IWA&B26

anthocyanins and betalains

12th International Workshop on
Anthocyanins and Betalains

ABSTRACT BOOK

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WELCOME

We are pleased to welcome you to the 12th International Workshop on Anthocyanins & Betalains (IWA&B 2026). This event is hosted by the University of Salamanca (USAL), one of the oldest universities in Europe—founded in 1218—and organized by the Polyphenol Research Group of the USAL (GIP-USAL).

Bringing together researchers from around the globe, IWA&B 2026 offers a unique platform to share and discuss the latest scientific advances in the study of anthocyanins and betalains. These plant pigments span nearly the entire visible spectrum—from yellow and orange to purple and blue—and contribute not only to the vivid colors of plants and foods, but also to relevant biological functions and potential health benefits. Over the years, significant research efforts have been devoted to understanding the biosynthesis, properties, bioactivities, and potential applications of these fascinating compounds. IWA&B 2026 aims to foster collaboration among academics and industry professionals on these topics, providing a valuable opportunity for networking and knowledge exchange.

On behalf of the organizers, we would like to wish you an inspiring and fruitful scientific meeting, as well as an enjoyable stay in Salamanca.

María Teresa Escribano-Bailón

Celestino Santos-Buelga

CONVENERS

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TOPICS

Topic 1. Biosynthesis and Genetics

Topic 2. Ecology, Function and Evolution

Topic 3. Health and Nutrition

Topic 4. Phytochemistry and Analysis

Topic 5. Applications in Food and Industry

IWA&B 2026 Program

Wednesday July 15th 2026

08:00 – 09:00	*Registration*
09:00 – 09:20	**Welcome and opening**
Session 1: Topic 5. *Chairs: M. Teresa Escribano-Bailón, Erika Salas*	
09:20 – 10:20	**Opening plenary** Professor **Olivier Dangles** (Research Unit SQPOV Avignon University – INRAE, France) *The multifaceted chemistry of anthocyanins illustrated by a journey from plant to food, and finally to human*
10:20 - 10:35	**Oral communication O5.1** **Ana Fernandes** (University of Porto, Portugal): Formation of insoluble anthocyanins-λ-carrageenan biopolyelectrolyte complexes: insights into structural morphology, thermal stability, and release dynamics
10:35 - 10:50	**Oral communication O5.2** **María Fernanda López-Molina** (University of Sevilla, Spain): Color preservation of anthocyanins using grape seed peptides: Evaluation of binding affinity and molecular mechanisms of stability
10:50 - 11:00	**Short oral communications** PT5.1. **Jin Huang** (China Agricultural University, China): Effect of freeze and thermal concentration techniques on grape juice anthocyanin profiles PT5.2. **Berta María Cánovas** (CEBAS-CSIC, Spain): Does sugar influence anthocyanin content during fermentation? PT5.3. **Sara F. Vieira** (CIMO, LA SusTEC, Portugal): Physicochemical and bioactive characterization of elderberry musts and concentrates from the Portuguese food industry
11:00 – 11:30	Coffee Break
Session 2: Topic 5. *Chairs: Cristina García-Viguera, Belén Gordillo*	
11:30 – 12:15	**Keynote** **Richard S. Blackburn** (University of Leeds, UK) *Extraction of anthocyanins from food industry waste and their application as dyes*
12:15-12:30	**Oral communication O5.3** **Mariana Cunha** (University of Porto, Portugal): Spectroscopic characterization of dynamic complexes between anthocyanins and a boronic acid-chalcone receptor
12:30-12:45	**Oral communication O5.4** **Susana Soares** (University of Porto, Portugal): From pigment to mouthfeel: anthocyanins for designing silky astringency
12:45-13:00	**Oral communication O5.5** **Iván Puerta-García** (University of Salamanca, Spain): Grape pomace-derived polysaccharides as modulators of anthocyanin oxidative fate in wine systems
13:00– 14:45	*Lunch break* + **Poster session**
Session 3: Topic 4. *Chairs: Celestino Santos-Buelga, Joana Oliveira*	
14:45 – 15:30	**Keynote** **Cristina Alcalde-Eon** (University of Salamanca, Spain) *Structural diversity of red grape and wine pigments: From established markers to the visible unknowns*
15:30-15:45	**Oral communication O4.1** **Inés Sampedro-Marigómez** (ITACYL, Spain): Anthocyanin profiles of wines from red grape varieties resistant to fungal diseases

15:45-16:00	**Oral communication O4.2:** **Benno Zimmermann** (University of Bonn, Germany): Purple tea and how to avoid pitfalls in anthocyanin identification
16:00-16:10	**Short oral communications** PT4.1. **Raquel Rosales** (CIDE-CSIC, Spain): Anthocyanins profile changes in grape fruit skin treated with ABA receptor agonists PT4.2. **Lesly L. Torres-Díaz** (ICVV, Spain): Effect of foliar application of grape pomace extract on the anthocyanins profile of Tempranillo grapes
16:10 – 16:30	*Coffee Break*
Session 4: Topic 1. *Chairs: Celestino Santos-Buelga, Joana Oliveira*	
16:30-16:45	**Oral communication O1.1** **Lennart Malte Sielmann** (Bielefeld University, Germany): A revised regulatory model for flavonoid biosynthesis in *Arabidopsis thaliana*
16:45-17:00	**Oral communication O1.2** **Dor Haim (**Agricultural Research Organization, Israel): elucidating the genetic regulation of anthocyanin biosynthesis underlying peel color change during avocado ripening
17:00-17:15	**Oral communication O1.3** **Serge Antonczak** (University of Côte d'Azur, France): On the road to anthocyanin biosynthesis: insights into the synthesis of naringenin chalcone by Chalcone Synthase.
17:15 – 18:00	**Poster session**
18:30 – 20:30	*Welcome reception*

Thursday July 16th 2026

08:30 – 09:00	*Registration*
Session 5: Topic 3. *Chairs: Ana M. González-Paramás, Lillian Barros*	
09:00 – 09:45	**Keynote** **Tuba Esatbeyoglu** (Leibniz University Hannover, Germany) *From pigmented plants to bioactivity: Impact of targeted fractionation on functional bioactivities*
09:45 - 10:00	**Oral communication O3.1** **Hélder Oliveira** (University of Porto, Portugal): Effect of anthocyanin-rich edible flowers extracts in the modulation of metabolic disease parameters
10:00 - 10:15	**Oral communication O3.2** **Mary Ann Lila** (North Carolina State University, USA): Just don't believe everything you read: Sorting out the quagmire of evidence on anthocyanins and human health
10:15 - 10:30	**Oral communication O3.3** **Margarida Teixeira** (University of Porto, Portugal): How polyacylation shapes the stability and digestive fate of butterfly pea flower anthocyanins
10:30 - 10:40	**Short oral communications** PT3.1. **Mabel Guevara-Terán** (University of Salamanca, Spain): Phenolic characterization and bioactivity of Andean blackberry: Effects on oxidative stress resistance and aging in *Caenorhabditis elegans* PT3.2. **Marcelo C. Acúrcio** (University of Coimbra, Portugal): Anthocyanin-rich extract from Portuguese red wine attenuates neurodegeneration and intestinal dysfunction in a mouse model of Parkinson's disease PT3.3. **Sara Silva** (Universidade Católica Portuguesa,Portugal): Port and Red Wine lees' extracts anti-inflammatory effect in Caco-2 cells.
10:40 – 11:15	Coffee Break

Session 6: Topics 4-5. *Chairs: Fernando Gandía-Herrero, Ignacio García-Estévez*	
11:15 – 12:00	**Keynote** **Slawomir Wybraniec** (Cracow University of Technology, Poland) *Analytical passage through betacyanin diversity*
12:00-12:15	**Oral communication O4.3** **Jesús de las Heras Roger** (University of La Laguna, Spain): Beyond total anthocyanins: copigmentation and matrix effects driving stable red–blue hues in wine
12:15-12:30	**Oral communication O4.4** **Nikolai Kuhnert** (Constructor University, Germany): Ion mobility mass spectrometry as tool for the investigation of pH dependent equilibria of anthocyanins
12:30-12:45	**Oral communication O5.6** **María Oyón-Ardoiz** (University of Salamanca, Spain): Structural features of mannoproteins from different wine yeast and their influence on the formation and stability of A- and B-type vitisins
12:45-12:55	**Short oral communications** PT5.4. **Aylin Ruiz-Guerrero** (University of Chihuahua, Mexico): Effect of ethanol concentration on ultrasound-assisted antisolvent encapsulation of anthocyanins in Zein/Gum arabic and their thermal stability PT5.5. **Sayantani Dutta** (University of Salamanca, Spain): Enhancement of stability of grape pomace anthocyanins by microencapsulation PT5.23. **Pu Jing** (Shanghai Jiao Tong University): Heat-Induced Beta-lactoglobulin Aggregates: Stabilizing Anthocyanins through Hydrophobic Interactions and Dynamic Structural Remodeling
12:55 –14:15	Lunch break
14:15 – 15:00	**Poster session**
Session 7: Topics 2-5. *Chairs: Nuno Mateus, Susana González-Manzano*	
15:00 – 15:45	**Keynote** **Fernando Gandía** (University of Murcia, Spain) *Redesigning natural pigments: betalains as molecular tools for health-promoting foods*
15:45 - 16:00	**Oral communication O3.4** **Wen Tao** (University of Porto, Portugal): From characterization to bioactivity: zein/polysaccharide composite nanoparticles enhance digestive stability and intestinal barrier function protection of anthocyanins
16:00 - 16:15	**Oral communication O2.1** **Selene Niveyro** (National University of La Pampa, Argentina): Beyond Color: Deciphering the divergent dynamics of betacyanin in *Amaranthus* plants under insect herbivory pressure
16:15 - 16:25	**Short oral communications** PT3.4. **Irene Besné-Eseverri** (Universtiy of the Basque Country, Spain): Effects of *Opuntia ficus-indica* var. colorada pulp extract on diet-induced hepatic steatosis and associated changes in gut microbiota in rats PT5.6. **María Jesús Cejudo Bastante** (University of Sevilla, Spain): Semisynthesis of betalains as a strategy to enhance color stability PT4.3. **Boris Nemzer** (VDF FutureCeuticals, USA): Untargeted serum metabolomics profiling of betalains intake markers
16:25 – 16:50	Coffee Break

Session 8: Topic 1. *Chairs: Heidi Halbwirth, Mary Ann Lila*

16:50 – 17:35	**Keynote** **Massimo Iorizzo** (NC State University, USA) *Toward a genetic framework to optimize anthocyanin properties as bioactive and natural colorant*
17:35 - 17:50	**Oral communication O1.4** **Henrik Brinch-Pedersen** (Aarhus University, Denmark): Modulating the colour palette of black carrots by new genomic techniques
17:50 - 18:05	**Oral communication O1.5** **Ingrid Lykke Mohr** (Technical University of Denmark, Denmark): Production of anthocyanin food colorants using yeast cell factories
18:05 - 18:15	**Short oral communications** PT1.1. **Alejandro Brand** (Leibniz Institute of Plant Biochemistry, Germany): Coloring from within: using Cas9 fused exonucleases to engineer cis-genic purple tomatoes PT1.2. **Matthias Hackl** (Technical University Vienna, Austria): Divergent polyphenol oxidases determine L-DOPA accumulation in faba bean
20:00 – 23:00	*Gala dinner*

Friday July 17th 2026

08:45 – 09:00	*Registration*

Session 9: Topics 1,2,5. *Chairs: Erika Salas, Richard S. Blackburn*

09:00 – 09:45	**Keynote** **Joana Oliveira** (University of Porto, Portugal) *Stabilizing blue anthocyanin colors: From molecular mechanisms to industrial applications*
09:45 - 10:00	**Oral communication O5.7** **Fei Lao** (China Agricultural University, China): Antioxidant of bamboo leaves as high-efficacy copigments for anthocyanin stabilization in beverages
10:00 - 10:15	**Oral communication O5.8** **Mário Pinho** (University of Porto, Portugal): Understanding the molecular interaction mechanisms of wine-waste pigments and biomordants to produce dyed sustainable wool textiles
10:15 - 10:30	**Oral communication O5.9** **Xinyue Fan** (The Ohio State University, USA): Development of anthocyanin-based blue colorant for acidic food applications by simulating the Blue Hydrangea complex
10:30-10:40	**Short oral communications** PT5.7: **Liandra Gracher-Teixeira** (CIMO, LA SusTEC, Portugal): Black carrot anthocyanin stabilization via W1/O/W2 double emulsions for yogurt and 3D-printed food applications PT1.3. **Laura Jaakola** (University of Norway, Norway): Light quality-mediated regulation of anthocyanin biosynthesis in *Vaccinium* berries PT2.1. **Billy Williams-Marland** (University Pablo de Olavide, Spain): Heatwaves under climate change alter flower colour: linking flavonoid biosynthesis to pollinator signalling
10:40 – 11:10	Coffee Break

Session 10: Topic 2. *Chairs: Kevin Davies, Cristina Alcalde-Eon*

11:10 – 11:55	**Keynote** **Heidi Halbwirth** (Technische Universität Wien, Austria) *Exploring the anthocyanin colour palette: A guided biochemical journey*
11:55 - 12:10	**Oral communication O2.2** **Fernando Pina** (University NOVA of Lisboa, Portugal): Plant-derived flavylium cations: exploring their potential as evolutionary molecular clocks
12:10 - 12:25	**Oral communication O2.3** **José Carlos del Valle** (University of Sevilla, Spain): The colors of speciation: Pigment transitions towards hummingbird pollination
12:25 - 12:40	**Oral communication O2.4** **Nancy Choudhary** (University of Bonn, Germany): Cucurbitaceae: A novel model system for pigment biosynthesis
12:40 – 13:00	Closing words + Awards to the best presentations
13:00 – 13:30	Lunch boxes and end of conference
Afternoon	**Social events** Optional excursion (winery visit) Optional guided visit to historical area of Salamanca

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OPENING
PLENARY
LECTURE

The multifaceted chemistry of anthocyanins illustrated by a journey from plant to food, and finally to human

Olivier Dangles

Research Unit SQPOV Avignon University - INRAE, Avignon, France

Anthocyanins (ACNs) are a pH-dependent mixture of colored forms and colorless water adducts in equilibrium, each species having its specific reactivity toward electrophiles, nucleophiles, oxidants...
In plant, ACNs typically interact with other phenols and with metal ions to express their rich color palette, while restricting the accumulation of the colorless forms and degradation products.
In food, additional interactions with biopolymers modulate their stability upon processing and storage. ACN derivatives, colored or colorless, also form, especially in alcohol-containing beverages.
As part of the human diet, ACNs undergo extensive modifications in the digestive tract, as a consequence of several mechanisms, e.g. the dissociation of ACN-stabilizing complexes, the sensitivity of ACNs to autoxidation under near neutral conditions, their possible involvement as antioxidants in fighting postprandial oxidative stress, the extensive microbial catabolism experienced by the major fraction of dietary ACNs reaching the colon. Considering the large set of simple phenolic compounds thus produced and the very low circulating concentrations of native ACNs (and derivatives still retaining the typical tricyclic polyphenolic nucleus), the way ACNs express their effects on health remains puzzling.
A booming field of research now aims at preparing supramolecular assemblies (liposomes, emulsions, gels and capsules combining proteins and polysaccharides), in which ACNs display improved stability during processing, storage, and along the digestive tract, in hope of eventually enhancing their bioavailability for use as additives in functional foods.
These diverse and versatile chemical properties of ACNs will be illustrated through recent examples. Emphasis will be laid on:
- the mechanisms of blue color development in plant and in potential food colorants,
- the redox chemistry of ACNs and its relevance to human health.
Possible perspectives for future research will be outlined.

