugars.

VM extract has been shown in several reports to have an effect on eye fatigue. However,

the pharmacokinetics of VM anthocyanins has not been confirmed in correlated between its dosage and its plasma concentration.

Objectives

We conducted a single-group crossover test to show the pharmacokinetics of *Vaccinium Myrtillus* Anthocyanin (VMA) after single dose administration with bilberry extract against healthy adult men and women.

Methods

Each single oral intake of 160 mg, 320 mg or 480 mg with bilberry extract was proceeded before blood samples, and samples were collected at 7 points (0.25, 0.50, 0.75, 1.00, 1.50, 2.00 and 3.00 h) after intake. Determination of anthocyanin in blood was performed using HPLC.

Results and conclusions

The AUC of total anthocyanin were dose-dependently increased about 2.2 times at double dosage and about 3.5 at triple dosage. Result suggested that 160mg – 480mg oral VM extract intake might be recommendable dosage in clinical studies to see physiological effects, too.

Notes

P31

Solutions for development of bioeconomics-use of food wastes to obtain products with added value

Linards Klavins

University of Latvia
linards.klavins@lu.lv

Linards Klavins[1], Arturs Viksna[2], Maris Klavins[1]

[1] University of Latvia, Faculty of geography and earth sciences, laboratory of natural products research, Jelgavas street 1, Riga, Latvia, LV-1004;
[2] University of Latvia, Faculty Chemistry, Jelgavas street 1, Riga, Latvia, LV-1004

The use of wild and cultivated berries have increasing popularity, which in turn creates large quantities of left over waste- berry press residues. *Vaccinium spp.* berries (bilberries, blueberries, cranberries, lingonberries) are used to produce juice, the residual pomace has low calorific value and the material is highly acidic. At the same time berry press residues contain high amounts of effective antioxidants- polyphenolics and substance groups like lipids, waxes and phytosterols. Using environmentally friendly approach, the extraction of this material can be done using ethanol, carbon dioxide coupled with extraction methods like ultrasound, microwave or supercritical fluid extractions. For the analysis of obtained hydrophilic berry press residue extracts UPLC-PDA-MS/MS was used, while for the analysis of hydrophobic extracts GC/MS was used. Optimisation of extraction was done using ethanol, methanol and trifluoroacetic acid (TFA) or formic acid as additives, optimal extraction conditions for analytical work were found to be 70% ethanol with 1% TFA. The extracts of press residues were characterized and approximately 150 different polyphenols and 95 compounds belonging to lipids were found. Biological activity of these extracts were tested on various food pathogens and it was found that 0.25mg/L of polyphenolic extracts show significant inhibition properties. Antioxidant extracts were also tested to show the hypoglycaemic and hepatoprotective properties. The prepared extracts show many possible applications, however, these extracts must be purified to remove carbohydrates, which can be done by using sorption chromatography or by stabilizing the extracts by using encapsulation matrices. The potential use of these extracts can be attributed to the high contents of biologically active substances; the main applications can include food industry, bio pharmacy, functional foods, cosmetics and others.

Notes

P32

A cisgenesis and target editing approach to improve health properties of citrus fruits combining anthocyanins and lycopene

Angelo Ciacciulli

CREA Research Centre for Olive, Citrus and Tree Fruit
concetta.licciardello@crea.gov.it

Angelo Ciacciulli[1], Fabrizio Salonia[1,2], Lara Poles[1], Helena D. Pappalardo[1], Marco Caruso[1], Paola Caruso[1], Maria P. Russo[1], Chiara Catalano[1,2], Giuseppe Russo[1], Concetta Licciardello[1]

[1] CREA Research Centre for Olive, Citrus and Tree Fruit, Corso Savoia 190, 95024, Acireale (CT), Italy;
[2] Department of Agriculture, Food and Environment, University of Catania, Via Valdisavoia 5, 95123 Catania, Italy

In the last years, several studies emphasize the importance of fruits and vegetables rich in healthy compounds. Anthocyanins and lycopene represent the most interesting and deeply studied pigments due to their protective effects, on treating chronic pathologies and lowering the risk of cancer and cardiovascular diseases.

Among citrus, there are several sweet orange (*Citrus sinensis*) varieties with high content of anthocyanins in the flesh, while only a limited number of grapefruit (*C. paradisi*), pummelo (*C. maxima*) and sweet oranges varieties contain exclusively lycopene. To our knowledge there are no references reporting the coexistence of both pigments in the same citrus cultivars.

The goal of several breeding programs is to obtain fruits that contain both pigments, even though conventional strategies in citrus are hampered by long juvenility, high heterozygosity and nucellar embryony. The advent of new breeding techniques (NBTs) and the knowledge of the function of specific genes represent an efficient alternative to conventional breeding once the regeneration protocol of the transformed variety has been optimized.

In the framework of BIOTECH program, funding by MIPAAFT to CREA Institution, the CITRUS (CITRus improvement by sUStainable BIOTECHnologies) project is addressed to the development of citrus fruit containing both anthocyanins and lycopene. Taking advantage from the knowledge of Ruby, the gene controlling anthocyanin pigmentation in blood oranges, we plan to use it in a cisgenesis experiment to transform lycopene-rich citrus varieties. On the other hand, in anthocyanin-rich varieties we are planning to perform a target editing of a putative GTP binding protein, likely associated with lycopene pigmentation. The activation of lycopene accumulation in a subset of blood varieties will be used to potentially reach our goal and combine both healthy pigments in one fruit.

Notes

P33

Increase of gut-protection bioactivity induced by wild strawberry anthocyanins after *in vivo* digestion

Massimiliano Fontana

Ulster University
massimiliano.fontana@guests.fmach.it

Massimiliano Fontana[1,3], Cheryl Latimer[1], Nigel G. Ternan[1], L. Kirsty Pourshahidi[1], Roger Lawther[2], Gloria O'Connor[2], Lorenza Conterno[3,4], Francesca Fava[3], Kieran Touhy[3] and Chris I. R. Gill[1]

[1]Nutrition Innovation Centre for Food and Health (NICHE), Ulster University, Coleraine, N. Ireland, UK
[2]Altnagelvin Area Hospital, Western Health and Social Care Trust, Londonderry, UK
[3]Department of Food Quality and Nutrition, Fondazione Edmund Mach, San Michele all'Adige, Italy
[4]Fermentation and Distillation Group, Laimburg Research Centre, Laimburg, Italy

Berries are one of the most consumed sources of bioactive polyphenols, including anthocyanins, and these compounds exert protective effects against initiation of colorectal cancer (CRC) by reducing DNA damage. Some varieties of freshly collected wild strawberry can reach up to 200mg/100g of anthocyanins. We hypothesised that physiologically relevant levels of Italian wild strawberry metabolites exiting the ileum would be both bioavailable and would exert positive effects on gut health. Five ileostomists completed a wild strawberry feeding study (11/NI/0112), ileal fluid was collected pre (0 h) and post (8 h) consumption of strawberries (225 g) and assessed for phytochemical composition by LCMSn. We simulated the interaction of the ileal fluids with colonic microbiota over a 24 h period (0, 5,10, 24 hr) using in vitro gut fermenter models. Nutri-kinetic analysis using LCMSn demonstrated significant increases in the concentration of gut microbiota-mediated polyphenolic metabolites over time. While changes in the bacterial composition of the gut fermenter models were monitored using fluorescent in situ hybridisation analysis with validated probes for Total bacteria, Bifidobacterium genus, *Clostridium histolyticum/perfringens* group, *Faecalibacterium prausnitzii*, Eubacterium rectale group, *Bacteroides*, Lactobacilli and Enterobacteria; limited changes observed. Bioactivity of the post-berry consumption ileal fermentates was assessed on two colonocyte cell lines (HT29 and CCD841 CON (normal)) using COMET assay. Post-berry ileal fermentate from all five ileostomists significantly ($p<0.01$) decreased DNA damage in both HT29 cells and CCD841 cells compared to untreated controls. To conclude, strawberry phytochemicals were available for colonic fermentation following ileal digestion and human microbiota-mediated fermentation which subsequently increased overall levels of polyphenolic metabolites, the post berry fermentates were demonstrated to reduce DNA damage in colonocytes.