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KEYNOTES

Extraction of anthocyanins from food industry waste and their application as dyes

Richard Blackburn[a,b]

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[b]*Keracol Limited, Nexus, Discovery Way, Leeds, LS2 3AA (United Kingdom)*

Growing concern over the environmental and toxicological impact of synthetic colorants has accelerated the search for sustainable alternatives across the cosmetics, textile, and food industries. Anthocyanins represent a promising renewable solution, particularly when recovered from agro-industrial waste streams. Anthocyanins were successfully extracted and purified from waste epicarp generated by the fruit pressing industry, transforming an underutilised by-product into a high-value natural colorant source. The pigment composition and chemical constituents were comprehensively characterised and quantified.

The resulting anthocyanin-rich extracts demonstrated remarkable multifunctionality across diverse applications. In cosmetic formulations, intense blue hair dyeings with excellent resistance to repeated washing were achieved, highlighting their potential as safe, bio-based alternatives to conventional synthetic “purple shampoo” toning products used to neutralise yellowing in bleached hair [1]. In textile applications, regenerated protein (casein) fibres dyed with anthocyanins exhibited medium colour depths and good wash fastness under optimised conditions. Maximum dye uptake occurred at pH 4, where anthocyanins exist predominantly as a mixture of neutral quinonoidal base and flavylium cation forms [2]. The inclusion of alum further enhanced dye sorption through the formation of aluminium–anthocyanin complexes, while higher pH and dyeing temperatures improved wash durability. To further reduce environmental impact, a novel *in-situ* coloration approach was developed by incorporating anthocyanin dyes directly into a wet-spinning process for regenerated protein fibres [3]. This strategy halved water consumption and significantly reduced energy demand compared with conventional dyeing systems.

Enzymatic acylation was shown to enhance anthocyanin stability without compromising bioactivity or chromatic performance, expanding future application potential. In scaling-up this procedure, a flow reactor was developed where the immobilised enzyme acts as the stationary phase and can be used multiple times, and reduced reaction time with high yields.

References

1. Rose et al. (2018). *J. Agric. Food Chem*. 66, 6790.
2. Blackburn et al. (2024). *Color. Technol*. 140, 393.
3. Houghton et al. (2024). *ACS Sus. Chem. Eng*. 12, 2130.

Structural diversity of red grape and wine pigments: From established markers to the *visible unknowns*

Cristina Alcalde-Eon

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Red wine is a complex and dynamic matrix, whose colouring matter is a mixture of grape native anthocyanins and anthocyanin-derived pigments formed during winemaking and/or ageing after their reaction with other grape phenolics, yeast metabolites or wood compounds. The development of HPLC-DAD-MS^n techniques in the last decades has allowed a gradual identification of grape and wine pigments. Nevertheless, despite this increasing knowledge, further research is still required to fully characterize grape and wine pigment composition.

The objective of this communication is to review the structural diversity of grape and wine pigments, ranging from well-defined compounds (established markers) to compounds detected in most samples, but whose identification still remains uncertain (*visible unknows*).

The native anthocyanins in *Vitis vinifera* L. grapes derive from 5 main anthocyanidins (delphinidin, cyanidin, petunidin, peonidin and malvidin). Most of them are non-acylated monoglucosides, but monoglucosides acylated with acetic, *p*-coumaric or caffeic acids are also present. Pelargonidin derivatives or diglucosides of these anthocyanidins, which were thought to be restricted to non-*V.vinifera* grapes, have been nowadays reported in some *V.vinifera* varieties. In our current research on minority Spanish *V.vinifera* varieties, these unusual compounds have been clearly identified. In addition, pentosides of malvidin and of peonidin, neither usually reported in grapes, have also been detected. Grape anthocyanin profile is characteristic of each variety and for this reason its determination can be used for chemotaxonomic purposes.

In wines, two main families of anthocyanin-derived pigments can be synthesised, the pyranoanthocyanins and the products of condensation between flavanols and anthocyanins. Considering the large variety of grape native anthocyanins and of compounds able to react with them, more than 800 of potential pigments could be present in wines. For this reason, it is important to establish (and review in this communication) the identification criteria in well-identified compounds to have clues for identifying the *visible unknowns*.

From pigmented plants to bioactivity: Impact of targeted fractionation on functional bioactivy

Tuba Esatbeyoglu

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Anthocyanin-rich plants are widely investigated as sources of natural pigments and health-relevant phytochemicals. However, translating pigmented plant materials into nutrition-relevant functionality remains challenging, because crude extracts contain complex mixtures of anthocyanins, phenolic acids, flavonols, tannins and polymeric compounds. Therefore, observed bioactivities cannot always be attributed to anthocyanins alone.

This presentation will discuss how targeted enrichment and fractionation strategies can help clarify composition-activity relationships in anthocyanin-rich plant matrices. Examples from purple sweet potato, black carrot, pomegranate, eggplant and red fruit juice extracts will be used to illustrate how separating anthocyanin-rich fractions from copigment-rich fractions changes the interpretation of functional effects. Across these studies, XAD-based enrichment, adsorptive membrane chromatography and chromatographic profiling were combined with *in vitro* and cellular bioactivity assays, including antioxidant capacity, protection against oxidative stress, inhibition of carbohydrate-digesting enzymes, glucose uptake, antimicrobial activity and proliferation-related endpoints. The findings show that anthocyanin-rich fractions can contribute to bioactivity, but copigments and whole-extract interactions are often equally or more relevant depending on the biological endpoint. For example, phenolic-acid-rich copigment fractions may strongly support antioxidant defence, whereas whole extracts may show stronger activity than isolated fractions due to combined effects among phytochemical subclasses. Targeted fractionation therefore provides a valuable bridge between food chemistry and nutrition by moving beyond total phenolic content or colour intensity toward mechanism-oriented interpretation.

Such approaches are essential for the rational development of anthocyanin-rich ingredients and for understanding how plant pigment complexity contributes to functional bioactivities.

Analytical passage through betacyanin diversity

Sławomir Wybraniec

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Betacyanins, water-soluble plant pigments, are present mostly in fruits, flowers, leaves and roots in plant families of the Caryophyllales [1]. They are extensively used in industry as food colorants. In addition, their chemopreventive and strong antioxidant properties stimulate research on their new structures, derivatives and especially their influence on health. Continuous research brings new discoveries of unique betacyanins, especially with new acyl moieties, at times, in previously investigated plants [1-3].
Most betacyanins can be conveniently divided into 2 basic structural groups of betanin- and gomphrenin-type pigments [1]. Betacyanins based on the betanin backbone (5-*O*-glucosylated-betanidin) form several groups of amaranthin-, melocactin-, oleracin-, and apiocactin-type derivatives. To gomphrenin-type pigments (based on 6-*O*-glucosylated-betanidin), a huge group of glabranin-type derivatives can be attributed with their unique and extremely complex profile of acylated betacyanins tentatively detected in the only one plant species, *Bougainvillea glabra* Choisy, including nine definitively identified betacyanins by NMR analysis. Recent studies on *Basella alba* L. fruits [2] revealed further not reported acylated gomphrenin pigments from which malonylated and 3''-hydroxy-butyrylated gomphrenins were tentatively identified. The most noteworthy was a detection of four unusual gomphrenins acylated with unique nitrogenous acyl moieties. Furthermore, for the malonylated gomphrenins, additional isomeric acyl migration products were tentatively indicated. The acyl migration phenomenon is always anticipated in plant extracts for betacyanins acylated with dicarboxylic acids, especially malonic acid [1]. This reaction proceeds fast in alkaline environment, however, its rate at slightly acidic pH is significant enough to detect characteristic chromatographic profiles of the acyl migration product regioisomeric distribution (6'-*O*-, 4'-*O*-, 3'-*O*-). Other novel gomphrenin derivatives, 6'-*O*-(3,4-dimethoxy-*E*-cinnamoyl)-gomphrenin and 6'-*O*-(3,4,5-trimethoxy-*E*-cinnamoyl)-gomphrenin, have been recently isolated from purple leaf extracts of *Basella alba* L. var. 'Rubra' as abundant pigments [3].

References

4. Kumorkiewicz-Jamro et al. (2021). *Nat. Prod. Rep.*, 38, 2315.
5. Sutor-Świeży et al. (2022). *Int. J. Mol. Sci.*, 23, 1.
6. Kozioł et al. (2024). *Food Chem.*, 445, 138714.

Redesigning Natural Pigments: Betalains as Molecular Tools for Health-Promoting Foods

Fernando Gandía-Herrero

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Betalains are natural pigments widely used as food colorants and increasingly recognized as bioactive compounds with potential applications in the development of health-promoting foods. Traditionally, the study of betalains has focused on a relatively restricted group of natural structures. However, our research has demonstrated that the chemical space of betalains is far broader than previously assumed. Through the generation of extensive libraries of tailor-made pigments, we have expanded betalain diversity by modifying both the amino acid or amine moieties involved in pigment formation and the core structure of betalamic acid itself. This strategy led to the discovery and characterization of unprecedented betalain families, including decarboxylated and methylated derivatives, revealing new opportunities for structure-guided design of functional food pigments [1–3].

Beyond molecular diversity, this work has focused on understanding how structural modifications affect physicochemical and technological properties relevant to food applications. We have systematically characterized the color, fluorescence, and stability of these novel pigments, establishing structure-property relationships that facilitate the rational development of improved natural colorants. Importantly, molecular redesign has been coupled to the evaluation of biological activity. Using integrated *in vitro*, *in silico* and *in vivo* approaches, including studies in the model organism *Caenorhabditis elegans*, we have explored how subtle structural changes influence antioxidant, anti-inflammatory, neuroprotective and chemopreventive capacities. These studies are beginning to reveal structure-activity relationships that may support the future development of betalain-derived ingredients with enhanced functionality. Altogether, results illustrate how the combination of chemistry, biotechnology and biological evaluation can transform betalains into versatile molecular tools for the next generation of health-promoting foods.

Acknowledgments

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References

1. Martínez-Rodríguez et al. (2025). *Trends Food Sci. Technol.* 105150.
2. Martínez-Rodríguez et al (2024). *Plant Physiol.* 146, 446-460.
3. Martínez-Rodríguez et al. (2025). *Food Front.* 5, 2137–2154.

Toward a Genetic Framework to Optimize Anthocyanin Properties as Bioactive and Natural Colorant

Massimo Iorizzo[a], Muhammad Ali Abid[a], Romit Seth[a], Molla Fentie Mengist[b], Chelsey Fiecke[c], Pablo Cavagnaro[d], Giuseppe Valacchi[a], Colin Kay[c], Mario Ferruzzi[e], Mary Ann Lila[a]

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Demand for fruit and vegetable crops as sources of anthocyanins is increasing due to their health benefits and their use as natural colorants. However, within each crop, the level of anthocyanin diversity in terms of chemical structure and content can directly affect biological activity and colorant properties. This presentation will highlight recent work on carrot and blueberry that assessed how genetic variants controlling anthocyanin diversity influence their properties as bioactive compounds and natural colorants. Genes controlling anthocyanin content, glycosylation, and acylation were identified, and methods to test their functions were developed. In-depth analysis of these genes enabled the identification of target polymorphisms for use in anthocyanin selection, as well as sequences that can modulate anthocyanin gene expression and content. Studies using in vitro models indicated that anthocyanin acylation in blueberry can significantly increase stability and biological activity. Similarly, when used as natural colorants, acylated anthocyanins from carrot were more stable under different pH and temperature conditions. Together, this work indicates that a DNA-based approach can be used to select for or modulate anthocyanin content and profiles that maximize health benefits and improve their use as natural colorants. Future in vivo studies will be essential to validate and build upon these findings.

Acknowledgments

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Stabilizing Blue Anthocyanin Colors: From Molecular Mechanisms to Industrial Applications

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Obtaining stable blue hues from anthocyanins remains one of the main challenges limiting the broader application of these pigments as natural colorants. Since anthocyanin color and stability strongly depend on structural features, environmental conditions, and intermolecular interactions, several complementary stabilization strategies have been explored.

One of the most established approaches involves the stabilization of the blue quinoidal base through metal complexation, promoting the formation of blue metalloanthocyanin-type complexes and enhancing color stability [1, 2]. However, many metals traditionally associated with these systems present limitations regarding food compatibility and industrial applicability.

Alternative strategies have therefore focused on the development of more compatible blue systems. Insights from wine chemistry enabled hemi-synthetic modifications of native anthocyanins, improving resistance to pH variation and degradation while enhancing blue tonalities [3, 4]. In parallel, structurally complex anthocyanins, namely polyacylated anthocyanins from *Clitoria ternatea*, highlighted the role of acylation and intramolecular stacking in chromophore stabilization and color modulation [5]. More recently, interactions between malvidin derivatives and proteins such as bovine serum albumin (BSA) demonstrated the potential of protein–polyphenol associations to stabilize anthocyanin structures and tune their optical properties in complex systems [6].

Together, these approaches contribute to a broader understanding of blue anthocyanin stabilization, linking metal complexation, chemical transformation, structural complexity, and supramolecular interactions with the development of more stable naturally derived blue colorants for industrial applications.

Acknowledgements

J.O. would like to thank FCT for her research contract (2022.00042.CEECIND/CP1724/CT0017).

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Exploring the Anthocyanin Colour Palette: A Guided Biochemical Journey

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Colouration represents a prominent and biologically significant trait in plants, with floral pigmentation constituting a major determinant of visual signalling and aesthetic value. The red, purple, and blue colour spectrum is predominantly conferred by anthocyanins, a diverse class of flavonoids whose structural variability underlies both chromatic diversity and functional versatility. In addition to their optical roles, anthocyanins contribute to plant adaptation by mediating responses to environmental stress, including protection against ultraviolet radiation and modulation of plant–pollinator interactions under changing environmental conditions.

Anthocyanidin biosynthesis is among the best-characterised plant secondary metabolic pathways, with a clearly defined enzymatic framework and extensive genetic understanding. Central to colour determination is the hydroxylation pattern of the B-ring: one hydroxyl group typically produces orange to red hues (pelargonidin), two dark red to pink tones (cyanidin), and three generate blue to violet colours (delphinidin).

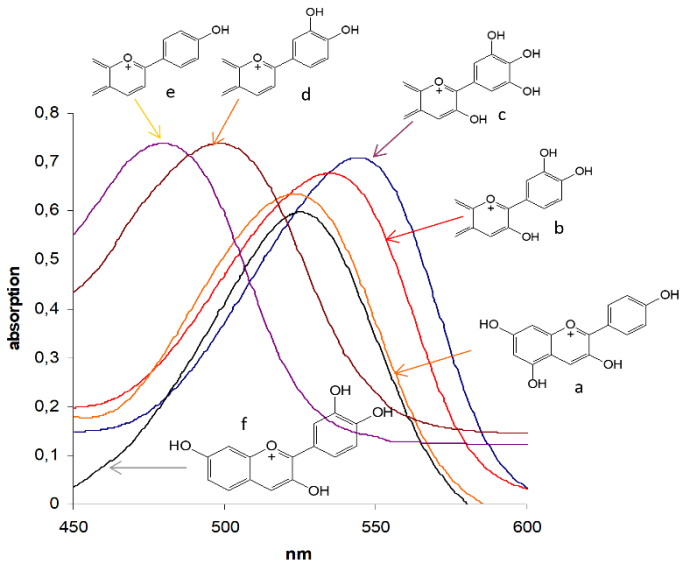

Figure: Part of the absorption spectra of (a) pelargonidin, (b) cyanidin, (c) delphinidin, (d) luteolinidin, (e) apigeninidin, (f) robinetinidin. Source: https://doi.org/10.3390/ijms11020595

While these primary anthocyanidins have been extensively studied, varying hydroxy patterns on the other rings can lead to additional, often overlooked dimensions to the anthocyanin palette. A lack of hydroxyl groups on the C-ring (3-deoxyanthocyanins) and A-ring (5-deoxy-anthocyanins) result in shifts in absorption spectra (Figure). These modifications expand the theoretical and practical colour space achievable through anthocyanin chemistry.

This presentation gives an overview of the biosynthesis of anthocyanins, giving special focus to these less prevalent groups.

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ORAL COMMUNICATIONS

TOPIC 1. BIOSYNTHESIS AND GENETICS

O1.1. A Revised Regulatory Model for Flavonoid Biosynthesis in *Arabidopsis thaliana*

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Flavonoid biosynthesis has been extensively studied in the model plant *Arabidopsis thaliana*. Its regulation serves as an example for understanding transcriptional control of specialized metabolism in plants. In *A. thaliana*, the pathway is predominantly regulated at the transcriptional level by a complex network of transcription factors (TFs) from multiple families, among which R2R3–MYB proteins serve as key determinants of specificity. Genetic and molecular studies in vegetative *Ath* plants have shown that early biosynthetic genes (EBGs), which direct the formation of non-visible flavonoids, are regulated by three R2R3–MYBs: PRODUCTION OF FLAVONOL GLYCOSIDE1-3 (PFG1-3). In contrast, the late biosynthetic genes (LBGs), responsible for the formation of visible anthocyanin and proanthocyanidin (PA) pigments, are regulated by the R2R3–MYBs, PRODUCTION OF ANTHOCYANIN PIGMENT1-4 (PAP1-4) and TRANSPARENT TESTA2 (TT2), respectively.

Accumulating evidence suggests that this regulatory model may be incomplete. To reassess the transcriptional control of flavonoid biosynthesis, especially regarding regulation of EBGs by PAP1-4 and TT2, we generated multiple combinatorial *r2r3-myb* mutant lines. We characterized *pfg1-3*, *pfg1-3 tt2*, and *pfg1-3 pap1-4* mutants using comparative metabolite profiling and transcriptomic analyses.

Our results demonstrate that PA accumulation is abolished in the *pfg1-3 tt2* mutant, whereas anthocyanin accumulation is impaired in the *pfg1-3 pap1-4* mutant. Correspondingly, genes required for PA and anthocyanin biosynthesis were significantly downregulated in the *pfg1-3 tt2* and *pfg1-3 pap1-4* mutant, respectively. Notably, these genes include not only the canonical LBGs but also EBGs. These findings show that PAP and TT2 R2R3-MYB TFs are not restricted to branch-specific regulation of LBGs, but rather regulate all structural genes required for anthocyanin and PA biosynthesis, respectively. We propose a revised regulatory model for flavonoid biosynthesis in *A. thaliana*, in which the different TFs not only regulate their respective branches, but also the “core flavonoid biosynthesis pathway”, comprising the EBGs *CHS*, *CHI*, *F3H*, and *F3’H*, leading to the formation of dihydroflavonols.

O1.2. Elucidating the Genetic Regulation of Anthocyanin Biosynthesis Underlying Peel Color Change During Avocado Ripening

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Peel color change during fruit ripening is an important quality trait influencing consumer preference in avocado (*Persea americana Mill.*). In some cultivars, ripening is accompanied by anthocyanin accumulation, resulting in purple–black peel coloration, whereas in others the peel remains green. This study aimed to elucidate the genetic mechanisms underlying avocado peel color change during ripening to facilitate the development of molecular markers for marker-assisted breeding. Samples from two avocado cultivars exhibiting contrasting peel color change during ripening, 'Ettinger' (green peel) and 'Hass' (purple–black peel), were collected at three time points from harvest to full ripening. Quantitative profiling using HPLC-PDA showed a sharp increase in cyanidin 3-*O*-glucoside during ripening exclusively in 'Hass' peel, whereas 'Ettinger' exhibited higher levels of upstream phenylpropanoid metabolites, suggesting limited flux toward anthocyanin biosynthesis. In agreement with these findings, comparative transcriptomic analysis revealed strong upregulation of flavonoid biosynthesis–related genes in 'Hass', while their expression in 'Ettinger' was completely repressed. By screening of differentially expressed regulatory genes, including transcription factors associated with flavonoid and anthocyanin biosynthesis, we identified several transcription factors that were specifically upregulated in 'Hass' during the transition from green to purple–black peel. One of these genes emerged as a primary candidate following expression profiling in a segregating 'Hass' × 'Ettinger' hybrid population. Furthermore, allele isolation and genomic sequencing revealed that 'Hass' is heterozygous for this gene, carrying one functional and one putatively non-functional allele. In contrast, disruptive mutations were detected in both alleles of 'Ettinger', likely resulting in the complete loss of function in this gene. These findings provide new insights into the transcriptional regulation underlying anthocyanin accumulation in avocado peel during ripening and identify potential targets for developing genetic markers to support avocado breeding for peel color traits.

O1.3. On the road to anthocyanin biosynthesis: insights into the synthesis of naringenin chalcone by Chalcone Synthase

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In the anthocyanin biosynthesis pathway, Chalcone Synthase (CHS) is the first enzyme involved after the phenylpropanoid biosynthesis steps and is common to the ways also leading to flavonols, flavonones, and catechins. Even though the specialization towards anthocyanins in the biosynthetic cascade begins with the involvement of Dihydroflavonol Reductase (DFR), the role of CHS remains central since it leads to the production of the first secondary metabolite common to these polyphenol families, the naringenin chalcone, which is formed from cofactors (based on CoEnzyme A) and the initial substrate (L-Phenylalanine). Moreover, in the framework of the formation within the cell of transient multi-enzyme complexes derived from enzymes of the same biosynthetic pathway, called metabolons, it is accepted that CHS and the Chacone Isomerase (CHI) interact sufficiently strongly for the transfer of the CHS product to the CHI active site to occur via a substrate channeling event.
In this work [1], we experimentally confirmed the interaction of these two enzymes using BiFC and Split-GAL4-Ruby methods and, through the use of numerous theoretical methods ranging from protein/protein docking to QM-MM/DM hybrid calculations, we investigated the mechanism of naringenin chalcone formation and its transfer to the chalcone isomerase active site. Studying the reaction mechanism of Chalcone Synthase which involves chain-growth steps made of the condensation of one p-coumaroyl-CoA molecule and three malonyl-CoA molecules onto the catalytic cysteine, followed by cyclization of the chain polyketide to form naringenin chalcone, we clearly demonstrated the roles of the amino acids in the active site, particularly the protonation state of histidine near the sulfur atom of cysteine during these successive reaction steps. Even more interestingly, the study of putative CHS-CHI complexes revealed the existence of possible pathways for product transfer between the two active sites and here we report the best CHS-CHI complexes candidates together with the most plausible channeling events.

Acknowledgments

This project has benefited from financial support from the CNRS and provision of computing time has been granted by the French GENCI-Jean-ZAY national center and by the Azzurra university computing center.

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O1.4. Modulating the colour palette of black carrots by new genomic techniques

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Anthocyanins from black carrot (Daucus carota ssp. sativus var. atrorubens Alef.) are valuable natural colorants, but their composition is dominated by cyanidin derivatives, limiting the achievable colour range. Expanding the spectrum to include pelargonidin (orange–pink) and delphinidin/malvidin (blue–bluish purple) would increase the utility of carrot-derived colorants. We applied New Genomic Techniques to redirect anthocyanin biosynthesis in the purple cultivar CH5544.

To generate pelargonidin-producing carrots, a CRISPR/Cas9 multiplex construct was designed to target two flavonoid 3′-hydroxylase genes (F3′H-B and F3′H-C). Plants carrying homozygous or biallelic edits in F3′H-B, with or without concomitant edits in F3′H-C, developed reddish taproots. Metabolite analyses confirmed a near-complete shift from cyanidin to pelargonidin, with pelargonidin levels comparable to cyanidin in unedited controls.

For delphinidin biosynthesis, we built two transgenic constructs: (a) pansy (Viola x wittrockiana) F3′,5′-hydroxylase (F3′5′H) plus pansy dihydroflavonol 4-reductase (DFR); and (b) pansy F3′5′H plus carrot DFR3 from the Deep Purple line. Both constructs produced transgenic calli that accumulated exclusively delphinidin-type anthocyanins. Regeneration of delphinidin-colored carrot roots from these calli is in progress.

These results demonstrate that targeted gene editing and heterologous expression of key pathway enzymes can effectively reprogram anthocyanin profiles in black carrot, enabling an expanded natural color palette for food applications.

O1.5. Production of anthocyanin food colorants using yeast cell factories

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BRIGHT, Technical University of Denmark, (Denmark)

Anthocyanins are flavonoid-type blue, purple, and red colors in fruits, flowers, and vegetables. Besides being effective coloring agents in foods and beverages, anthocyanins offer health benefits due to their antioxidant, anti-inflammatory, and other beneficial properties. However, anthocyanins extraction from natural sources is complicated by their low abundance and environmental variability [1]. To address this, we aim to develop a sustainable method for producing anthocyanins through yeast fermentation from renewable feedstocks.

The oleaginous yeast *Yarrowia lipolytica* was selected as the host due to its ability to produce high-titer tyrosine-derived secondary metabolites, such as resveratrol [2] and betanin [3]. *Y. lipolytica* was initially engineered to produce the flavonoid precursor naringenin, achieving a titer of 223.59 ± 9.79 mg/L. We then constructed combinatorial gene expression libraries comprising gene variants from different plants to produce cyanidin-3-O-glucoside (library size ~120) and pelargonidin-3-O-glucoside (library size ~60). These libraries were transformed into the naringenin platform strain, and the best-producing yeast isolates were identified by absorbance and confirmed by LC-MS. This resulted in two yeast strains capable of producing 15 mg/L cyanidin-3-O-glucoside and 27 mg/L pelargonidin-3-O-glucoside respectively. The gene variants present in the best isolates were identified by sequencing.

Establishing the biosynthesis of anthocyanins in *Y. lipolytica* is the first step towards developing an efficient cell factory for fermentative production of anthocyanins.

Acknowledgments

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ORAL COMMUNICATIONS

TOPIC 2. ECOLOGY, FUNCTION, AND EVOLUTION

O2.1. Beyond Color: Deciphering the divergent dynamics of betacyanin in *Amaranthus* plants under insect herbivory pressure

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Despite extensive industrial characterization of betacyanins, their evolutionary role in mediating plant–insect interactions remains a largely understudied dimension of their ecological chemistry. While pigment-rich *Amaranthus* cultivars maintain a competitive advantage, manifesting as reduced susceptibility to herbivory, the underlying quantitative dynamics remain elusive. This study addresses this knowledge gap by assessing phytochemical plasticity under varying herbivore pressures to determine whether pigment induction constitutes a generalized defensive strategy or a tailored response modulated by insect specialization. A two-way factorial design (N = 40 total; n = 5 biological replicates per group) was implemented, treating cultivar (green DJ vs. red 280) and herbivore specialization (polyphagous vs. monophagous) as fixed effects. Betacyanin concentrations (mg/100g DW) and amaranthin relative abundance (a.u.) were quantified via LC-DAD-ESI-MS/MS at baseline (0 h) and 72 h post-herbivory. While amaranthin and isoamaranthin were exclusively detected in the red cultivar, their accumulation profiles were strongly modulated by herbivore specialization. Polyphagous infestation resulted in the total attenuation of basal signals by 72 h, suggesting a potential immune evasion mechanism. Conversely, the monophagous insect induced substantial inter-individual variability; while most replicates showed declining pigment levels, a subset exhibited a distinct inductive response. Statistical analysis using Spearman's rank correlation revealed that herbivory magnitude does not linearly dictate total betacyanin accumulation ($\rho = 0.10$, $p = 0.87$). However, the amaranthin fraction displayed a notable, albeit non-significant, positive correlation with leaf damage ($\rho = 0.73$, $p > 0.05$), suggesting that induced stress may preferentially modulate isomeric ratios rather than total concentration. These findings underscore a complex genotype-by-herbivore interaction governing *Amaranthus* chemical plasticity, highlighting the need to further characterize the regulatory mechanisms underlying this multifaceted defense.