Notes

P34

In vitro antioxidant activity and α-glucosidase inhibition of red and yellow prickly pear fruits (*Opuntia* spp)

Sumali Fernando

University of Leeds
fsgsnf@leeds.ac.uk

Sumali Fernando[1,2], Aisha Jamil[1,2], Estefania Valero-Cases[3], Natalia Sergeeva[2], Lisa Marshall[1], Maria Jose Frutos Fernandez[3], Christine Boesch[1]

[1] School of Food Science and Nutrition, Faculty of Mathematics and Physical Sciences, University of Leeds, United Kingdom;
[2] School of Chemistry, Faculty of Mathematics and Physical Sciences, University of Leeds, United Kingdom;
[3] Agro-food Technology Department, Miguel Hernández University of Elche, Alicante, Spain

Prickly pear fruits are considered one of the richest sources of plant bioactives containing different polyphenols and betalain compounds. Betalain pigments can be classified into two major groups, red coloured betacyanins and yellow coloured betaxanthins, which have demonstrated high radical scavenging potency and been associated with health benefits although research into biological mechanisms is scarce. This project aimed to evaluate the phytochemical composition, antioxidant and α-glucosidase inhibitory properties of red and yellow prickly pear varieties. Total betalain content, total phenolics, antioxidant capacity (Trolox-equivalent antioxidant capacity, TEAC), Ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) assays) as well as α-glucosidase inhibition were analysed. Qualitative analysis of betalain pigments in red and yellow prickly pear was conducted with reverse phase high-performance liquid chromatography-diode array detection (HPLC-DAD) coupled with mass spectrometry (MS). Total betalain and total polyphenol contents of red prickly pear were 0.77 ± 0.2 mg/g and 2.28 ± 0.2 mg/g whereas yellow prickly pear showed 0.71 ± 0.2 and 2.58 ± 0.2 mg/g, respectively. Antioxidant activity did not differ between the samples as well as the dose-dependent inhibition of α-glucosidase enzyme activity which was similar in both prickly pear types. HPLC-MS results revealed that indicaxanthin is the major betalain compound present in both yellow and red prickly pear fruits whereas red prickly pear contains only betanin. In summary, despite differences in betalain pattern, both red and yellow prickly pear fruits can be considered comparable with regards to antioxidant and potential anti-diabetic properties which requires further investigation.

Notes

P35

Methyl jasmonate as a tool to improve anthocyanins on grapes grown on subtropical climate

Panagiotis Arapitsas

Fondazione Edmund Mach
panagiotis.arapitsas@fmach.it

Laís Moro[1], Alessio Da Ros[2], Renata Vieira da Mota[3], Eduardo Purgatto[1, 2],Fulvio Mattivi[2,4], Panagiotis Arapitsas[2]

[1] FORC – Food Research Center, University of São Paulo, São Paulo, Brazil;
[2] Department of Food Quality and Nutrition, Research and Innovation Center Fondazione Edmund Mach, San Michele all'Adige, Italy;
[3] Empresa de Pesquisa Agropecuária de Minas Gerais – EPAMIG - Núcleo Tecnológico Uva e Vinho;
[4] Center Agriculture Food Environment, University of Trento, Trento, Italy

Vitis labrusca L. grapes are largely cultivated in Brazil, but the tropical climate affect negatively the phenols content, especially anthocyanins. In the future, due to the global climate changes, such phenomenon could expand in more regions. Therefore, many researches focus on increasing grape phenols content, and methyl jasmonate (MeJa) treatment could be a good alternative. Since studies on *Vitis vinifera L.* grapes gave promising results, the aim of this study was to evaluate anthocyanin changes caused by the MeJa pre-harvest application on two *Vitis labrusca L.* cultivars grape, both grown in two Brazilian regions. Isabel Precoce and Concord grapes cultivated under subtropical climate on south and southeast of Brazil, received MeJa pre-harvest treatment. Grape metabolites were extracted and analyzed with a MS based metabolomics protocol by UPLC-HRMS-QTOF. Unsupervised data analysis reveals clear separation between the two regions and the two cultivars, while supervised data analysis revealed biomarkers between MeJa and control group. Among the varieties, Concord grapes were more responsive to the treatment, while Isabel Precoce cultivated on the south was the least responsive one. We annotated 35 anthocyanins, such cyanidin, peonidin, delphinidin, malvidin and petunidin in the caffeoyl, acetyl and p-coumaroyl mono and diglucoside derivatives. We observed, that MeJa promoted higher amounts of anthocyanins 3' hydroxylated, acetylated and coumarylated derivatives forms, with exception of Isabel Precoce grapes cultivated on southeast region. Our results suggested that MeJa could be used as elicitor to secondary metabolism in grapes grown even under subtropical climate, affecting anthocyanin biosynthesis.

Notes

P36

Natural Blues: Structure meets function in plant natural products

Alan Houghton

John Innes Centre
alan.houghton@jic.ac.uk

Alan Houghton

Department of Metabolic Biology, John Innes Centre, Norwich, United Kingdom

Butterfly pea (*Clitoria ternatea*) produces intensely blue-coloured flowers pigmented by highly decorated delphinidin-based anthocyanins. Petals from these plants are used to colour popular Asian dishes, such as blue tea which changes colour based on the pH of substances added to it.

Choices of blue food pigments are extremely limited, with only two options in the US, synthetic blue No. 1 and No. 2, and a third available in Europe, patent blue V. Confectionery manufacturers have invested a great deal in finding a naturally derived replacement, with little success.

To date, only one naturally derived blue colourant has been developed, the dried biomass of cyanobacterial species Anthospira platensis and A. maxima. This colourant is limited in usefulness, producing a more greenish blue that degrades upon heating and exposure to light.

Anthocyanins of butterfly pea have remarkable stability stored in solution, even at room temperature and resistance to high-temperature processing. However, the anthocyanins in extracts from the flower petals represent a complex mixture of intermediates with different chemical decorations and different functionalities.

Understanding how decoration and interaction with co-pigments influence the stability and colour characteristics are an important consideration when developing an industrially reliant food pigment. Preliminary results have shown increased stability of ternatins at neutral pH's over a three-month period compared to other blue anthocyanin. In further work using state-of-the-art natural product analysis techniques, purified ternatins will be analysed and their structural characteristics related to their function as pigments.

Notes

P37

Betalains in Australian *Portulaca oleracea* populations with bioactive potential

Colette Geier

Charles Sturt University
cgeier@csu.edu.au

Colette A Geier[1], Paul D Prenzler[2], Danielle Ryan[2], Lachlan J Schwarz[1]

[1] School of Agriculture and Wine Science, Charles Sturt University, Wagga Wagga, Australia,
[2] Graham Centre for Agricultural Innovation, Wagga Wagga, Australia.

Portulaca oleracea (PO) is a widely utilised medicinal plant with considerable ethnobotanical use worldwide. Multiple studies (both animal and human) have supported the use of PO in the treatment of a range of conditions, however the isolation and identification of compounds responsible for these activities is ongoing.

PO is a source of betalain pigments, which are currently receiving significant attention in other plants due to their bioactivity, including anti-inflammatory, anti-oxidant, neuroprotective and insulin regulating action. The betalains in PO were first examined in the 1970's, but have received little attention since, with most profiling focused on phenolic compounds rather than betalains.

This project involved testing of 3 distinct Australian PO populations chosen due to differing levels of visual pigmentation. Each population underwent a variety of in vitro bioactivity tests and chromatographic profiling with diode array and mass spectral detection (LC-QTOF-MS and LC-QQQ-MS) for compound characterisation. The lack of commercial betalain standards available to aid compound identification was overcome by preparing extracts from plant species with known betalains.

Eight betacyanins were detected in all PO populations; the majority of which are reported here for the first time (in PO). They consist of 4 pairs of isomers including betanin and isobetanin, plus 3 more complex pairs containing additional sugar and phenolic moieties.

A crude extract of the PO population with the highest abundance of betalains also exhibited the highest levels of bioactivity in a range of assays, including antioxidant activity and glucose metabolism inhibition.

Notes

P38

Exploring new anthocyanin sources: from the underutilized *Prunus mahaleb* and local landraces of purple carrot, to the new anthocyanin-rich tomato genotype, 'Sun Black'

Federica Blando

National Research Council (CNR)
federica.blando@ispa.cnr.it

Federica Blando[1], Gabriele Maiorano[1], Helge Berland[2], Giovanni Mita[1], Øyvind. M. Andersen[2]

[1] Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy
[2] Department of Chemistry, University of Bergen, Allegt 41, 5007 Bergen, Norway

The identification of novel plant source of bioactive components with antioxidant function has recently become important to the food and nutraceutical industries.

Over the last decades, a huge amount of research has been focused on the possible health effects of anthocyanins, relatively to the consumption of fruit and vegetables containing these bioactive phytochemicals. Many of these health activities have been attributed to the intense antiradical and antioxidant activity of anthocyanin. The relationship between anthocyanin chemical structure and their corresponding chemical and biological activity is a challenging area of research which has been tackled in several studies.

Here we report on the structure-activity relationship of anthocyanin compounds from unusual fruit and vegetables source.

Prunus mahaleb L. is used as rootstock for sweet cherry, producing dark-purple drupes, rich in non-acylated cyanidin 3-glycosides. Black carrot (*Daucus carota L. ssp. sativus* var. atrorubens Alef.), is an anthocyanin-rich carrot producing mostly cyanidin 3-glycosides acylated with various cinnamic acid derivatives, and it is present in Apulia with local landraces (purple carrot). 'Sun Black' tomato is a new genotype of tomato synthesizing anthocyanins in the peel, based mainly on petunidin and malvidin 3,5-diglycosides acylated with p-coumaric acid.