O2.2. Plant-Derived Flavylium Cations: Exploring Their Potential as Evolutionary Molecular Clocks

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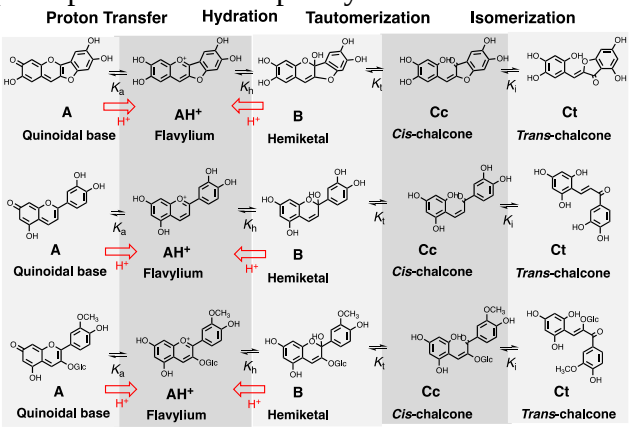
The classification of organisms based on chemical compounds was pioneered by botanist Rolf Hegnauer in the-1950-1960s. Later researchers, including Markham, emphasized that the distribution of chemical compounds reveals phylogenetic relationships and evolutionary trends, rather than serving merely as catalogues of compounds. Although the terms "chemosystematics" and "chemotaxonomy" are sometimes viewed negatively today, we can build on their valuable principles in a contemporary context.

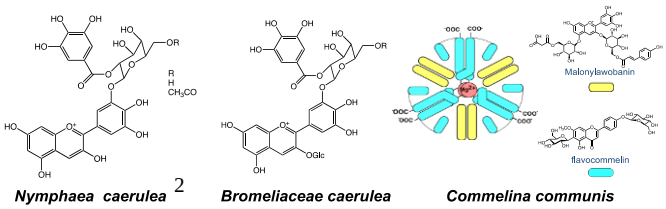
Previous studies have traced evolutionary patterns using compounds such as alkaloids, terpenoids, flavonoids, phenolics, glucosinolates, and iridoids. Here, we focus on the compounds underlying color systems derived from flavylium cations in liverworts, (furanoflavylium), mosses and ferns, (3-deoxyanthocyanins) and angiosperms (anthocyanins).



Scheme 1

The color systems based on flavylium cations exhibit in acidic medium identical chemical species interconnected by identical chemical reactions (Scheme 1) [1]. However, there is an evolution in the rate of interconversion and only anthocyanins exhibit blue colors.

While these color systems may be shaped by environmental adaptation, their evolution remains constrained by their phylogenetic origins. The flavylium-based color systems observed in extant plants reflect not only the chemistry of the ancestral species but also subsequent adaptations to environmental change. Our thesis is that in the case of angiosperms the adaptation to environment changes can be limited by their evolutionary origins. The following plants were used to illustrate the concepts.



Nymphaea caerulea [2] *Bromeliaceae caerulea* *Commelina communis*

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O2.3. The colors of speciation: Pigment transitions towards hummingbird pollination

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In animal-pollinated plants, differences in pollinator sensory systems impose strong selective pressures on floral traits [1]. Floral color in particular plays a central role in shaping pollinator fidelity and reproductive isolation, yet its evolutionary consequences ultimately depend on the biochemical pathways that generate floral pigments and on how these signals are perceived by different pollinators [2]. Understanding how shifts in pollinator identity translate into predictable biochemical and visual changes is therefore key to linking pigment evolution with pollinator-mediated diversification.
Here, we examined how repeated shifts toward hummingbird pollination affect floral pigment composition, reflectance properties, and pollinator perception in Californian plant lineages. We compared ten pairs of closely related bee- and bird-pollinated species across multiple families. Transitions toward bird pollination were consistently associated with shifts from pink-blue anthocyanins to red pelargonidin-based anthocyanins, often accompanied by increased UAP levels. Carotenoids were more common in bird- than bee-pollinated species, although the difference was not statistically significant. These biochemical modifications enhance red reflectance while reducing UV brightness, rendering flowers more conspicuous to birds but less detectable to bees, simultaneously supporting both the bird-preference and the bee-avoidance hypotheses.
Overall, our results indicate that red coloration in hummingbird-pollinated species evolved primarily through reduced anthocyanin hydroxylation, a relatively simple biochemical shift that may facilitate repeated convergent evolution of red flowers and contribute to pollinator-driven speciation in the Californian flora.

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O2.4. Cucurbitaceae: A novel model system for pigment biosynthesis

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Anthocyanins, part of the flavonoid biosynthesis, are responsible for red, purple, and blue coloration and are nearly ubiquitous across angiosperms. Exceptions are rare and have largely been restricted to betalain-producing lineages within Caryophyllales. Here, we report a previously unrecognized, family-wide loss of anthocyanin biosynthesis in the Cucurbitaceae, one of the most economically important plant families, characterized by predominantly white or yellow flowers and carotenoid-based pigmentation. Using a phylogenomics framework encompassing 258 genomic and transcriptomic datasets spanning all 15 Cucurbitaceae tribes, we systematically surveyed the presence, synteny, and evolutionary history of genes involved in anthocyanin and proanthocyanidin biosynthesis. We detected the consistent absence of key structural genes (DFR, ANS, arGST, LAR, and ANR) as well as anthocyanin-related MYB transcription factors across the family. Synteny and phylogenetic analyses support that these absences reflect true gene loss rather than annotation or assembly artifacts. Our results support an evolutionary scenario involving a stepwise loss of anthocyanin pigmentation early in the evolution of Cucurbitaceae, likely accompanied by partial functional replacement through carotenoid-based pigmentation. This discovery challenges the long-held assumption that anthocyanin biosynthesis is universally conserved in flowering plants and identifies Cucurbitaceae as a powerful natural system to identify novel anthocyanin-biosynthesis genes by contrasting Cucurbitaceae (anthocyanin-deficient) and anthocyanin-producing lineages.

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ORAL COMMUNICATIONS

TOPIC 3. HEALTH AND NUTRITION

O3.1. Effect of anthocyanin-rich Edible Flowers extracts in the modulation of Metabolic Disease parameters

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Edible flowers (EF) have been part of human diet for centuries, in ancient Greece and Rome, extending through Europe and Asian countries like China and Japan. EF are emerging as promising functional food products, due to the heightened consumer awareness for healthy diets. Although still a niche segment, these flowers and their derived ingredients offer a rich source of bioactive compounds. Among the different species of EF, some are rich in anthocyanins, especially in acylated anthocyanins, known as having an overall higher stability when compared to the non-acylated ones. In today's society there is a significant emphasis on exploring natural food sources as potential preventive/therapeutic agents to tackle major health issues, and metabolic disease (MetD) is no exception. This disease is characterized by multiple altered metabolic function including, insulin resistance, low-grade inflammation and fatty acid accumulation.
Therefore, in this work we aimed to assess the role of the selected EF extracts on inflammatory and adipose biology parameters using a multidisciplinary *in vitro* approach. For that, THP-1 derived macrophage (for inflammation studies) and SGBS human adipocyte (for adipose biology studies) cells were used and a set of metabolic pathways were evaluated using different techniques including qPCR, ROS or ELISA.
The results showed that all the extracts from *Viola tricolor*, *Cosmos bipinnatus* and *Centaurea cyanus* were effective to modulate LPS-induced inflammation in the THP-1. Furthermore, *Centaurea cyanus* was able to increase glucose uptake in SGBS with concomitant increase of GLUT4 gene expression that seemed to result from an upregulation of insulin receptor activation. *Viola tricolor* decreased lipoprotein lipase gene expression which may explain the observed decrease in lipid accumulation. Interestingly, the effects were not concentration dependent for none of the extracts.
The results highlight the importance of these parental anthocyanin structures in shaping metabolic outcomes. Given that these compounds are well known to be highly degraded upon ingestion, there is a clear need to explore delivery systems that ensure the preservation of their original form is important.

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O3.2. Just don't believe everything you read: Sorting out the quagmire of evidence on anthocyanins and human health

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Anthocyanins' multifaceted roles in human health maintenance, including cardiovascular, vision, age-related and metabolic health protection, are well-established in preclinical, epidemiological and clinical research. This accumulated evidence has fostered the popular notion that *all* anthocyanins are health promoting, and "more is better". But what are the particulars, and how does this work mechanistically? A score of years ago, it was assumed that anthocyanins were not very bioavailable, as their presence was not detected in post-consumption blood draws, but more recently the critical role of the gut microbiota in catabolizing anthocyanins and generating bioavailable circulating phenolic metabolites has come to light. In other work, structurally diverse anthocyanins have demonstrated prominent discrepancies in biological activity; for example, delphinidins and malvidins are markedly more potent than cyanidins to mitigate metabolic syndrome. And what about acylated anthocyanins, which are notably more heat, light, and alkaline stable in food products? Past studies claimed that acylation diminishes the bioavailability of anthocyanins, a conclusion consistently perpetuated in the literature. However, recent reports suggest that because acylated anthocyanins are more stable in the human gastrointestinal tract, they may be *more* readily delivered to the microbiome and catabolized to produce bioavailable phenolic metabolites. As such, the old dogma may need to be revisited. We have acquired a unique model system to explore the acylation question – a selection of seven purple carrot cultivars and accessions with a common plant matrix but accumulating differing levels of acylated anthocyanins (mostly cyanidins; 53-95% acylated) and differing levels of sinapic and/or ferulic acid acylation. We cross-compare these genotypes in terms of 1) phytochemical characterization 2) stability and bioaccessibility after in vitro gastrointestinal digestion, 3) stability and production of phenolic acid catabolites in a fecal fermentation, and 4) bioactivity assays, to conclusively ascertain the impact of anthocyanin acylation of bioaccessibility and health-relevance.

O3.3. How Polyacylation Shapes the Stability and Digestive Fate of Butterfly Pea Flower Anthocyanins

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Among natural sources of anthocyanins, Clitoria ternatea (butterfly pea flower, BPF) is an edible flower traditionally recognized for its agricultural and medicinal relevance. In recent years, growing scientific interest has focused on its intense and stable blue pigmentation and unique anthocyanin composition. This characteristic hue arises from complex anthocyanins known as ternatins, delphinidin-based compounds derived from delphinidin-3,3′,5′-triglucoside. These anthocyanins are highly polyacylated and polyglycosylated, a rare structural feature that confers exceptional properties compared with simple glycosylated anthocyanins [1,2]. This positions BPF as an interesting model to study structure-driven anthocyanin stability and gastrointestinal behavior. This work provides an integrative characterization of BPF anthocyanins, exploring how their structural features influence stability, bioaccessibility, and bioavailability. Full and purified BPF extracts were characterized by UHPLC-DAD-MS, confirming polyacylated ternatins as the dominant anthocyanins, with ternatin B2/B3 as the major compound. Chemical stability of BPF anthocyanins was assessed under varying pH, temperature, and time conditions. pH exerted the strongest influence on ternatin stability, while temperature and time had negligible effects, highlighting the intrinsic stability of these highly acylated anthocyanins. Chromatic stability analysis by UV-visible spectroscopy demonstrated sustained blue coloration over 14 days across a wide pH range, with minimal degradation, consistent with enhanced self-stabilization conferred by ternatin polyacylation. Simulated digestions following the INFOGEST guidelines revealed an increase in anthocyanin content after the intestinal phase. In vitro cytotoxicity assays showed no substantial cytotoxicity across the tested concentrations in gastric and intestinal cell models (NCI-N87 and Caco-2/HT29-MTX, respectively). Transepithelial transport studies revealed that both gastric and intestinal absorption followed a timedependent pattern. Altogether, these findings indicate that the highly polyacylated structure of ternatins underlies their exceptional stability and modulates anthocyanin release during simulated digestion, emphasizing the role of structure in anthocyanin stability and bioaccessibility.

Acknowledgments

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O3.4.From characterization to bioactivity: zein/polysaccharide composite nanoparticles enhance digestive stability and intestinal barrier function protection of anthocyanins

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Anthocyanins (ACNs) are a group of water-soluble flavonoids widely distributed in plant cell vacuoles. In addition to their coloring properties, ACNs present a variety of beneficial effects in human health. Nevertheless, their high sensitivity to external factors and low bioavailability restrict their widespread application in food industry [1]. To address these limitations, protein/polysaccharide composite systems have emerged as an effective strategy not only to improve ACNs stability, but also to overcome their gastrointestinal instability and further boost their bioavailability. Previously, we have fabricated ACNs-loaded zein/polysaccharide composite nanoparticles comprising zein and anionic polysaccharides (pectin, chondroitin sulfate, λ-carrageenan) with improved environmental stability using a combined antisolvent precipitation/electrostatic deposition method [2]. In this study, digestion fate and release behavior was explored through *in vitro* digestion conditions. Results indicated that these composite nanoparticles showed increased digestive stability and intestinal bioaccessibility compared to free ACNs. Moreover, the composite nanostructures enabled controlled ACNs release in the intestine and enhanced antioxidant activity throughout the gastrointestinal tract. Furthermore, a Caco-2/HT29-MTX (9:1) coculture cell monolayer model was established to evaluate intestinal barrier function protection under lipopolysaccharide induction. Results indicated that ACNs-loaded zein/polysaccharide composite nanoparticles provided higher protection against intestinal epithelial barrier dysfunction compared to free ACNs by increasing TEER value, regulating tight junction protein expression, relieving oxidative stress, and inflammation response. Overall, zein/polysaccharide composite nanoparticles are promising delivery carriers of ACNs in the gastrointestinal tract and can effectively prevent the LPS-induced intestinal barrier injury.

Acknowledgments

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ORAL COMMUNICATIONS

TOPIC 4. PHYTOCHEMISTRY AND ANALYSIS

O4.1. Anthocyanin profiles of wines from red grape varieties resistant to fungal diseases

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Vitis vinifera L. varieties are highly susceptible to fungal diseases, such as downy and powdery mildew, which cause serious losses in most wine regions. Control typically relies on fungicides application. However, it can affect wine quality. Therefore, resistant or PIWI varieties have been developed through crosses with other *Vitis spp.* to reduce the need for phytosanitary treatments, which is advantageous both economically and environmentally. Some of these varieties are now officially registered, and because anthocyanins are key in wine color development, their characterization is essential for assessing the enological potential and market acceptance of these new cultivars.

This study evaluated the anthocyanin profile of red wines elaborated from four PIWI varieties (Cabernet Volos, Cabernet Eidos, Merlot Khorus and Merlot Kanthus) over two vintages, and compared it with Cabernet Sauvignon and Merlot. Individual anthocyanins were quantified using a UPLC-DAD method adapted from Pérez-Magariño et al. [1] and identified by LC-MS.

The anthocyanin profiles of C. Volos, M. Khorus and M. Kanthus differed greatly from that of *V. vinifera* due to their higher proportion of anthocyanin diglucosides, as observed in hybrid grape wines [2]. In contrast, C. Eidos showed a profile comparable to *V. vinifera* wines. C. Volos and M. Kanthus exhibited higher total anthocyanin content due to elevated diglucosides content, but lower colorimetric intensity. M. Khorus had lower total anthocyanin content and diglucosides levels, with intermediate color intensity. This distinct anthocyanin composition may influence color development and stability during aging, as diglucosides have a slower polymerization rate than monoglucosides, resulting in reduced stabilization [3].

A detailed characterization of the anthocyanin profile in PIWI wines is essential for tailoring winemaking strategies. C. Eidos and M. Khorus have a more favorable profile for producing high-quality red wines with improved color evolution, characterized by higher concentration of anthocyanin monoglucosides and lower levels of diglucosides.

Acknowledgments

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O4.2. Purple Tea and how to avoid pitfalls in anthocyanin identification

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Purple Tea is the common name for tea plant varieties (*Camellia sinensis* [L.] KUNTZE) with purple leaves caused by anthocyanins. The first scientific publication on the chemical characterization of anthocyanins in Purple Tea dates back only to 2011, identifying galactosides, glucosides, coumaroylgalactosides and coumaroylglucosides of delphinidin and cyanidin, as well as petunidin coumaroylgalactoside, using LC-MS and NMR spectroscopy [1]. Subsequent publications confirmed anthocyanins containing delphinidin and cyanidin as the main components. Furthermore, low concentrations of anthocyanins built with the other common aglycones were detected. Surprisingly, high percentages of free anthocyanidins have been described in Purple Tea [2]. We hypothesized that the free anthocyanidins are artifacts formed during sample preparation. We extracted fresh and processed Purple Tea leaves and applied published methods of sample preparation. The extracts were analyzed by UHPLC-UV/Vis-MS[2]. Extraction with 5% aqueous fuming hydrochloric acid (HCl) in methanol (MeOH) at 35 °C for 10 min, as well as solvent evaporation in a rotavapor at 35 °C after extraction with 1% HCl in MeOH resulted in unintended hydrolysis, mainly of the bond between the coumaroyl moiety and the sugar. This led to a sharp decrease in coumaroylglycosides and an increase in non-coumaroylated glycosides and free anthocyanidins. Thus, high anthocyanidin levels have been identified as analytical artifacts, while small amounts were detected in the processed Purple Tea leaves. In contrast, the extraction of Purple Tea leaves with 2% formic acid or 1% HCl in MeOH at room temperature or at 35 °C did not cause hydrolysis within 45 min. Additionally, we found that MS data reporting the presence of anthocyanidin rutinosides and other diglycosides in several publications seem to be questionable. Our results are of particular importance in food authentication studies because acylated anthocyanins have been demonstrated to be characteristic markers for specific fruits [3].

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O4.3. Beyond Total Anthocyanins: Copigmentation and Matrix Effects Driving Stable Red–Blue Hues in Wine

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Wine color stability is often attributed to anthocyanin concentration, yet this parameter alone fails to capture the complex molecular interactions governing the persistence of red–blue hues. Recent advances highlight that color arises from a dynamic balance between anthocyanins, phenolic cofactors, and other matrix constituents such as metals, ethanol, and polysaccharides. Understanding how these components interact to modulate pigment expression is essential for developing a realistic, matrix-driven view of copigmentation in real wines.

This study evaluated how matrix effects determine anthocyanin expression and color stability in wines, moving beyond the simplistic measure of total anthocyanins. A total of 250 red wines from diverse cultivars and vintages were analyzed under actual enological conditions. Copigmentation was quantified spectrophotometrically at 520 nm following the Boulton dilution protocol, while phenolic and mineral profiles were determined by HPLC and atomic absorption spectroscopy. Statistical relationships were explored using Pearson correlation and ANOVA to identify the main compositional drivers of copigmentation and overall color intensity.

Results showed that anthocyanin concentration alone explained little of the color variability. The pigment/copigment molar ratio, rather than absolute pigment content, was the best predictor of hue stability. Hydroxycinnamic acids and flavonols—particularly caffeic, coumaric, rutin, and myricetin—exhibited strong positive correlations with copigmentation, whereas gallic acid, quercetin, and protocatechuic acid had inverse or neutral effects. Ethanol and volatile acidity acted as dissociating cosolvents, while magnesium and potassium enhanced total color but not directly copigmentation, revealing complex ionic influences.

These findings demonstrate that wine color stability emerges from the synergy among anthocyanins, cofactors, alcohol, metals, polysaccharides, and SO_2 rather than from anthocyanin concentration alone. The proposed “realistic copigmentation” framework integrates the full matrix as the key determinant of visible color expression, offering predictive tools for enology and other anthocyanin-rich systems.

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O4.4. Ion mobility mass spectrometry as tool for the investigation of pH dependent equilibria of anthocyanins

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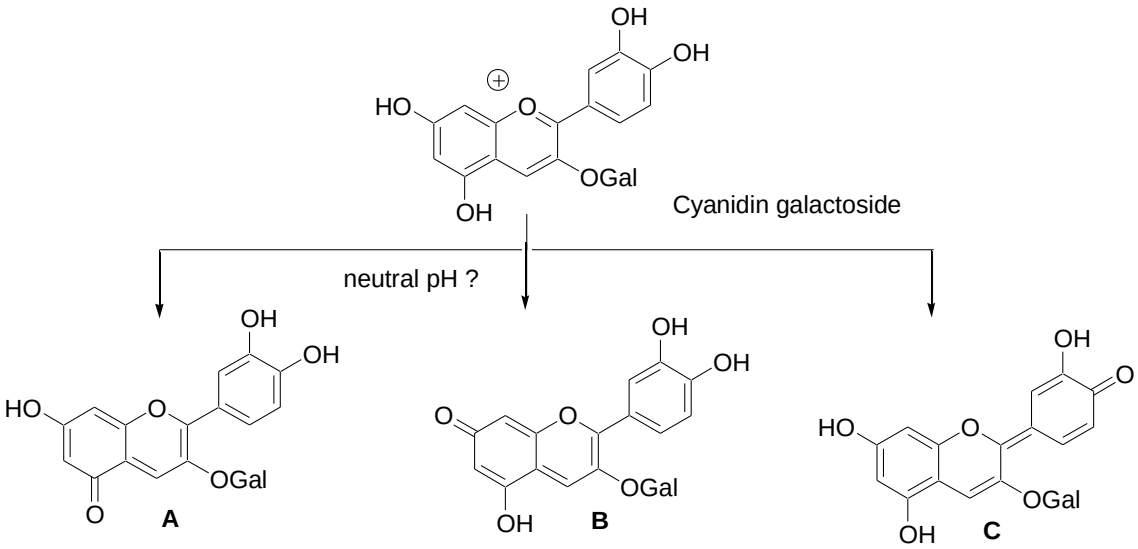
Anthocyanins, possessing beyond doubt a variety of beneficial health effects undergo structural changes when exposed to aqueous solutions at different pH values. When consumed by humans they encounter pH 6.5-7.5 in the mouth, transferring to the stomach at pH 2-3.5, to the duodenum at pH 6.0-7.0 finally reaching the small and large intestine at pH 6.0-7.5, undergoing structural changes in each passage [1].



The equilibrium species present at each pH in each organ defines the bioavailability of the anthocyanin and its structure is predisposed to undergo gut microbial or human hepatic metabolism, ultimately producing bioavailable metabolites responsible for biological activity and health effects [2].

In this contribution we describe our efforts to clarify equilibrium structures of anthocyanins at different pH values using a combination of high-resolution tandem mass spectrometry and ion mobility mass spectrometry [3]. By determination of experimental collisional cross sections and fragment spectra of solution species, we argue for example in favour of one of the three quinoid species **A-C** formed from cyanidin-galactoside by deprotonation. Additionally, we address products of nucleophilic attack on the electrophilic anthocyanin C-ring ultimately leading to ring opened structures.

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ORAL COMMUNICATIONS

TOPIC 5. APPLICATIONS IN FOOD AND INDUSTRY

O5.1. Formation of insoluble anthocyanins-λ-carrageenan biopolyelectrolyte complexes: insights into structural morphology, thermal stability, and release dynamics

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Anthocyanins are multifunctional natural pigments with recognized antioxidant and chromatic properties; however, their industrial translation into food and biobased materials remains limited by their intrinsic instability toward pH, temperature, light, and ionic strength. Structuring anthocyanins into solid-state delivery systems represents a promising strategy to overcome these constraints. Biopolyelectrolyte-like complexes (bioPECs), formed through electrostatic interactions between oppositely charged molecules, provide a rational strategy to enhance stability while enabling controlled and stimuli-responsive release. Despite extensive research on soluble complexes, insoluble anthocyanin-based bioPEC materials remain largely unexplored. This work investigates the formation, mechanisms, and functional performance of insoluble complexes formed between blackberry anthocyanin extract (BAE) and λ-carrageenan (λ-CRG), aiming to develop a stable anthocyanin-based material platform for food and industrial applications. The extract was characterized in terms of total phenolics, total anthocyanins, antioxidant capacity, and anthocyanin profile, with cyanidin-3-O-glucoside identified as the predominant compound. Complexation was driven by ionic interactions between the positively charged flavylium form of anthocyanins (at acidic pH) and the negatively charged sulfate groups of λ-CRG. Optimal insoluble complex formation occurred at pH 3 and a λ-CRG/BAE weight ratio of 0.05. FTIR-ATR analysis confirmed electrostatic interactions and structural rearrangements upon complexation. SEM revealed a porous, hydrogel-like network, suggesting the formation of a three-dimensional matrix capable of entrapping anthocyanins. Thermal analysis demonstrated enhanced thermal resistance compared to free extract, evidencing improved physicochemical stability. Release studies showed limited anthocyanin diffusion over 8 h, following a Fickian diffusion model, with the diffusional exponent (n) modulated by pH and ionic strength. Overall, these findings highlight insoluble anthocyanin–carrageenan bioPECs as a versatile and technologically promising platform for stabilized, stimuli-responsive anthocyanin delivery systems.

Acknowledgments

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O5.2. Color preservation of anthocyanins using grape seed peptides: Evaluation of binding affinity and molecular mechanisms of stability

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Anthocyanins are highly sensitive to factors such as temperature, pH, and nucleophilic hydration, which limit their stability in food systems[1]. In matrices such as wine, this instability promotes the formation of colorless or yellowish forms of the flavylium cation, thereby compromising sensory quality, commercial value, and reducing their bioactivity. In this context, peptides isolated from grape seeds, a by-product of the wine industry, are gaining attention as functional compounds. Their aromatic residues exhibit a natural affinity for anthocyanins enabling the formation of non-covalent complexes [2] that provide the chemical and structural basis for stabilizing the flavylium cation. These interactions promote stacking mechanisms and generate hydrophobic cavities that create a microenvironment of reduced polarity around the chromophore, enhancing its bioactivity while improving chromatic stability and antioxidant effectiveness. To understand how a grape seed peptide contributes to color stabilization, a multidisciplinary approach is necessary. Accordingly, the anthocyanin-peptide complexes were studied in controlled solutions using increasing molar ratios of the peptide while maintaining fixed anthocyanin concentrations. Visible spectra were recorded to detect hyperchromic and bathochromic shifts, color differences were calculated using CIELAB coordinates, and the estimation methods proposed by Boulton and Gonnet were compared to determine their copigmentation constants[3]. These analyses were complemented by accelerated degradation assays and molecular simulations to further characterize the systems. The results showed copigmentation constants comparable to those of traditional phenolic copigments and supported the non-covalent nature of these complexes. Higher peptide to anthocyanin molar ratios exhibited greater resistance to degradation and increased color intensity. These outcomes highlight the role of grape seed peptides as promising stabilizers of the flavylium cation through stacking interactions.

Acknowledgments

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O5.3. Spectroscopic Characterization of Dynamic Complexes between Anthocyanins and a Boronic Acid-Chalcone Receptor

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Boronate–diol interactions have emerged as powerful tools in molecular recognition, controlled drug delivery, and sensing applications. Plant-based polyphenols are relevant bioactive molecules that could benefit from this chemistry for the development of smart systems [1]. However, studies investigating their interactions, particularly those involving pH-responsive anthocyanin dyes and boronic acid derivatives, remain limited in the literature [2].

In this work, we conducted systematic spectroscopic studies (UV–Vis, fluorescence, NMR) to evaluate the binding behavior between a synthesized boronic acid–chalcone receptor and structurally diverse anthocyanins. The preliminary results enabled the estimation of association binding constants and demonstrated a dynamic, pH-dependent, and structure-driven interaction mode between the receptor and the anthocyanins.