Purified anthocyanin from the abovementioned plant materials have been evaluated for their anthocyanin content and composition, in addition to the antioxidant capacity using TEAC and ORAC assays.

Our findings suggest that these new anthocyanin sources could serve as a source of bioactive compounds and therefore find interest from the functional food and nutraceutical industries, as a natural food colorant and antioxidant ingredient in the formulation of functional foods. Moreover, the new utilization of these crops plant can protect plant biodiversity from erosion.

Notes

P39

Anthocyanin profile of eleven table grape cultivars grown in Slovenia

Maja Mikulic-Petkovsek

University of Ljubljana
maja.mikulic-petkovsek@bf.uni-lj.si

Maja Mikulic-Petkovsek[1], Andreja Skvarc[2], Denis Rusjan[1]

[1] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Chair for Fruit, Vine and Vegetable Growing, Jamnikarjeva 101, SI-1000 Ljubljana, Slovenia
[2] Chamber of Agriculture and Forestry of Slovenia, Agricultural and forestry institute Nova Gorica, Pri hrastu 18, SI-5000 Nova Gorica, Slovenia

Table grapes are enlarging an importance place on the European market, from the economic and production points of view. In addition to content sugars and acids, phenolic compounds are also important for fruit quality. Skin colour varies mainly due to the composition and content of anthocyanins, which contribute to the red, blue or violet skin colour. The content of anthocyanins in grapes is genetically conditioned, but it also depends on a number of environmental factors, such as soil properties, water, temperature, nutrient content, presence of pathogens etc. Consumers also demand that grapes should be cultivated with a low quantity of pesticides, so tolerant grape cultivars are selected for production, those that can be cultivated in an environmentally friendly way. Very little information is available on tolerant table grape cultivars. It therefore seemed to us important to try to discover differences in the quality parameters of different table grape cultivars and to discover which cultivars would meet consumer demands, as well as be suitable for intensive cultivation.

The composition and content of anthocyanins were analysed by HPLC-MS in eleven table grape cultivars: 'Esther', 'Cardinal', 'Lival', 'Muscat de Hambourg', 'Michele Palieri', 'Muscat blue', 'Nero', 'Ribol', 'Perlon', 'Presentabil' and 'Chasselas rouge'. In grapes has been identified different glycosides of malvidin, cyanidin, delphinidin, petunidin and peonidin. Malvidin glycosides were the most abundant anthocyanins in all cultivars. In grapes were predominantly detected anthocyanin monoglucosides, two diglucosides, one caffeoyl glycoside, five coumaroyl derivatives and four acetyl derivatives. Main anthocyanins by their contents were malvidin 3-O-glucoside, peonidin 3-O-glucoside, malvidin 3-O-glucoside-5-O-glucoside, peonidin 3-O-glucoside-5-glucoside, malvidin 3-O-(6-O-coumaroyl)-glucoside and peonidin 3-O-(6-O-coumaroyl)-glucoside.

Notes

P40

Anthocyanins in berries of various *Sambucus* species and their interspecific hybrids

Robert Veberic

University of Ljubljana
robert.veberic@bf.uni-lj.si

Maja Mikulic-Petkovsek[1], Anton Ivancic[2], Robert Veberic[1]

[1] Department of Agronomy, Chair for Fruit, Wine and Vegetable Growing, University of Ljubljana, Biotechnical faculty, Ljubljana, Slovenia
[2] Institute for Genetics, University of Maribor, Faculty of Agriculture and Life Sciences, Hoce, Slovenia

Genotypic and phenotypic variations within the *Sambucus nigra* species appear to be insufficient to satisfy all specific demands, especially those associated with yield, chemical composition of berries and uniformity of ripening. Our studies indicate that interspecific hybridisation efficiently increases the variation in chemical composition of elderberries.

The aim of the present study was to analyze the variations associated with the levels of various anthocyanidin derivatives in different elderberry genotypes.

The biochemical investigation included five species of the *Sambucus genus* (1 - *Sambucus cerulea* (CER), 2 – *S. ebulus* (EB), 3 - S. *javanica* (JA), 4 - *S. nigra* (NI), 5 - *S. racemosa* var. miquelli (MIQ)), and three groups of interspecific hybrids (1 – hybrids of *S. cerulea*, 2 - hybrids of S. nigra and 3 – hybrids of *S. racemosa*). In five elderberry species and eight hybrids were detected 20 different anthocyanins. *Sambucus nigra* fruit have the highest content of cyanidin-3-sambubioside (344 mg/100 g FW) and cyanidin-3-glucoside (190 mg/100g FW). The major anthocyanins detected in fruit of different species and interspecific hybrids were cyanidin-3-O-sambubioyl-5-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-sambubioside, cyanidin-3-glucoside and cyanidin-3-(E)-p-coumaroyl-sambubioside-5-glucoside. Conversely, in S. javanica and S. racemosa hybrids cyanidin-3-(Z)-p-coumaroyl-sambubioside-5-glucoside and cyanidin-3-(E)-p-coumaroyl-sambubioside-5-glucoside prevailed. Total analyzed anthocyanins (TAA) varied greatly from hybrid to hybrid. Promising species or hybrids with TAA higher than 400 mg/100 g were (JA x NI) x CER, (JA x NI) x 'Black Beauty', *Sambucus nigra* and in particular, JA x RAC hybrid which contained 834 mg/100g of anthocyanins.

Notes

P41

Changes during development of red- and white-fleshed apple cultivars

Martina Kolarek

Technical University of Munich
kolarek@wzw.tum.de

Martina Kolarek[1], Vinicius Vilperte[2], Rares Lucaciu[3], Md Sahadat Ali[1], Michael Neumüller[4], Thomas Rattei[3], Thomas Debener[2], Thomas Hoffmann[1], Johannes Hadersdorfer[6], Heidrun Halbwirth[5], Wilfried Schwab[1]

[1] Biotechnology of Natural Products, Technical University of Munich, 85354 Freising, Germany;
[2] Department of Molecular Plant Breeding, Institute for Plant Genetics, Leibniz Universität Hannover, Germany;
[3] Division of Computational Systems Biology, University of Vienna, Vienna, Austria;
[4] Bavarian Centre of Pomology and Fruit Breeding, 85399 Hallbergmoos, Germany;
[5] Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Vienna, Austria;
[6] Associate Professorship of Fruit Science, Technical University of Munich, 85354 Freising, Germany

Red-fleshed apples are increasingly important for consumer preference and marketability, not only due to aesthetic reasons, but also to the health benefits associated particularly with red pigments. Due to their particular anthocyanin contents, red-fleshed apples have received increased awareness.

'Baya® Franconia' and 'Baya® Marisa' are modern and aromatic red-fleshed apple cultivars with an attractive bicolored fruit flesh. They were bred at the Bavarian Centre of Pomology and Fruit Breeding. The apple fruits of these cultivars develop a broad red colored stripe below the skin that turns into a marbled pattern towards the core of the fruit. These cultivars are progenies of a white flesh apple cultivar 'Pomona' and a red-fleshed apple cultivar 'Weirouge'.

The objective of this research was to evaluate the polyphenolic profile and the underlying gene expression between the cultivars and their parental lines during development by means of high performance liquid chromatography (HPLC) and reverse transcription quantitative PCR (RT-qPCR). The acquired detailed information on phenolic pattern could sustain future breeding efforts towards additional improvement of apple fruit quality and could support the potential of red-fleshed apples as functional food.

Notes

P42

One-step strategies to prepare natural anthocyanins and 3-deoxyanthocyanidins

Mora-Soumille

Université d'Avignon, INRA
nathalie.mora@univ-avignon.fr

Nathalie Mora, Manon Disdier, Hélène Bitard, Pascale Goupy, Maxence Rosa, Olivier Dangles

UMR408, UAPV-INRA, SQPOV/Safety & Quality of Plant Products. Equipe Micronutriments: Réactivité & Digestion/Reactivity & Digestion. Université d'Avignon, UFR-ip STS Campus Jean-Henri Fabre, Pôle Agrosciences. 301, rue Baruch de Spinoza, BP 21239 84911 Avignon Cedex 9.

Although ubiquitous, anthocyanins are generally found in low amounts in plant and are difficult to extract in high yield. Thus, in some instances, strategies involving chemical synthesis and hemisynthesis come up as interesting alternatives. Two such strategies will be presented:

- The synthesis of red sorghum 3-deoxyanthocyanidins (3-DAs) apigeninidin and luteolinidin in a one-step acid-catalyzed aldol condensation from cheap commercially available phenolic precursors. The optimal conditions maximize the yield (70 – 80%) while suppressing the side reaction leading to *xanthylium* pigments (< 5%).

As 3-DAs are typically poorly water-soluble, the pigments were solubilized by interaction with food-compatible biopolymers (food proteins, pectins), thereby allowing the determination of the thermodynamic constants of proton transfer (color variation) and water addition (color loss) in the absence of organic solvents.