These findings provide valuable insights for the development of smart biomaterials, such as films and membranes, designed to host and release polyphenol-like bioactive molecules in a self-regulated manner triggered by pH variations. Such systems could have promising applications in innovative food packaging solutions.

Acknowledgments

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O5.4. From Pigment to Mouthfeel: Anthocyanins for Designing Silky Astringency

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Anthocyanins are widely valued for colour, but they also shape mouthfeel, often aligning with positive astringency subqualities such as velvety and silky. Astringency is traditionally linked to polyphenol–salivary protein interactions, yet recent evidence indicates that different subqualities may be driven by distinct oral interactions, including binding to the mucosal pellicle and oral epithelium. Red wine and butterfly pea flower tea phenolics (monoglucosidic anthocyanins, ternatins (polyacylated anthocyanins), flavanols, and flavonols) were investigated using cell-based oral models combining saliva, an oral mucosal pellicle, and oral epithelial cell lines from tongue (HSC3) and buccal mucosa (TR146) [1,2]. Across epithelia, phenolics within a family displayed similar binding patterns, supporting family-specific interaction signatures. Under the most physiologically relevant condition (saliva + pellicle + cells), flavanol-rich extracts showed the strongest overall interaction, largely mediated by salivary proteins; epicatechin derivatives were the most interactive compounds. Conversely, anthocyanin-rich extracts exhibited reduced interaction in the full model and higher interaction mainly with oral cells; among delphinidin-, peonidin-, petunidin-, and malvidin-3-glucosides, interaction differences were minimal. In butterfly pea tea, ternatins and flavonols interacted significantly with HSC3 cells, but salivary proteins inhibited binding. Structural anthocyanin motifs tuned retention: p-coumaroyl residues enhanced interactions, whereas rhamnosyl residues diminished them. pH shifted the dominant ternatin forms and binding propensity: acidic conditions favoured neutral quinoidal-base species with greater membrane affinity, while near-neutral pH increased anionic forms disfavoured by electrostatic repulsion. The data indicate that saliva, pellicle, and epithelium can dominate at different phases of intake, helping explain shifts from harsher dryness toward silkier astringency.

Acknowledgments

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O5.5. Grape pomace-derived polysaccharides as modulators of anthocyanin oxidative fate in wine systems

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Oxygen critically affects wine key organoleptic properties, such as color and astringency. These characteristics are mainly related to anthocyanin and flavanol evolution in wines. Thus, oxygen management and understanding of oxidative mechanisms are vital in the winemaking industry [1]. Grape pomace, a major winemaking by-product, could become a valuable oenological adjuvant thanks to its polyphenolic and polysaccharidic content [2, 3]. Accordingly, the objective of the present study was to characterize the oxidation pathway of wine anthocyanins and flavanols in a wine-like medium (4 mg/L $FeCl_3$, 0.4 mg/L $CuSO_4$, 13% EtOH; pH=3.6) under periodic oxygenation (90 days). Additionally, the effect of soluble polysaccharide extracts (PS) from white and red grape pomace (0.4 mg/mL) was studied. Oxygen consumption was monitored with an optical fiber sensor and polyphenols and oxidation products were characterized by HPLC-DAD-MS [3]. Color was periodically measured and CIELAB parameters were obtained. Furthermore, antioxidant capacity evolution was evaluated in terms of radical scavenging activity (ABTS) and ferric reduction ability (FRAP) [2]. The results showed a decrease of native anthocyanin concentration over oxidation, initially accelerated by the presence of PS. Oxidative degradation products such as syringic acid, vanillic acid and 2,4,6-trihidroxybenzaldehyde appeared within the first days of oxidation before stabilizing or decreasing due to subsequent degradation. A similar trend was observed for acetaldehyde-mediated derived pigments, reaching a peak concentration to then equally decay. In the case of PS-added samples, these pigments reached higher concentrations, likely due to the prooxidant effect of PS in oxygen saturated media, facilitating the transformation of ethanol to acetaldehyde [3]. While antioxidant capacity decreased over time, PS-added samples exhibited higher ABTS values than the control samples. Regarding color, oxygenation led to lower values of lightness and chroma while hue increased. PS impact (particularly red pomace-PS) was most pronounced toward the end of the assay in terms of higher chroma and hue. In conclusion, grape pomace PS modulated anthocyanin evolution by promoting the oxidative transformation of the pigments. This can provide a basis for a targeted use of pomace-derived adjuvants in oenology, contributing to the circular economy of winemaking by-products.

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O5.6. Structural features of mannoproteins from different wine yeast and their influence on the formation and stability of A- and B-type vitisins

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Mannoproteins (MPs) are one of the main groups of polysaccharides found in wines. They are proteoglycans of the yeast cell wall that are characterized by being heavily glycosylated with mannose residues. Several works have attributed beneficial effects to MPs such as protein, tartaric and color stabilization, among others. For this reason, the addition to wine of MPs is authorized in enology. Recently, some studies point to the possible influence of MPs in the formation of anthocyanin-derived pigments, such as pyranoanthocyanins (PAs) [1-3]. PA formation in wine is relevant because they show different chromatic features than anthocyanins and enhanced chemical stability. However, some discrepancies have been reported regarding the effects of MPs on wine color, which may be strongly influenced by their structural characteristics, in turn conditioned by the yeast species and the method of extraction, among other factors [3]. Therefore, the objective of this work was to obtain and characterize MPs from several wine yeasts and evaluate their influence on the formation and stability of PAs in model wine. MPs were obtained from *Saccharomyces cerevisiae*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Torulaspora delbrueckii* and *Pichia kluyveri* by induced autolysis and enzymatic hydrolysis. PA formation was assessed in a model solution obtained from the fermentation of glucose by *S. cerevisiae*, ensuring the presence of pyruvic acid and acetaldehyde, which are required for the formation of A- and B-type vitisins. Characterization of MP extracts by several techniques (GC-FID, GC-MS, particle charge detector and HPSEC-MALLS-QELS) showed important differences that depended on both the yeast species and the method of extraction. The addition of the MPs to the model wine systems had different effects on the stability of anthocyanins and in the accumulation of A- and B-type vitisins. Moreover, these effects were related to the compositional and structural characteristics of the MP extracts.

Acknowledgments

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O5.7. Antioxidant of Bamboo Leaves as High-Efficacy Copigments for Anthocyanin Stabilization in Beverages

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Anthocyanin degradation during processing and storage remains a critical challenge for the beverage industry. This study demonstrates that antioxidant of bamboo leaves (AOB) functions as an effective natural copigment to enhance the color stability of grape juice. Incorporation of 0.5 g/L AOB induced significant hyperchromic (31.6% increase) and bathochromic (6.0 nm) shifts to pasteurized grape juice, transforming the juice color from ruby red to purplish-red. AOB extended anthocyanin half-life from 12.8 to 15.2 days at 25°C and improved sensory acceptability scores from 15.9/20 to 17.4/20 by introducing a desirable cooling taste. Mechanistic investigation using a malvidin-3-glucoside (Mv3G) matrix identified AOB flavones (AOBF), particularly isoorientin (Iso) and orientin (Ori), as the principal active constituents. At a 1:4 Mv3G:copigment molar ratio, AOBF prolonged Mv3G half-life from 25.4 to 29.1 days and maintained stable purple coloration throughout storage. Molecular docking revealed that Ori and Iso exhibited superior binding affinities with Mv3G (-4.3 kcal/mol each) compared to classical copigments gallic acid (-2.8 kcal/mol) and hydroxybenzoic acid (-2.8 kcal/mol), attributable to enhanced π–π stacking and hydrophobic interactions with the anthocyanin structure. These findings establish AOB as a clean-label, multifunctional ingredient that simultaneously addresses color stability and sensory quality, offering significant commercial potential for naturally preserved anthocyanin-rich beverages.

O5.8. Understanding the Molecular Interaction Mechanisms of Wine-Waste Pigments and Biomordants to Produce Dyed Sustainable Wool Textiles

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The wine industry generates massive quantities of solid waste annually1, posing environmental challenges while offering significant opportunities for biotechnological valorization. This work focuses on repurposing red grape pomace (*Vitis vinifera* L.) as a source of anthocyanins—natural phenolic pigments with high coloring potential. Aligned with the European Green Deal, this research contributes to the development of sustainable materials through innovative, biodegradable, and ecological solutions.
Since the textile industry is one of the most polluting in the world [1-2], with synthetic dyeing being a major contributor, there is an urgent need for sustainable alternatives. Given the high dyeing potential of compounds in wine by-products, this work is the first systematic study on the use of these residues as natural dyes and biomordants. As wool is the best fiber for natural pigments [3], it serves as the primary focus of this investigation.
The research started by extracting these compounds from various grape varieties, followed by characterization using HPLC-DAD, HPLC-ESI/Orbitrap-MS, and NMR. This allowed for the identification of known monomeric anthocyanins and new polymeric colored species. Furthermore, the interaction between these extracts and wool fibers was investigated using Isothermal Titration Calorimetry (ITC) to assess chemical affinities and the underlying mechanisms.
To optimize color fixation and ensure a fully circular process, natural mordants derived from wine industry residues were explored, namely white wine extracts and tartaric acid (tartar) recovered from barrels and vats. Preliminary results indicate that tartaric acid effectively modulates the dyeing pH and enhances the chemical bonding between anthocyanins and wool keratin, improving colorfastness.
This project demonstrates the feasibility of transforming winery residues into high-value resources, providing a bio-based alternative to synthetic dyes while establishing new methods to optimize natural dyeing within a circular economy framework.

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O5.9. Development of anthocyanin-based blue colorant for acidic food applications by simulating the Blue Hydrangea complex

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Production of blue food colorants as alternatives to artificial ones has remained a challenge for decades, since edible blue colors are rare in nature. Anthocyanin pigments, widely distributed in fruits and vegetables, produce blue colors under alkaline conditions. However, they have poor stabilities and there are limited food applications of alkaline pH. Blue Hydrangea flowers have been known to form blue complex which consists of delphinidin-3-glucoside, aluminum and neochlorogenic acid in pH4 [1]. Our goal was to stabilize anthocyanin blue colors under acidic pH by simulating the Hydrangea Blue-complex using edible materials.

Delphinidin and petunidin derivatives were extracted from American eggplant (AE), Chinese round eggplant and Black goji (BG) were semi-purified and used intact or saponified. Neochlorogenic acid was concentrated from a Yerba mate hot water extract (YMF). Anthocyanins and YMF were mixed with aluminum or iron in pH4. Blue color stability was evaluated in pH 2.5 to 7. UV-vis spectra (380-650 nm) was collected on a plate reader, and the spectral data was converted to colorimetric data (CIELAB) by ColorBySpectra software. Blue complex structure was elucidated by circular dichroism, density functional theory computation, Raman spectroscopy and high-resolution mass spectrometry.

All anthocyanins tested successfully complexed with Al^{3+}/Fe^{3+} and YMF, producing blue color in pH 4. Colors remained blue at pH as low as 3 with saponified BG showing bluer hue than other anthocyanins. All samples turned greener when the pH was increased above 5. Our results showed that the glycosylation and acylation position and patterns tested did not prevent the blue complex formation. The density functional theory computation confirmed that the blue complex was octahedral with one molecule of anthocyanin and neochlorogenic acid attached to a central Al^{3+}, but provided a slightly different arrangement as compared to the previously proposed Hydrangea Blue-complex.

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SHORT ORAL COMMUNICATIONS

TOPIC 1. BIOSYNTHESIS AND GENETICS

PT1.1. Coloring from within: Using Cas9 fused exonucleases to engineer *cis*-genic purple tomatoes

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Anthocyanins are multifunctional pigments with potential health benefits for consumers. Purple tomato fruits enriched with anthocyanins have been genetically engineered using a transgenic approach; however, their commercialization remains limited due to the extensive regulation of GM crops. A recent study demonstrated that the use of viral 5′-exonucleases fused to the Cas9 endonuclease (Exo-Cas9) significantly enhanced homology-directed repair (HDR) in plants, facilitating the scar-free integration of several kilobases in both transient and stable assays [1], and expanding the application of gene editing far beyond short DNA sequences. We aim to exploit this innovative tool to engineer *cis*-genic purple tomatoes by inserting fruit-specific regulatory elements from the tomato genome into the promoters of genes regulating anthocyanin biosynthesis. Previous studies indicate that constitutive overexpression of certain R2R3-MYB transcription factors (TFs) on chromosome 10 induces anthocyanin accumulation in tomato fruits, making them promising targets for promoter editing. However, reporter lines expressing, for example, the MYB TF SlAN2 driven by the E8 promoter fail to accumulate significant amounts of these pigments, suggesting that additional components—such as the bHLH TF partner and removal of the MYB repressor ATV—are also required. Alternatively, promoter editing in already bred purple-skinned tomato varieties could be used to redirect MYB TF expression to fruit flesh. Because this approach relies exclusively on endogenous tomato DNA sequences, it enables the generation of *cis*-genic purple tomatoes with enhanced nutritional value and improved shelf life, while bypassing the stringent regulations applied to their GM counterparts.

Acknowledgments

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PT1.2. Divergent polyphenol oxidases determine L-DOPA accumulation in faba bean

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L-3,4-dihydroxyphenylalanine (L-DOPA) is a non-proteinogenic amino acid that accumulates in many plants. In animals, L-DOPA has a critical role as the precursor of catecholamines such as dopamine and is the first-line pharmaceutical treatment for Parkinson's disease. In plants, L-DOPA is an allelochemical and putative pheromone, and it serves as an important precursor, for instance, of betalains and dopamine-derived alkaloids. Overall, however, L-DOPA's function is poorly understood.
The legume, faba bean (*Vicia faba*), accumulates high levels of L-DOPA and while early studies already established that L-DOPA is derived from L-tyrosine, the enzyme catalysing this oxidation reaction has been unknown. Using RNA-seq across faba bean tissues, we identified two closely related polyphenol oxidase (PPO) enzymes, VfPPO9 and VfPPO10, as candidate L-tyrosine oxidases. Heterologous expression in *Nicotiana benthamiana* showed that both enzymes produce L-DOPA, as well as dopamine. A loss-of-function ppo10 mutant confirmed the in vivo role of this enzyme in L-DOPA biosynthesis and suggests a role in melanin pigmentation. Using isotopically labelled substrates, we further demonstrated that VfPPO10 is capable of oxidising L-tyrosine to L-DOPA as well as tyramine to dopamine, however dopamine does not accumulate in faba bean due to the loss of L-tyrosine decarboxylase from the genome. VfPPO9/10 come from a legume-specific clade of PPOs that are distinct from canonical PPOs, enzymes responsible for browning in damaged plant tissue. Canonical PPOs are localised to the chloroplast whereas VfPPO9/10 localise to the endoplasmic reticulum where we hypothesise L-DOPA biosynthesis is occurring. We also tested putative VfPPO9/10 orthologues from other legumes finding that L-tyrosine oxidase activity likely evolved in an ancestor of the legume Faboideae subfamily. Polyphenol oxidases were long hypothesised to catalyse L-DOPA formation in betalain-producing species but were ruled out following the discovery of the cytochrome P450 enzymes, CYP76AD5/6, from beet (*Beta vulgaris*). Here, we show that, in legumes, PPOs are the critical enzymes catalysing this reaction, a finding that will support the studies needed to better understand L-DOPA function in plants as well as to inform research into plant-derived sources of this important pharmaceutical.

Acknowledgments

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PT1.3. Light quality-mediated regulation of anthocyanin biosynthesis in *Vaccinium* berries

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Vaccinium berries, including blueberries (*V.* spp.), bilberries (*V. myrtillus*), cranberries (*V. macrocarpon*) and lingonberries (*V. vitis-idaea*), are one of the best sources of anthocyanins. Lingonberries and cranberries represent red-coloured *Vaccinium* berries, in which the anthocyanin profile includes cyanidin and peonidin glucosides from the cyanidin-branch. In contrast, the blue-coloured bilberries and blueberries contain more complex anthocyanin profile, additionally containing delphinidin, malvidin, and petunidin glucosides characteristic of the delphinidin-branch of the anthocyanin pathway. Our studies have shown that light conditions, i.e. light irradiance, day length and light quality, collectively modulate the biosynthesis of anthocyanins in *Vaccinium* berries. Controlled studies with specific light quality spectrum on bilberry and lingonberry have revealed differential responses between red- and blue-coloured berries. In bilberry, both red (660 nm) and blue light (460 nm) induced anthocyanin biosynthesis in ripening berries compared to far-red (735 nm) or control white light or dark treatments, even 12-fold under red light, 4-fold under blue light, with highest increase in delphinidin branch of anthocyanins and related gene expression. Interestingly, differential responses were detected if berries were detached from the parent plant for the experiment [1,2]. In lingonberry, only blue light increased anthocyanin content during ripening, albeit with less marked increase compared to bilberry experiment [3]. These results, in addition to other earlier studies, indicate that delphinidin branch of anthocyanin biosynthesis pathway existing in blue-colored berries reacts more sensitively to light spectrum changes.

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SHORT ORAL COMMUNICATIONS

TOPIC 2. ECOLOGY, FUNCTION, AND EVOLUTION

PT2.1. Heatwaves under climate change alter flower colour: linking flavonoid biosynthesis to pollinator signalling

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Floral colour in angiosperms has traditionally been interpreted as an adaptation to pollinator preferences. However, increasing evidence indicates that environmental conditions can plastically modify floral coloration by altering pigment composition and concentration. Temperature, in particular, affects anthocyanins and other flavonoids through the regulation of key genes in the flavonoid biosynthetic pathway (FBP). Under current climate change scenarios, where heatwave frequency and intensity are expected to increase, temperature-driven shifts in floral pigmentation may have important fitness consequences if they alter pollinator perception. Here, we investigate how environmental temperature influences (i) the expression of genes involved in pigment biosynthesis and (ii) the perception of resulting colour changes by pollinators.
We used *Lysimachia monelli* (L.) U. Manns & Anderb., a blue-flowered Mediterranean perennial, sampling eight ramets from each of 20 genotypes across two populations differing in thermal environment. Ramets were exposed to two contrasting temperature regimes (20/11 °C and 38/20 °C day/night; simulating spring and summer temperatures, respectively) under controlled growth chamber conditions. We quantified petal spectral reflectance and modelled colour in pollinator visual spaces, measured flower–air temperature differentials, and characterized flavonoid composition using UHPLC-HRMS/MS. In addition, transcriptomic analyses were conducted to identify differentially expressed genes in the FBP across treatments. Preliminary results indicate that elevated temperature increases the concentration of malvidin-3-O-rhamnoside and quercetin-3-O-rhamnoside in petals. By integrating biochemical and transcriptomic data, this study aims to elucidate the mechanistic basis of temperature-induced shifts in floral pigmentation and their potential ecological consequences.


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TOPIC 3. HEALTH AND NUTRITION

PT3.1. Phenolic characterization and bioactivity of Andean blackberry: Effects on oxidative stress resistance and aging in *Caenorhabditis elegans*

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Andean blackberry (*Rubus glaucus* Benth) is a rich source of phenolic compounds with antioxidant and health-promoting properties. Both growing altitude and fruit ripeness are key determinants of phytochemical composition and biological activity [1], as high-altitude cultivation, characterized by intense UV radiation, temperature fluctuations, and low oxygen availability, enhances the accumulation of these secondary metabolites.

This study investigates how these variables influence the qualitative and quantitative profile of phenolic compounds in Andean blackberry and evaluates their bioactivity using *Caenorhabditis elegans* as an in vivo model. Furthermore, it explores the molecular mechanisms underlying the observed biological effects.

Phenolic compounds from fruits collected at different altitudes and ripeness stages were characterized by HPLC-DAD-ESI/MS, leading to the identification of thirty compounds, mainly anthocyanins, flavonols, and ellagitannins. High-altitude extracts showed higher levels of bioactive compounds than low-altitude ones across ripening stages. Total polyphenols and flavonoids were highest at early stages, whereas anthocyanins reached maximum at full ripeness.

Bioactivity was assessed in wild-type and mutant *C. elegans* strains through oxidative stress resistance and lifespan assays, together with RT-qPCR analysis of genes related to insulin/IGF-1 signaling and oxidative stress response [2, 3], revealing stage-dependent effects. In vivo, extracts improved oxidative stress resistance and extended both mean and maximum lifespan in wild-type worms, whereas gene expression patterns differed with ripeness: immature extracts were associated with lower *daf-16* and higher *hsp-70*, while mature extracts increased *daf-16* and *sod-3*, suggesting modulation of antioxidant defense pathways. The absence of protection in mutant strains suggests that these transcription factors are required for mediating the observed effects.

Overall, these findings highlight the antioxidant potential of Andean blackberry phenolics and support their relevance as bioactive ingredients for functional foods applications. Notably, unripe fruits also exhibited beneficial effects, expanding their potential applications.

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PT3.2. Anthocyanin-Rich Extract from Portuguese Red Wine Attenuates Neurodegeneration and Intestinal Dysfunction in a Mouse Model of Parkinson's Disease

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Parkinson's disease (PD) is a common age-related neurodegenerative disease characterized by the progressive loss of dopaminergic neurons in the substantia nigra (SN), leading to motor and cognitive impairments. Although PD aetiology remains unclear, it is believed to arise from a complex interplay between endogenous and environmental factors that converge on interconnected mechanisms of neurodegeneration, including mitochondrial dysfunction, nitroxidative stress and neuroinflammation. Emerging evidence also implicates intestinal dysbiosis and gut–brain axis dysfunction in PD pathogenesis. Currently, PD has no cure, and the development of effective neuroprotective strategies is urgently needed. Notably, dietary polyphenols have shown beneficial effects, potentially through modulation of redox signalling, gene expression, and gut microbiota.

This study investigated the potential protective effects of an anthocyanin-rich polyphenolic extract obtained from a Portuguese red wine (RWE) in a rotenone-induced mouse model of PD, using C57BL/6 mice treated with low-dose rotenone (i.p.), with or without RWE supplementation, focusing on neurodegeneration and intestinal dysfunction.

RWE supplementation attenuated rotenone-induced motor deficits, as evidenced by improved performance in rotarod and vertical pole tests, along with increased exploratory behaviour in the open field and rearing assays. In addition, RWE partially prevented the loss of tyrosine hydroxylase (TH)-positive neurons in the SN induced by rotenone.

Rotenone impaired intestinal function, reducing faecal output and water content, whereas RWE improved these parameters. Histological analysis of colon samples showed increased spacing between colonic crypts, along with vascular congestion and focal haemorrhage in rotenone-treated animals, suggestive of tissue oedema and inflammatory alterations, which were effectively prevented by RWE supplementation.

Overall, these findings suggest that RWE exerts neuroprotective effects in PD, likely involving modulation of neurodegenerative processes and gut–brain axis-related mechanisms.

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PT3.3. Port and Red Wine lees' extracts anti-inflammatory effect in Caco-2 cells

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Introduction: Wine lees are one of the main by-products generated during winemaking and represent a promising source of bioactive compounds with potential health-promoting properties. The present work aimed to evaluate the cytotoxicity, antioxidant activity, and anti-inflammatory potential of extracts obtained from Port and red wine lees using an intestinal epithelial cell model.
Methods: Ethanolic extracts' cytotoxicity was evaluated in Caco-2 cells using the PrestoBlue viable dye. The anti-inflammatory capacity of non-cytotoxic concentrations was evaluated via IL-6 and TNF-α production in CaCo-2 while intracellular reactive oxygen species (ROS) accumulation was evaluated using the DCFH-DA fluorescent probe following tert-butyl hydroperoxide (TBHP)-induced oxidative stress.
Results: Both Port and red wine lees extracts showed no cytotoxic effects in Caco-2 cells at concentrations up to 1 mg/mL, demonstrating their suitability for subsequent biological evaluation. Furthermore, treatment with the extracts reduced intracellular ROS accumulation in cells exposed to oxidative stress, indicating a protective antioxidant effect. Preliminary results also suggested that the extracts exert anti-inflammatory activity, as evidenced by the modulation of inflammatory markers in stimulated Caco-2 cells.
Conclusion: Overall, the results show wine lees' potential as a sustainable source of bioactive compounds capable of protecting intestinal epithelial cells against oxidative stress and inflammation. These results support the valorization of winery by-products within a circular bioeconomy framework and encourage further investigation into the mechanisms underlying their biological effects and their potential application as functional ingredients for gut health promotion.

Acknowledgments

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PT3.4. Effects of *Opuntia ficus-indica* var. *colorada* pulp extract on diet-induced hepatic steatosis and associated changes in gut microbiota in rats

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Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by excessive hepatic fat accumulation, which may be prevented by plant-derived bioactive compounds. This study evaluated the effect of a polyphenol- and betalain-rich extract from *Opuntia ficus-idica* var. *colorada* on diet-induced steatosis and its impact on microbiota composition. Forty male Wistar rats were allocated into 4 groups and fed either a standard (C) or high-fat high-fructose (HFHF) diet. Two HFHF-fed groups were treated with 25 or 100 mg/kg/day of an *Opuntia ficus-indica* var. *colorada* pulp extract (L-OFI and H-OFI, respectively), for 8 weeks. Hepatic and serum triglycerides, and serum cholesterol levels, were measured spectrophotometrically. For microbiota composition analysis faecal DNA was extracted and 16S rRNA gene amplification was carried out. The HFHF group developed hepatic steatosis. In the L-OFI group, hepatic triglyceride levels were significantly lower than in the HFHF group. Both L-OFI and H-OFI groups decreased serum triglyceride concentrations relative to HFHF-fed rats. The steatogenic diet caused an increase in total cholesterol and non-HDL cholesterol, which was prevented with H-OFI. Both treatments augmented HDL cholesterol compared to HFHF group. Regarding microbiota, differences were observed in alpha and beta diversity among control and HFHF groups. A small but significant difference was detected between HFHF and L-OFI group in beta diversity ($R = 0.15$, $p = 0.023$), along with taxonomic changes across several bacterial levels. Serum triglycerides showed multiple significant correlations with gut microbiota taxa, particularly with members of the genus *Faecousia*, which exhibited the strongest associations. In contrast, cholesterol-related parameters displayed limited associations, with only the order *Erysipelotrichales* positively correlating with total and non-HDL cholesterol. Overall, *Opuntia ficus-indica* var. *colorada* extract attenuated HFHF-induced steatosis, improved serum lipid profile, and modulated gut microbiota composition, mainly at the low dose.

Acknowledgments

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TOPIC 4. PHYTOCHEMISTRY AND ANALYSIS

PT4.1. Anthocyanins profile changes in grape fruit skin treated with ABA receptor agonists

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The wine industry plays a major economic, social, and territorial role in Spain, which hosts the largest vineyard area worldwide (>930,000 ha; ~13% of the global vineyard surface) and generates close to €24 billion annually [1]. Mediterranean viticulture is increasingly threatened by climate change, and up to 90% of traditional lowland and coastal wine regions in southern Europe may become unsuitable for viticulture by the end of the century [2]. Heat and drought accelerate sugar accumulation while reducing organic acids, phenolic compounds, and volatile metabolites, leading to unbalanced wines and economic losses. Abscisic acid (ABA) is a key phytohormone involved in grapevine responses to water stress and has been shown to regulate ripening, phenolic accumulation, and sugar–acid balance in grapes [3]. However, the exogenous application of ABA is not agronomically viable due to its rapid degradation. In contrast, emerging ABA-receptor agonists exhibit longer half-life and persistence after application, offering new perspectives for climate-resilient viticulture. In this study, grape bunches of 6-year-old vines of *Vitis vinifera* L. cv. Monastrell, were sprayed four times from veraison to ripening with 30 µM ABA or the ABA receptor agonist, iSB09, to the point of drip off. Total phenolic compounds were extracted with acidic methanol from berry skins, and twenty-three anthocyanins were identified and quantified by HPLC-DAD-MS. Principal component analysis revealed an effect of ABA and iSB09 treatments on the anthocyanin profile, with ten anthocyanins showing significant differences among treatments. The most abundant anthocyanin in red grapes, malvidin-3-glucoside, was significantly higher in iSB09-treated berries than in control or ABA treated ones. Exogenous application of iSB09 also increased acetylated and caffeoylated derivatives of petunidin and peonidin. Further research is underway to assess the persistence of applied ABA and a different ABA receptor agonist in berry tissues, and their effects on phenolic extractability during winemaking.