- The one-step conversion of flavonols (abundant in plant and food, e.g. in yellow onions) into anthocyanins by two-electron reduction of the flavonols' carbonyl group followed by dehydration. Some methods have been described in the literature, using different reducers, including metals and metal amalgams. In this study, zinc and magnesium were used as biocompatible reducers under acidic conditions. Our strategy was:

a) to optimize the reaction conditions (metal/flavonol molar ratio, acid concentration, solvent, temperature, reaction time, sonication, second step of stirring under air to regenerate the flavylium ion from over-reduced products) using the commercial flavonol rutin (up to 40% conversion into cyanidin 3-O-rutinoside with minimal amounts of by-products resulting from hydrolysis of the glycosidic bonds),

b) to apply the best experimental conditions to onion extracts rich in flavonols for the production of cyanidin 4'-O-glucoside and cyanidin 3,4'-O-diglucoside.

Notes

P43

Generalized additive models reveal the dynamic changes of anthocyanins in *Vitis vinifera* cv. Gamay cell suspension cultures in response to methyl jasmonate

Pietro Franceschi

Fondazione Edmund Mach
nmmtsaw@gmail.com

Nay Min Min Thaw Saw, Claudio Moser, Stefan Martens, Pietro Franceschi

Research and Innovation Centre, Fondazione Edmund Mach, 38010 San Michele all'Adige, Italy

Plant cell cultures are model systems for the study of plant biosynthetic pathways and to aid the investigation of which factors influence metabolism. Unlike whole organisms, they can be grown under controlled and reproducible conditions and this makes them ideal to collect robust datasets and to develop new methods of data analysis. Nowadays, high throughput metabolomics allow us to investigate the cellular metabolic response over time, so we used targeted metabolomics to obtain a detailed metabolic characterization of anthocyanins over twenty days of methyl jasmonate (MeJA)-elicited Gamay cell cultures. To fully capture the whole information within this type of data, we implemented a new approach using generalized additive models (GAMs). GAMs are a powerful statistical analysis for time resolved experiments to model and compare the "trends" of different metabolites. We obtained time series from Vitis vinifera cell suspension cultures for 20 time points during the growing period. The control and MeJA treated samples were extracted by methanol/chloroform/water (2:2:1 v/v) solvent mixture and anthocyanins were analysed by ultra-performance liquid chromatography mass spectrometry (UPLC-MS). Afterwards, we applied GAMs to infer the time dependents profile of anthocyanins in VGR cell cultures in relation to MeJA elicitation. The general finding of our investigation is that the concentration and pattern of polyphenols are changing in time to a different extent depending on their position in the biosynthetic pathway and that the elicitation by MeJA selectively changes these trends.

Notes

P44

Study of the Chilean Cabernet Sauvignon wines metabolomic fingerprint

Vania Sáez

Fondazione Edmund Mach
vania.saezpulgar@guests.fmach.it

Vania Sáez[1], Alvaro Gonzalez[2], Panagiotis Arapitsas[1]

[1] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach, San Michele all'Adige, Italy
[2] Research and Investigation Center of Concha y Toro Vineyards, Talca, Chile

Chile is one the biggest exporters and producers of wine with an annually production of 12 million of hectoliters [1]. The vineyards in Chile covers a surface of 135.907 hectares including diverse valleys from Elqui to Malleco. The special geography of Chile can provide diverse climatic influences for the viticulture valleys ruled by the Andes mountains range in the east and Coastal range in the west. The north of Chile is very hot and dry, whereas in the south are colder and wet. Chilean wine production is focalized in Maule, O'Higgins and Metropolitan region. The 65.8% of Chilean wines with appellation of origin produced are red wines and the 28.7% of this production are Cabernet Sauvignon wines [2], thus mean is the more exported and commercialized Chilean wine. With the purpose to study the metabolomic fingerprint of the Chilean Cabernet Sauvignon, 50 wine samples were produced by grapes of different geographic origin (Maule, Colchagua, Maipo, Bío-Bío, Curicó, Cachapoal, Limarí and Valparaiso) and were analyzed by an untargeted LC-MS protocol [4]. In order to exclude the impact of the winemaking, the same standardized winemaking protocol was applied to all the samples. The analysis of the data is focused on the investigation of the behavior of a) the metabolomic fingerprint with a holistic way, and b) specific metabolites known for their importance in wine quality. Between the studied metabolites, were several anthocyanins derived by the grapes or produced during the winemaking.

Acknowledgment: Research and innovation Center (CRI), Talca, Chile and CONICYT, Beca Chile.

References:

1. World wine production OIV 2018.

2. Servicio Agrícola y Ganadero, Informe Ejecutivo, producción de vinos, SAG, Chile, 2018.

3. Montes et al., Australian Journal of Grape and Wine Research,18, 20–28, 2012

4. Arapitsas P & Mattivi F., Metabolic Profiling, Methods in Molecular Biology, vol 1738, 2018.

Notes

P45

Improved delphinidin stability and cellular absorption by chitosan-based nanoparticles entrapment

Federica Tramer

University of Trieste

ftramer@units.it

Odeta Kalaja[1], Andreja Vanzo[2], Sabina Passamonti[1], Federica Tramer[1]

[1] Dept. Of Life Sciences, University of Trieste, Via Weiss 2, 34128 Trieste, Italy;
[2] Central Laboratory, Agricultural Institute of Slovenia, Hacquetova 17, 1000 Ljubljana, Slovenia

Several studies demonstrated that anthocyanidins exert intense biological activity at the cellular level. In intestinal cell lines, delphidin has been shown to cause selective cytotoxicity in transformed LoVo cells, leaving intact normally differentiated intestinal Caco2 cells. These observations can however hardly be replicated in vivo, because of extensive biotransformation within the gut. We aimed at improving delphinidin (DEL) stability and cellular absorption by means of a chitosan-based nanodelivery system.

Different chitosan/tripolyphosphate (TPP) nanoparticle formulations were prepared by the ionotropic gelation method and subsequently characterized. Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS) were used to measure size, dispersity and morphology. Encapsulation efficiency and delphinidin stability were determined by HPLC. Free radical scavenging activity was determined using ABTS assay. Improved cellular uptake of nanoencapsulated delphindin (DNPs) with respect to the free form was observed in Caco2 cells.

Among the different formulations, we established that both the amount and the relative proportion of chitosan/TPP/delphinidin are involved in particles charge and size as well in anthocyanidin stabilization and encapsulation. Moreover nanoparticle formulations increase the stability of DEL over time with respect to the pure compound also in physiological solution, so improving its scavenging activity.

We further evaluated uptake of DNP into cells, finding that intracellular DEL concentration was significantly higher when cells were treated with DNPs in comparison to those treated with free compound.

In conclusion, entrapment of delphinidin in chitosan-based nanoparticles improves the stability of the molecules over the time and increases its absorption in intestinal cells. Further studies are needed to evaluate the biological function exerted.

Notes

P46

Effects of lactic acid fermentation on the anthocyanin content of juices from *Citrus sinensis* L. Osbeck cv. 'Tarocco'

Salvatore Multari

Fondazione Edmund Mach
salvatore.multari@fmach.it

Salvatore Multari[1], Ilaria Carafa[1], Marco Caruso[2], Concetta Licciardello[2], Kieran Tuohy[1] and Stefan Martens[1]

[1] Fondazione Edmund Mach, Research and Innovation Centre, San Michele All' Adige (TN), Italy
[2] CREA, Centro di Ricerca Olivicoltura, Frutticoltura e Agrumicoltura, Acireale (CT), Italy

Citrus fruits have a great importance in the agricultural sector of Southern Italy. *Citrus sinensis L.* Osbeck cv. 'Tarocco' is a common orange variety produced in the region of Sicily, and in general in the Mediterranean area. The interest toward this cultivar is due to its organoleptic characteristics and content in bioactive compounds. Anthocyanins are a group of phenolic compounds found in cv. 'Tarocco'. Since lactic acid bacteria (LAB) have been rarely used to ferment juices from Citrus fruits, the aim of the present work was to evaluate in influence of lactic acid fermentation on the anthocyanin profile of juices obtained from the sweet orange cv. 'Tarocco'.

For this purpose, four LAB were selected, *L. plantarum*, *L. rhamnosus*, *L. paracasei*, and *L. brevis*. Following pasteurization at 81 °C for 40 sec, juices were inoculated and fermented at 30 °C for 48 h. Then, juices were centrifuged (5 min; 1800×g; 4°C) and filtered through 0.22 µm PTFE membranes. Anthocyanins were detected by UHPLC-MS/MS.

Fifteen different anthocyanins were identified in the studied juices, with the glucoside derivatives being the most abundant metabolites. The unfermented juice showed cumulative anthocyanin content of 5.28 ± 0.15 mg L-1. Petunidin 3-O-glucoside resulted the main compound (1.69 ± 0.09 mg L-1), followed by delphinidin 3-O-glucoside (1.04 ± 0.07 mg L-1). All the lactobacilli generated a significant decrease ($p < 0.01$) in cumulative anthocyanins. The fermentation with *L. brevis* gave the greatest reduction, approx. 45% of decrease (2.95 ± 0.09 mg L-1). On the contrary, L. plantarum produced the lowest degradation, i.e., approx. 30% of reduction (3.77 ± 0.13 mg L-1). Results from this investigation indicated that the lactic acid fermentations reduced the anthocyanin content of orange juices from cv. 'Tarocco'. This metabolic modification might have contributed greatly to the color alteration observed in the fermented juices.

Notes

P47

Effect of curing and low-temperature storage on anthocyanins biosynthesis and accumulation

Lourdes Carmona López

Fundo de defesa da citricultura (Fundecitrus)
lourdes.carmona@fundecitrus.com.br

Lourdes Carmona[1], Berta Alquézar[1,2], Tatiane Malara[1], Gianfranco Diretto[3], Maria Teresa Lafuente[4] & Leandro Peña[1,2]

[1] Fundo de defesa da citricultura (Fundecitrus). Avenida Dr. Adhemar Pereira de Barros, 201. Araraquara. São Paulo. Brazil.
[2] Instituto de Biología Molecular y Celular de Plantas (IBMCP), Consejo Superior de Investigaciones Científicas (CSIC), Universidad Politécnica de Valencia (UPV). C/ Enginiero Fausto Elio, s/n, València, España.
[3] Agenzia nazionale per le nuove tecnologie, l'energia e lo sviluppo economico sostenibile (ENEA) Centro Ricerche Casaccia. Via Anguillarese, 301. 00123 Santa Maria di Galeria RM, Italia.
[4] Instituto de Agroquímica y Tecnología de los Alimentos (IATA), Consejo Superior de Investigaciones Científicas (CSIC). C/ Catedrático Agustín Escardino Benlloch, 7, València, España.

Blood oranges (BO) are the only citrus fruits accumulating anthocyanins, which provide a distinctive purple coloration to the fruit and juice. The effects of BO juice consumption on health have been investigated in recent years, denoting its beneficial influence on alleviating several diseases as well as the synergistic effects between anthocyanins and other orange juice phytonutrients on health. Despite these benign effects, a reliable global market of BO juice is limited due to the cold dependence of anthocyanins biosynthesis and accumulation and thus the pigmentation process. An alternative practice in tropical areas (where the most important orange juice industries are located) to avoid this is the storage of the harvested fruit at low temperature. Storage at 9 ºC for 15-30 days of BO fruits has been established as proper to enhance anthocyanin production via the induction of the anthocyanin biosynthesis genes and their products as well as the accumulation of proteins related with stress response, secondary metabolism, defense and oxidative stress, suggesting a possible role of these metabolic pathways in anthocyanins yield. On the other hand, these pathways together with the phenylpropanoid and flavonoids metabolism are stimulated through high temperature conditioning or curing of the fruit (using hot humid air at 37 °C for 3 days during post-harvesting), a very effective method in citrus for inducing fruit tolerance to low temperatures. The aim of this work has been to investigate the potential additive effect of curing before the low temperature storage on enhancing anthocyanins production in fruit from two different BO cultivars. Results showed not only a darker purple coloration due to the higher induction of anthocyanins biosynthesis in treated fruit, but also a higher content of total flavonoids, suggesting a synergistic effect of heat followed by cold exposure in the accumulation of general flavonoids and anthocyanins in BO fruit.

Notes

P48

Extraction of anthocyanins from *Aronia melanocarpa* skin waste as a sustainable source of natural colorants

Muhammad H Wathon

University of Leeds
cmmhw@leeds.ac.uk

Muhammad H. Wathon[1,2], Nicholas Beaumont[1], Meryem Benohoud[3], Richard S. Blackburn[2,3], Christopher M. Rayner[1,3]

[1] School of Chemistry, University of Leeds LS2 9JT, UK;
[2] Sustainable Materials Research Group, School of Design, University of Leeds LS2 9JT, UK;
[3] Keracol Limited, University of Leeds, Leeds LS2 9JT, UK

Berries from *Aronia melanocarpa (Michx.)* Elliott (commonly known as black chokeberry) contain anthocyanins (ANC) in high concentrations and have a particularly simple ANC profile compared to other ANC-containing berries. ANC content in *A. melanocarpa* skin is found to be higher than in the juice; the raw material used herein is waste of *A. melanocarpa* skins generated as a by-product following pressing of the berries juice, which represents a potential natural, sustainable and renewable resource if it can be shown that useful products can be obtained from it in an efficient manner. Solid-liquid extraction is a classical technique to recover ANCs from natural resources, but this method is not very selective because many other molecules are typically co-extracted along with ANCs. Solid-phase extraction may be employed as an additional step to purify and separate selected analytes from co-extracted compounds.

Herein, *A. melanocarpa* skin waste was extracted, isolated and characterised. Optimal extraction conditions for yield and extract quality using a batch process were 60 °C, 3 h, 0.1% v/v HCl, biomass-solvent ratio of 1:16, and biomass-SPE resin ratio of 1:1. These optimum extraction parameters were then applied in an integrated method where extraction and adsorption were performed simultaneously. Higher ANC yields were obtained when the process was performed for 3 h without cooling and a flow rate was 1.3 mL s-1. The integrated method increased extraction yield by 20% and the ANC content by 40%, compared to the batch method; the method also simplified the process, saving time and energy. Batch method: Cy3gal (38.8%), Cy3ara (6.4%), Cy3glu (3.6%), Cy3xyl (0.5%) and Cy (50.7%). Continuous method: Cy3gal (45.7%), Cy3ara (16%), Cy3glu (3.6%), Cy3xyl (2.7%) and Cy (32%). Extraction under acidic conditions promotes hydrolysis to the aglycon and this effect is reduced with the continuous method.

Notes

P49

Novel apple cultivars in Trentino, the case of the red-fleshed apple

Lara Poles

CIF, Innovation Fruit Consortium
lara.poles@gmail.com

Lara Poles, Luca Lovatti

CIF, Innovation Fruit Consortium, 38010 San Michele all'Adige, Italy

Among the different goals pursued in a breeding program for fruit quality in apple, fruit flesh colour is gaining interest.

Apple showing red flesh are already present in nature, the red colouring is due to an increase in anthocyanins content; these components play an important role as antioxidant. This trait, usually present in wild apple, was integrated into white-flesh apple showing good quality properties through traditional breeding approaches. A success example is represented by the red-flesh apple named Kissabel® developed by the IFORED breeding program.

This work is aimed to assess the performance of two of this novel varieties ('variety 1' and 'variety 2') in two environments within the Trentino region (north Italy) characterized by different altitudes and climate conditions. We evaluated the developing of the red colour of the flesh during the onset of fruit ripening from blooming to harvest. In addition, we evaluated the fruit storability; in particular, to test the post-harvest performance of these new varieties, we focused on the definition of the appropriate window for harvest through the assessment of the index of absorption difference (IDA) and the ethylene production. IDA is an indirect index to measure the chlorophyll content (being the latter inversely related to ripening).

Overall, the results pointed out that (i) apples of 'variety 1' showed a good flesh colouration at harvest and a high content of anthocyanins in both environments; (ii) the IDA index is a good predictor of apple ripening and can greatly help the definition of the appropriate harvest time for both type of red-fleshed apple. Nevertheless, apples of 'variety 1' showed non-optimal storability due to the occurrence of internal browning (IB). To this extent, further studies are required to better highlight the physiology of this phenomenon and to define the best agronomical practices that can guarantee higher storability.

Notes

P50

Pigmented maize cobs waste as an environmental friendly solution to dye natural fibers (PASTEL)

Michela Landoni

Università degli Studi di Milano
michela.landoni@unimi.it

Giulia Borlini[1], Gloria Brunoldi[1], Michela Landoni[2], Elena Cassani[1], Pietro Squillace[3], Patrizia Denisi[3], Fabrizio Adani[3], Roberto Pilu[1]

[1] Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy , Università degli Studi di Milano, Via Celoria 2, 20133, Milan, Italy
[2] Department of Biosciences , Università degli Studi di Milano, Via Celoria 26, 20133 Milan, Italy
[3] Gruppo Ricicla – DiSAA – Università degli Studi di Milano, via Celoria 2, 20133 Milan, Italy

The increased demand for textile products and the related increase in their production, as well as the use of synthetic dyes, contributed to make dye wastewater one of the main responsible of the severe pollution problems in current times. For these reasons and taking into consideration the importance of this sector in the Lombardy Region, the aim of the PASTEL project (Granted by Cariplo Foundation) is to develop a new natural dyeing process of natural fibers based on the use of anthocyanins (dyes) and tannins (mordanting) extracted from maize cobs, i.e. the residual waste coming from the cultivation of red corn. At present, anthocyanins are mainly extracted from red grape skin and other red berries, but since the cultivation of pigmented corn is growing in Lombardy, because of beneficial properties of contained anthocyanins, cobs-waste could become a cheaper source of natural dye.

With this purpose we studied new materials and traditional varieties to find the best candidates for anthocyanins production. Anthocyanins belong to the class of flavonoids, secondary metabolites synthesized by a complex metabolic pathway consisting of about 20 biosynthetic genes and regulated by two types of transcription factors encoded by the bHLH and MYB gene families. The varieties selected for anthocyanins production carry the two strong regulatory genes involved in flavonoids synthesis Pl (purple plant 1) and P1 (pericarp color 1), leading respectively anthocyanins and phlobaphene synthesis in pericarp.

We set up a quick and cheap extraction method starting from dried cobs to obtain the flavonoid pigments used to stain different natural fibers (wool, silk, cotton and flax).

HPLC-MS analyses have been performed on the different repartitions water/ethanol extracts to individuate the best composition for the staining process.

In this poster we'll present preliminary data regarding the entire process, from the waste colored cobs to the pigment extraction and natural fiber staining.

Notes

P51

Effect of sun exposure on the evolution and distribution of anthocyanins in interspecific red hybrid winegrapes

Catherine Dadmun

Cornell AgriTech
akm87@cornell.edu

Catherine H. Dadmun[1], Anna Katharine Mansfield[1], Hans Walter-Peterson[2]

[1] Department of Food Science, Cornell AgriTech, Geneva, NY, 14456 USA;
[2] Finger Lakes Grape Program, Cornell Cooperative Extension, Penn Yan NY 14527

Interspecific hybrid winegrapes are economically important in areas where environmental pressures inhibit traditional *Vitis vinifera* production. Red hybrid grapes, however, show great diversity in anthocyanin profile, and viticultural and winemaking techniques proven to optimize color in red *V. vinifera* wines are often ineffective for hybrid wine production.

Because the chemistry of hybrid grape anthocyanins is largely unknown, the reactions they undergo during ripening, wine production, and aging are poorly understood. To clarify the effect of vine microclimate on red hybrid wine color, anthocyanin profiles were assessed for shaded and unshaded fruit from three economically significant cool-climate hybrid cultivars (*Vitis spp*): Marquette, Maréchal Foch, and Corot Noir. Berry samples were collected throughout ripening from triplicate blocks of each cultivar grown in the New York Finger Lakes region, and skin extract anthocyanins were characterized via HPLC analysis. Light exposure and berry and air temperature were monitored in Corot noir throughout the season to represent generalized vine microclimate. In 2018, exposed fruit was about 0.40C warmer on average (SD=0.37) than shaded fruit during the period Sept 21 – Oct 15, and average daily temperatures for exposed vs shaded berries ranged from 0-1.2 C warmer. Anthocyanin profile changed for all cultivars as fruit matured, with Maréchal Foch showing the greatest variation between initial and final sample collection. Shaded fruit generally showed slower and lower anthocyanin evolution, but changes in individual anthocyanins varied by cultivar. Identification and quantification of key mono- and di-glucoside anthocyanins is currently underway. This work is the first step in defining the evolution of anthocyanin profiles during interspecific hybrid grape ripening to allow cool-climate wine grape growers to optimize viticultural production methods for high-quality red hybrid wines.

Notes

P52

Preparative separation of anthocyanins from red wine extracts using high speed counter current chromatography

Baoshan Sun

Shenyang Pharmaceutical University
sun.baoshan@iniav.pt

Li Yuanyuan[1], Li Lingxi[2], Sun Baoshan[1]

[1] School of Functional food and Wine, Shenyang Pharmaceutical University, 110016 Shenyang, China
[2] Pólo Dois Portos, Instituto National de Investigação Agrária e Veterinária, I.P., Quinta da Almoinha, 2565–191 Dois Portos, Portugal

In this study, high-speed counter-current chromatography (HSCCC) was used for large-scale separation of anthocyanins from red wine extracts. With the solvent system of Methyl Tert-Butyl Ether (MTBE)-n-butyl alcohol-acetonitrile-water (1-40-1-50, acidified with 0.01% trifluoroaceticacid (TFA)) by one-step HSCCC of 100 mg of the red wine extracts, the major anthocyanins, i.e., malvidin-3-O-glucoside, delphinidin-3-O-glucoside and peonidin-3-O-glucoside, as well as two polymeric proanthocyanidin fractions were successfully separated one another within 320 min. The yields of malvidin-3-O-glucoside, delphinidin-3-O-glucoside and peonidin-3-O-glucoside were 12.12 mg, 1.78 mg and 11.57 mg with the purity of 92.74%, 91.03% and 91.21%, respectively. Thiolysis-UPLC analysis indicated that the two polymeric proanthocyanidin fractions presented high purity, with mean degree of polymerization of 7.66 ± 0.12 and 6.20 ± 0.09, respectively. The further experiments on the antioxidant activities by DPPH, FRAP and ABTS assays showed that all of the isolated anthocyanins and the two polymeric proanthocyanidin fractions possessed much greater antioxidant activities compared to standard Trolox and L-ascorbic acid.

Notes

P53

Biologically active compounds: properties, functions and application in food, pharmacy and cosmetics

Kulchenko Yaroslava

Hanze University of Applied Sciences

kulchenko.1992@mail.ru

YaYu Kulchenko[1,2], V.I. Deineka[1], Rob van Haren[2]

[1] Belgorod State National Research University

[2] Hanze University of Applied Sciences

Studies over the past decade have shown a lot of benefits arising from the consumption of fruit and vegetables for human health. In an effort to identify active health beneficial ingredients, many researchers have focused on the properties of flavonoids, a large class of phenolic compounds that is found in large quantities in such products. The most visible among the flavonoids are anthocyanins. Anthocyanins are different from other compounds of the extensive class of flavonoids not only by their high solubility in water, but also by the existence of several pH-dependent forms in aqueous solutions, some of which are colored. Flavylium form exists as a main form only in highly acidic conditions (pH < 4.5), it is red colored with shades depending upon solute structure. Meanwhile, high acidity is not in not consistent with pH of cell medium as well as with properties of food and cosmetics products, though namely flavylium form is the most stable one. On the other hand, the rise of pH may lead not only to color fading because of colorless pseudo base formation, but to also colored quinonoid uncharged and charged forms. These forms are not stable in solution. According to our experience in the form called encapsulated they are enough stable to be applied for some food and cosmetic purposes. Starting with acylated anthocyanins at different pH solutions after addition of natural polymeric matrices, differently colored (red, violet, blue, green or yellow) dry forms were prepared by spray or lyophilic drying. These colorants were utilized for cakes decoration, marmalade preparation, milk product coloration and preparation of lipsticks.

Notes

P54

Methods of thermal and non-thermal (UV-C) preservation on properties of red cactus pear juice

Martha Graciela Ruiz Gutiérrez

Universidad Autónoma de Chihuahua
mruizg@uach.mx

Gabriela Mesta-Vicuña, Martha G. Ruiz-Gutiérrez, Quintero-Ramos Armando and Carmen O. Meléndez-Pizarro

Departamento de Investigación y Posgrado, Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua, Circuito Universitario s/n Campus Universitario 2, Chihuahua 31125, México

Red cactus pear is a fruit rich in betalains, compounds with great potential for pigmentation and beneficial effects on health. UV-C is an alternative conservation method to preserve characteristic as color and chemical properties. A red cactus pear juice was elaborated (25%) with different pH (7 and 3.6) and processed in an UV-C radiation equipment (254 nm), with 4 radiation doses (0, 9.87, 15.13 and 31.78 mJ/cm2), and as well as thermally at 80°C, 30s (HTST) and 130°C, 3s (UHT). Microbiological analysis, color parameters, betalains content and antioxidant activity were performed. Regarding the microbiological analysis a logarithmic reduction (3.2-6.4) was obtained. The color parameters: L* (luminosity) showed values of 51.96-59.34, a* tends to red (31.38-63.98) and b* tends to yellow (9.98-33.92); the analyses shown that UV-C does not cause color changes, however between UV-C and thermal treatments a significant difference ($p<0.05$) was observed and by pH increase. In antioxidant activity, the UV-C doses not show difference, decreasing 12.68% and 14.06% for acidified and non-acidified treatments, respectively. The thermal treatments showed significant difference ($p<0.05$), with a decrease of 56.73% (HSTS) and 58.13% (UHT). In betacyanins a significant difference ($p<0.05$) was shown at doses of 15.13 and 31.78mJ/cm2, with reductions of 11.11% and 16.69% (acidified treatment) and 19.66% and 20.81% (non-acidified). In the betaxanthins, no differences were found by UV-C irradiation; however, the non-acidified treatment had the same behavior as the betacyanins, with reductions of 11% and 16.11%, at the doses of 15.13 and 31.78mJ/cm2. For HTST (80°C-30s) a significant reduction ($p<0.05$) of 14.83% for betacyanins and 14.39% for betaxanthins was obtained and for UHT, with a reduction of 63.09% and 45% for betacyanins and betaxanthins. An effective treatment with a dose of 9.91 mJ/cm2 and pH 3.6 was found.

Notes

P55

Thermal degradation kinetics of blue maize anthocyanins from tortillas produced using the extrusion nixtamalization process

María José Frutos

Universidad de Sonora
mj.frutos@umh.es

Mariela Menchaca-Armenta[1], María José Frutos-Fernández[2], Benjamín Ramírez-Wong[1], Raquel Muelas-Domingo[3], Estefanía Valero-Cases[2], Patricia Isabel Torres-Chávez[1], Armando Quintero-Ramos[4], Ana Irene Ledesma-Osuna[1], Olga Nydia Campas-Baypoli[5]

[1] Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Hermosillo, Sonora, 83000, México
[2] Grupo de Investigación en Calidad y Seguridad Alimentaria. Dep. de tecnología Agroalimentaria, Universidad Miguel Hernández, Ctra. Beniel, Km.3.2, 03312 Orihuela, Alicante, España
[3] División de producción animal. Departamento de tecnología agroalimentaria, Universidad Miguel Hernández, Ctra. Beniel, Km.3.2, 03312 Orihuela, Alicante, España
[4] Facultad de Ciencias Químicas, Universidad Autónoma de Chihuahua–UACH, Chihuahua, Chihuahua, 31125, México
[5] Departamento de Biotecnología y Ciencias Alimentarias, Instituto Tecnológico de Sonora, Cd. Obregón, Sonora, 85000, México

Anthocyanins are appreciated as health promoters due to their antioxidant properties. Blue maize is a rich source of anthocyanins. The nixtamalization process of maize for the production of tortillas consists of a series of unitary operations that involve heat and time. The study of anthocyanins degradation during nixtamalization process could contribute to the design of a kinetic model to predict the loss of quality during the thermal treatment, helping to establish the optimum process conditions. The aim of this study was to determine the kinetic and thermodynamic parameters of the thermal degradation of anthocyanins from raw maize, flour and tortilla of blue maize. Ground blue maize kernels were processed by extrusion with the following conditions: feed moisture content of 18.17%, extruder temperature in the fourth zone of 92.03 °C and screw speed of 76.61 rpm. The extrudates were dried and ground into flour that was used to produce the tortillas. The degradation kinetics of the anthocyanin extracts were studied during the heat treatment. A completely randomized factorial design was used. The factors were the process stage at three levels (grinding, extrusion, cooking) and thermal treatments at three levels (60, 70 or 90 °C). The analysis of variance was performed and Tukey test was used ($p \leq 0.05$). The mechanism of anthocyanins degradation followed a first-order reaction kinetics. The parameters t½, D, k and Q10 were highly dependent on temperature. As the temperature increased, the rate of degradation constant increased and t½, D and Q10 decreased. The activation energies for raw maize, flour and tortilla were 85.8, 73.4 and 74.2 kJ/mol, respectively. The activation energy of the anthocyanin extracts in blue maize tortilla revealed that only 13% of this value is affected by the heat treatment, indicating a high thermal stability. In conclusion, this study provides a valuable technological and scientific information to the tortilla manufacturing industry.

Note

LIST OF PARTICIPANTS

Nick Albert

Plant and Food Research
nick.albert@plantandfood.co.nz
New Zealand

Andrea Angeli

Fondazione Edmund Mach
Department Food Quality and Nutrition
andrea.angeli@fmach.it
Italy

Panagiotis Arapitsas

Fondazione Edmund Mach
Department Food Quality and Nutrition
panagiotis.arapitsas@fmach.it
Italy

Einar Bakstad

Biosynth AS
einar@biosynth.no
Norway

Gabrielel Ballistreri

CREA Research Centre for Olive, Citrus and Tree Fruit
gabriele.ballistreri@crea.gov.it
Italy

Meryem Benohoud

Keracol Ltd
m.benohoud@keracol.co.uk
United Kingdom

Hélène Bitard

Avignon University
INRA, UMR408
helenebitard@gmail.com
France

Richard Blackburn

University of Leeds
School of Design
r.s.blackburn@leeds.ac.uk
United Kingdom

Federica Blando

National Research Council (CNR)
Institute of Sciences of Food Production (ISPA)
federica.blando@ispa.cnr.it
Italy

Christine Bosch

University of Leeds
School of Food Science and Nutrition,
g.rawson@leeds.ac.uk
United Kingdom

Henrik Brinch-Pedersen

Aarhus University
Department of Molecular Biology and Genetics
hbp@mbg.au.dk
Denmark

Eugenio Butelli

John Innes Centre
Norwich Research Park
eugenio.butelli@jic.ac.uk
United Kingdom

Francesca Cappellini

Università degli Studi di Milano
Department of Biosciences
francesca.cappellini@guest.unimi.it
Italy

Maria Lourdes Carmona Lopez

Fundo de defesa da citricultura (Fundecitrus)
lourdes.carmona@fundecitrus.com.br
Brazil

Chiara Catalano

CREA Research Centre for Olive, Citrus and Tree Fruit
chiara1995catalano@hotmail.it
Italy

Chiara Cerletti

IRCCS Neuromed
Department of Epidemiology and Prevention
chiara.cerletti@moli-sani.org
Italy

Jean Chaudiere

University of Bordeaux
CBMN (UMR 5248)
jean.chaudiere@u-bordeaux.fr
France

Veronique Cheynier

University Montpellier
INRA - SupAgro
veronique.cheynier@inra.fr
France

Angelo Ciacciulli

CREA Research Centre for Olive, Citrus and Tree Fruit
angelo.ciacciulli@crea.gov.it
Italy

Ramona Ciuboatru

Fondazione Edmund Mach
Department Food Quality and Nutrition
ramona.ciubotaru@guests.fmach.it
Italy

Pasquale Crupi

CREA-VE
Viticoltura ed Enologia
pasquale.crupi@crea.gov.it
Italy

Luis Cruz

University of Porto
Department of Chemistry and Biochemistry
luis.cruz@fc.up.pt
Portugal

Catherine Dadmun

Cornell AgriTech
Department of Food Science
chd59@cornell.edu
United States

Maria Helena Damásio

CORALIM ADITIVOS
mhdamasio@coralim.com
Spain

Olivier Dangles

Avignon University
INRA, UMR408
olivier.dangles@univ-avignon.fr
France

Kevin Davies

Plant and Food Research
kevin.davies@plantandfood.co.nz
New Zealand

Dominik Durner

DLR Rheinpfalz
Institute for Viticulture and Enology
dominik.durner@dlr.rlp.de
Germany

Tsaneta Dzhanfezova

Chr. Hansen
dktsdz@chr-hansen.com
Denmark

Richard Espley

Plant and Food Research
richard.espley@plantandfood.co.nz
New Zealand

Simona Fabroni

CREA Research Centre for Olive, Citrus and Tree Fruit
simona.fabroni@crea.gov.it
Italy

Julie-Anne Fenger

Avignon University
INRA, UMR408
fengerja@gmail.com
France

Ana Luisa Fernandes

University of Porto
Department of Chemistry and Biochemistry
ana.fernandes@fc.up.pt
Portugal

Massimiliano Fontana

Ulster University
Nutrition Innovation Centre for Food and Health
massimiliano.fontana@guests.fmach.it
United Kingdom

Victor Freitas

University of Porto
Department of Chemistry and Biochemistry
vfreitas@fc.up.pt
Portugal

María José Frutos Fernández

Universidad Miguel Hernández
Departamento de tecnología Agroalimentaria
mj.frutos@umh.es
Spain

Sumali Fernando Ganwarige Nivanthi

University of Leeds
School of Food Science and Nutrition
fsgsnf@leeds.ac.uk
United Kingdom

Xiang Gao

Northeast Normal University
Key Laboratory of Molecular Epigenetics of MOE
and Institute of Genetics & Cytology
gaox891@nenu.edu.cn
China

Colette Geier

Charles Sturt University
School of Agriculture and Wine Science
cgeier@csu.edu.au
Australia

Kevin Gould

Victoria University of Wellington
kevin.gould@vuw.ac.nz
New Zealand

Claudia Gras

DOEHLER GmbH
Claudia.Gras@doehler.com
Germany

Laura Headley

Evonik Nutrition & Care GmbH
laura.headley@evonik.com
Germany

Silke Hildebrand

Symrise
Silke.Hillebrand@symrise.com
Germany

Sophie Hill

sophielouisehill@outlook.com
United Kingdom

Alan Houghton

John Innes Centre
Department of Metabolic Biology
alan.houghton@jic.ac.uk
United Kingdom

Nicole Hughes

High Point University
Department of Biology
nhughes@highpoint.edu
United States

Laura Jaakola

Arctic University of Norway
Department of Arctic and Marine Biology
laura.jaakola@uit.no
Norway

Tanja Karl

Victoria University of Wellington
tanja.karl@vuw.ac.nz
New Zealand

Katja Karppinen

University of Oulu
Department of Ecology and Genetics
katja.karppinen@oulu.fi
Finland

Anne Kivimäki

University of Helsinki
Medical Nutrition Physiology
anne.kivimaki@helsinki.fi
Finland

Linards Klavins

University of Latvia
Laboratory of Natural Products Research
linards.klavins@gmail.com
Latvia

Martina Kolarek

Technical University of Munich
Biotechnology of Natural Products
kolarek@wzw.tum.de
Germany

Tadao Kondo

Nagoya University
Graduate School of Bioagricultural Sciences
kondot@info.human.nagoya-u.ac.jp
Japan

Marie Kosehira

Omnica Co., Ltd.
kosehira@omnica.co.jp
Japan

Yaroslava Kulchenko

Belgorod State National Research University
kulchenko.1992@mail.ru
Serbia

S. Pradeep Kumar

Volcani Center
Department of Postharvest Science
pradeepkumar2k@gmail.com
Israel

Marco Landi

University of Pisa
Department of Agriculture, Food and Environment
marco.landi@agr.unipi.it
Italy

Michela Landoni

Università degli studi di Milano
Department of Agricultural and Environmental Sciences
michela.landoni@unimi.it
Italy

Alexander Leicht

DOEHLER GmbH
alexander.leicht@doehler.com
Germany

Yueqing Li

Northeast Normal University
Key Laboratory of Molecular Epigenetics of MOE and Institute of Genetics & Cytology
liyq339@nenu.edu.cn
China

Mary Ann Lila

North Carolina State University
Plants for Human Health Institute
mlila@ncsu.edu
United States

Ermes Lo Piccolo

University of Pisa
Department of Agriculture, Food and Environment
ermes.lopiccolo@gmail.com
Italy

Luca Lovatti

CIF
Innovation Fruit Consortium
lovatti@cif.tn.it
Italy

Mickael Malnoy

Fondazione Edmund Mach
mickael.malnoy@fmach.it
Italy

Anna Katharine Mansfield

Cornell AgriTech
Department of Food Science
akm87@cornell.edu
United States

Sylvestre Marillonnet

Leibniz Institute of Plant Biochemistry
Department of Cell and Metabolic Biology
smarillo@ipb-halle.de
Germany

Stefan Martens

Fondazione Edmund Mach
Department Food Quality and Nutrition
stefan.martens@fmach.it
Italy

Cathie Martin

John Innes Centre
Norwich Research Park
cathie.martin@jic.ac.uk
United Kingdom

Nuno Mateus

University of Porto
Department of Chemistry and Biochemistry
nbmateus@fc.up.pt
Portugal

Fulvio Mattivi

University of Trento
fulvio.mattivi@unitn.it
Italy

Nikitia Mexia

University of Leeds
School of Design
j.s.wise@leeds.ac.uk
United Kingdom

Maja Mikulič Petkovšek

University of Ljubljana
Department of Agronomy
maja.mikulic-petkovsek@bf.uni-lj.si
Slovenia

Daiki Mizuta

Nihon University
College of Bioresource Sciences
mizuta.daiki@nihon-u.ac.jp
Japan

Nathalie Mora-Soumille

Avignon University
INRA, UMR408
nathalie.mora@univ-avignon.fr
France

Paolo Morazzoni

Indena S.p.A.
scientificadvisor.pm@indena.com
Italy

Sarah Moss

Plant and Food Research
sarah.moss@plantandfood.co.nz
New Zealand

Sam Multari

Fondazione Edmund Mach
Department Food Quality and Nutrition
salvatore.multari@fmach.it
Italy

Michael Naeby

michael@naesby.fr
France

Dina Neves

University of Porto
Department of Chemistry and Biochemistry
dinaneves4@gmail.com
Portugal

Daria Nitarska

Technische Universität Wien
Institute of Chemical Engineering
daria.nitarska@tuwien.ac.at
Austria

Naonobu Noda

National Agriculture and Food Research Organization
Institute of Vegetable and Floriculture Science
naonobun@affrc.go.jp
Japan

Helder Oliveira

University of Porto
Department of Chemistry and Biochemistry
helder.oliveira@fc.up.pt
Portugal

Joanna Oliveira

University of Porto
Department of Chemistry and Biochemistry
jsoliveira@fc.up.pt
Portugal

Michal Oren-Shamir

Volcani Center
Department of Postharvest Science
vhshamir@agri.gov.il
Israel

Luisa Palmieri

Fondazione Edmund Mach
Department Food Quality and Nutrition
luisa.palmieri@fmach.it
Italy

Sabina Passamonti

University of Trieste
Department of Life Sciences
spassamonti@units.it
Italy

Joaquín Julian Pastor-Pérez

Universidad Miguel Hernández
Departamento de tecnología Agroalimentaria
mj.frutos@umh.es
Spain

Costantinos Patinios

N-Chroma
constantinos.patinios@n-chroma.com
The Netherlands

Virginie Pecher

LVMH Research Center
vpecher@research.lvmh-pc.com
France

Yongyan Angel Peng

The University of Auckland
School of Biological Science
yongyan.peng@plantandfood.co.nz
New Zealand

Katia Petroni

Università degli Studi di Milano
Department of Biosciences
katia.petroni@unimi.it
Italy

Lara Poles

CIF
Innovation Fruit Consortium
lara.poles@gmail.com
Italy

Amos Samkumar Rajan Premkumar

The Arctic University of Norway
Department of Arctic and Marine Biology
amos.s.premkumar@uit.no
Norway

Paolo Rapisarda

CREA Research Centre for Olive, Citrus and Tree Fruit
paolo.rapisarda@crea.gov.it
Italy

Lapo Renai

University of Florence
Department of Chemistry
lapo.renai@unifi.it
Italy

Elke Richling

Technische Universitaet Kaiserslautern
Department of Chemistry
richling@chemie.uni-kl.de
Germany

Thananunt Rojanasasithara

PepsiCo
thananunt.rojanasasithara@pepsico.com
United States

Martha Graciela Ruiz-Gutiérrez

Universidad Autónoma de Chihuahua
Departamento de Investigación y Posgrado
mruizg@uach.mx
Mexico

Vania Stephanie Saez Pulgar

Fondazione Edmund Mach
Department Food Quality and Nutrition
vania.saezpulgar@guests.fmach.it
Italy

Erika Salas-Muñoz

Universidad Autónoma de Chihuahua
Facultad de Ciencias Químicas
esalas@uach.mx
Mexico

Nobuhiro Sasaki

Toyo University
Department of Food and Nutritional Sciences
sasaki076@toyo.jp
Japan

Cédric Saucier

University Montpellier
INRA - SupAgro
cedric.saucier@umontpellier.fr
France

Akiyoshi Sawabe

Kindai University
Department of Applied Biological Chemistry
sawabe@nara.kindai.ac.jp
Japan

Kathy Schwinn

Plant and Food Research
kathy.schwinn@plantandfood.co.nz
New Zealand

Odette Shaw

Plant and Food Research
Food & Wellness Group
odette.shaw@plantandfood.co.nz
New Zealand

Domen Škrab

Fondazione Edmund Mach
Department Food Quality and Nutrition
domen.skrab@guests.fmach.it
Italy

Ralf Stracke

University of Bielefeld
Genetics and Genomics of Plants, CeBiTec
ralf.stracke@uni-bielefeld.de
Germany

Baoshan Sun

Pólo Dois Portos
Instituto National de Investigação Agrária e Veterinária
sun.baoshan@iniav.pt
Portugal

Ruiyan Tao

Zhejiang University
Department of Horticulture
753366394@qq.com
China

Teemu Teeri

University of Helsinki
Viikki Plant Science Centre
teemu.teeri@helsinki.fi
Finland

Vera Thole

John Innes Centre
Norwich Research Park
vera.thole@jic.ac.uk
United Kingdom

Federica Tramer

University of Trieste
Department of Life Sciences
ftramer@units.it
Italy

Camilla Valdesturli

Fondazione Edmund Mach
Department Food Quality and Nutrition
camilla.valdesturli@fmach.it
Italy

Robert Veberic

University of Ljubljana
Department of Agronomy
Robert.Veberic@bf.uni-lj.si
Slovenia

Urska Vhrovsek

Fondazione Edmund Mach
Department Food Quality and Nutrition
urska.vrhovsek@fmach.it
Italy

Hans Walter-Peterson

Cornell AgriTech
Department of Food Science
hcw5@cornell.edu
United States

Yunqing Wang

University of Leeds
School of Design
fs16y5w@leeds.ac.uk
United Kingdom

Muhammad Hizbul Wathon

University of Leeds
School of Chemistry
cmmhw@leeds.ac.uk
United Kingdom

Baoru Yang

University of Turku
Department of Geriatrics
baoru.yang@utu.fi
Finland

Aki Yoshida

Kochi University
b164p043@s.kochi-u.ac.jp
Japan

Kumi Yoshida

Nagoya University
Graduate School of Bioagricultural Sciences
yoshidak@i.nagoya-u.ac.jp
Japan

Lugang Zhang

Northwest A&F University
State Key Laboratory of Crop Stress Biology for Arid Areas
lugangzh@163.com
China

Debora Zorzan

Università degli Studi di Milano
Department of Biosciences
debora.zorzan@unimi.it
Italy