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PT4.2. Effect of foliar application of grape pomace extract on the anthocyanins profile of Tempranillo grapes

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Wine production has a high environmental impact due to the large volume of by-products generated during vinification. The main residue after alcoholic fermentation is pomace, consisting mainly of seeds, skins and pulp residues. In the case of red varieties, pomaces are particularly rich in phenolic compounds with high antioxidant and antimicrobial activities, which gives them considerable potential for recovery [1]. On the other hand, the increase in temperatures and decrease in rainfall associated with climate change are altering the thermal and water balance of the vineyard. These conditions accelerate phenological development, bring forward ripening, increase the potential alcohol content and can compromise the grape phenolic ripening, affecting the final wine quality [2]. In this context, the foliar application of an extract from Graciano pomace was proposed as a sustainable strategy for its revaluation and as a potential agronomic tool. This extract is rich in phenolic compounds and could act as elicitor, activating the plant's defence mechanisms and promoting the synthesis of secondary metabolites, especially phenolic compounds. These compounds play a key role in the colour stability of red wines through co-pigmentation, and contribute to sensory properties, such as bitterness and astringency; furthermore, with potential health benefits. The valorisation of pomace through its foliar application in the vineyard represents a promising alternative from both environmental and enological perspectives. The objective of this study was to assess the impact of foliar application of pomace extract on the anthocyanins profile of Tempranillo grapes. Identification and quantification of the phenolic compounds were performed by HPLC-DAD. Application of the extract did not significantly alter the total anthocyanins concentration in grapes, although slight variations were observed in some specific derivatives, particularly for delphinidin-3-p-coumaroyl-glucoside, cianidin-3-p-coumaroyl-glucoside and vitisin A. Overall, the foliar application of the extract did not produce a general modification of the anthocyanins composition of grapes.

Acknowledgments

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PT4.3. Untargeted serum metabolomics profiling of betalains intake markers

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Betalains, the water-soluble pigments found in plants such as beetroot, has significant potential as potent antioxidants, anti-inflammatory, antidiabetic, and cardioprotective effects. Traditional approaches to studying betalain bioavailability have focused on the identification and quantification of intact betalains. However, due to the inherent challenges in detecting intact betalains in human biofluids, particularly at lower dosages, the analytical focus in this area has shifted from a strictly "target-only" approach toward comprehensive profiling of the human metabolome. This strategy enhances our ability to detect and identify novel biomarkers associated with betalain intake. Accordingly, the main objective of the current study is to identify potential markers of betalain intake and explore their associated health benefits.

Serum samples were collected from four healthy human subjects before and after 1, 1.5, 2 and 3 hours post-supplementation of 68 mg, 70 mg and 31.25 mL of, respectively, red beet extract (RBE), red beet powder (RBP) and pure red beet juice (RBJ). Collected sera were subjected to protein precipitation, metabolite extraction, and untargeted metabolites screening using Thermo Scientific Q-Exactive Orbitrap MS coupled to Vanquish Neo UHPLC system. Data processing was carried out in Compound Discoverer 3.4 software program (Thermo Scientific), incorporating workflow nodes for peak detection, retention time alignment, compound identification.

Preliminary analysis, after excluding identifications with poor peak quality, identified a total of 178 serum metabolites that exhibited significant changes in abundance post-treatment with the three ingredients. RBE induced the most metabolic changes, with 112 metabolites affected, compared to 67 for RBP and 56 for pure RBJ. The compound classes showing the most significant changes were fatty acids, organic acids, short-chain peptides, and phenolic compounds. Furthermore, differential volcano plot analysis identified several metabolites unique to each treatment. These findings highlight that the three beet-derived treatments induce distinct metabolic signatures, with RBE eliciting the most pronounced metabolic response.


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SHORT ORAL COMMUNICATIONS
TOPIC 5. APPLICATIONS IN FOOD AND INDUSTRY

PT5.1. Effect of Freeze and Thermal Concentration Techniques on Grape Juice Anthocyanin Profiles

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Anthocyanins are responsible for the color and sensory quality of grape juice, but they are highly sensitive to processing conditions. This study explores the impact of different concentration technologies, including freeze concentration (FC), low-temperature vacuum concentration (LTVC) and evaporation concentration (EC) on the anthocyanin profile and retention in grape juice. Using non-targeted LC-MS metabolomics, we identified over 1300 metabolites, focusing on the changes in anthocyanins across all treatments.Multivariate analysis (PCA and PLS-DA) showed that FC preserved the anthocyanin profile most similar to fresh juice, while thermal treatments caused more significant changes. A total of 181, 208, and 293 differential metabolites were identified for FC, LTVC and EC, respectively, compared to fresh juice. Among the anthocyanins, cyanidin-3-glucoside, malvidin-3-glucoside, and delphinidin-3-glucoside showed the largest decreases under thermal treatments, with FC maintaining higher levels of these compounds. For example, cyanidin-3-glucoside levels decreased by 40% under LTVC and 50% under EC, while FC showed only a 15% decrease compared to fresh juice. In addition to anthocyanin changes, several other metabolites exhibited notable alterations. For example, phenylalanine and cinnamic acid, key precursors in anthocyanin biosynthesis, were significantly down-regulated in thermal treatments, while glucose and acetaldehyde, both potential precursors for anthocyanin degradation, were upregulated under EC. This suggests that thermal methods may not only reduce anthocyanin content but also promote the formation of degradation products. KEGG pathway enrichment analysis revealed that the flavonoid biosynthesis pathway was significantly enriched in all processing methods, with FC showing the highest rich factor (0.14). This suggests that FC better preserves the anthocyanin biosynthesis pathway, offering a potential solution for improving anthocyanin retention in grape juice processing. These findings provide valuable insights into the differential metabolic responses of grape juice during concentration and underscore the potential of freeze concentration for better preservation of anthocyanin stability and color in grape juice processing.

PT5.2. Does sugar influence anthocyanin content during fermentation?

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Kombucha is a fermented beverage whose consumption is associated with numerous health benefits, particularly in improving digestive function. This beverage is traditionally made by the fermentation of a sweetened tea infusion using a symbiotic culture of bacteria and yeast (SCOBY). In addition to the typical products of fermentation, these beverages contain a variety of potentially bioactive compounds, derived from both the fermentation process and the substrate used. In this regard, the use of alternative matrices, such as wine by-products, would provide bioactive compounds, such as anthocyanins, whilst also representing a strategy for the valorisation of these by-products. The present study evaluated the effect of sugar concentration (35 and 70 g/L) on the physicochemical parameters (pH, °Brix, acidity, sucrose, glucose, fructose, ethanol, and acetic acid) and the anthocyanin content during the fermentation process (days 0, 7, and 10) of kombucha-like beverages produced using wine lees. The values obtained fell within the typical ranges for these types of beverages and were safe for human consumption. Furthermore, both beverages exhibited a similar initial anthocyanin content (~1.60 mg/100mL). The five characteristic anthocyanins of wine were detected, being malvidin 3-*O*-glucoside the most representative. This content decreased during fermentation in both beverages, with no significant differences between them by day 10. However, a trend toward a lower reduction was observed in the beverage with higher sugar content. This phenomenon could be attributed to the increased availability of sugars for microbial metabolism, which resulted in a more acidic environment, promoting the flavylium cation form. These results revealed a low impact of sugar concentration on anthocyanin content, with the fermentation process being the main determinant, although the aforementioned trend must be taken into consideration. Additionally, this study demonstrated the potential of wine lees for the production of healthy fermented beverages with a bioactive profile distinct from traditional kombucha.

Acknowledgments

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PT5.3. Physicochemical and bioactive characterization of elderberry musts and concentrates from the Portuguese food industry

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Portugal has emerged as a relevant player in the European elderberry sector, particularly in the production of high-quality berries for industrial processing. These berries are commonly processed into must and subsequently concentrated to produce value-added ingredients used in juices, natural colorants, and functional formulations. In this study, twelve elderberry musts from different producers and three concentrates obtained from these musts were characterized in terms of physicochemical properties, nutritional composition, bioactivity, and phytochemical profile. Similar pH values were observed (≈4.0), and color parameters were comparable (a*≈2.66; b*≈2.49). As expected, total soluble solids were higher in concentrates (64.62 ± 0.16 °Brix) than in musts (14.79 ± 0.80 °Brix). This was accompanied by a decrease in water activity and moisture content, and an increase in ash and fat content, consistent with solid enrichment. Musts exhibited on average slightly higher carbohydrate content (96.89 ± 0.95 g/100 g fw) and energy values (401.65 ± 4.00 kcal/100 g) than concentrates (84.73 ± 5.76 g/100 g fw, 371.55 ± 27.56 kcal/100 g). Both matrices exhibited relevant antioxidant activity, with concentrates showing a 4.1-fold and 6.5-fold increase in lipid peroxidation inhibition and peroxyl radical scavenging, respectively. Regarding the phytochemical profile, as expected, concentrates showed a 2-fold increase in the levels of total anthocyanins. Cyanidin 3-sambubioside was the predominant compound in all samples (ranging between 610.439 ± 37.550 mg/100 mL and 1305.284 ± 106.326 mg/100 mL in concentrates and between 390.799 ± 13.27 mg/100 mL and 581 ± 019 mg/100 mL in musts). Rutin and chlorogenic acid were the main non-anthocyanin phenolic compounds in both musts and concentrates. Biological activity was further assessed in RAW 264.7 macrophages, and samples were cytocompatible at concentrations up to 10% (*v/v*) for musts and 5% (*v/v*) for concentrates. Overall, this study highlights elderberry musts and concentrates as valuable functional food ingredients.

Acknowledgments

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PT5.4. Effect of Ethanol Concentration on Ultrasound-Assisted Antisolvent Encapsulation of Anthocyanins in Zein/Gum Arabic and Their Thermal Stability

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Anthocyanins are bioactive compounds with recognized functional potential in food formulations due to their antioxidant and health-promoting properties [1]. However, their application remains limited by their low stability under different temperatures, pH, and light [2]. Additionally, anthocyanin extraction often relies on edible sources, highlighting the need to explore sustainable alternatives such as agro-industrial byproducts. In this context, purple Cacahuacintle corn cob (*Zea mays* L.) represents a promising source of anthocyanins with significant potential. Encapsulation is an effective strategy to enhance the stability and technological functionality of anthocyanins. Among encapsulating materials, zein is particularly attractive due to its amphiphilic nature and ability to self-assemble into nanoparticles via antisolvent precipitation, while gum arabic improves colloidal stability and prevents aggregation [3]. Furthermore, process variables such as ethanol concentration and ultrasound-assisted antisolvent precipitation have been reported to influence particle formation, enabling improved control over particle size and encapsulation efficiency. This study aimed to develop zein/gum arabic nanoencapsulation systems for anthocyanins extracted from purple corn cob and to evaluate the effects of ethanol concentration and ultrasound-assisted antisolvent precipitation on encapsulation efficiency and thermal stability. Anthocyanin-rich extracts were encapsulated using both conventional and ultrasound-assisted methods. The influence of sonication and ethanol concentration on nanoparticle formation and encapsulation performance was investigated. Particle characterization included encapsulation efficiency, particle size, and thermal degradation kinetics. Ultrasound-assisted processing and ethanol concentrations significantly affect encapsulation efficiency, and formation of smaller particles. Encapsulated anthocyanins exhibited improved thermal stability in the range of 80–160 °C compared with free compounds. Kinetic analysis showed reduced degradation rate constants and extended half-life values, confirming the protective effect of the zein/gum arabic matrix under thermal stress. This approach represents an effective strategy to enhance anthocyanin stability, facilitate their incorporation into food systems, and support the valorization of agro-industrial residues as sustainable sources of high-value bioactive compounds.

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PT5.5. Enhancement of stability of grape pomace anthocyanins by microencapsulation

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Application of anthocyanins in food products is primarily hindered by the instability of these compounds under several processing factors including temperature, pH and oxygen [1]. Encapsulation of anthocyanins is reportedly beneficial in protecting their stability. Grape pomace, a by-product of wine industry, consists of around 20-30% of the grape mass, and is a potential source of anthocyanins [2]. This study investigates the efficiency of microencapsulation involving different wall materials in enhancing the stability of anthocyanins extracted from grape pomace. Maltodextrin, gum arabic and whey protein concentrate, alone and in combinations (in the ratio of 7:3, w/w) were employed as wall materials (core:wall = 1:5) in freeze drying-based encapsulation process. Along with encapsulation efficiency, several physicochemical properties were evaluated to select the best wall material for anthocyanin encapsulation, including water activity, hygroscopicity, flow properties, color, antioxidant potential, surface morphology, thermal properties and stability at accelerated (60 °C) and room (20 °C) temperatures. Results indicated that all encapsulates exhibited fair flowability and intermediate cohesiveness, low moisture content, hygroscopicity and water activity. In SEM analysis, whey protein combined with other wall materials exhibited porous, spongy, breadcrumb-like morphology, whereas other combinations and whey protein alone produced particles with smooth, nonporous morphology. Moreover, whey protein incorporated encapsulates showed high stability (317 °C) in thermal analysis. The encapsulate prepared with combination of whey protein and maltodextrin provided the highest encapsulation efficiency (76.77±2.1%) and antioxidant potency (both in DPPH and ABTS methods). However, during storage studies at accelerated and room temperatures, combination of maltodextrin and gum arabic was found to be the best among all encapsulates. Compared to other encapsulates, combination of maltodextrin and gum arabic retained anthocyanins by 88.4% at 60 °C for 21 days storage, and 87.5% for 90 days storage at room temperature. Overall, the encapsulates prepared in this study were able to protect the anthocyanins present in the extract. The combination of whey protein and maltodextrin was beneficial for high content of anthocyanins in encapsulate and enhancing stability under high temperature; and maltodextrin combined with gum arabic was found to be useful for transportation and long-term storage of the grape extract.

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PT5.6. Semisynthesis of betalains as a strategy to enhance color stabilization

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Modern consumers increasingly recognize the health-promoting properties of natural compounds, such as betalains, leading to a rising demand for functional foods enriched with these bioactive compounds. Identifying novel betalain sources, particularly underutilized tropical fruits, is essential. However, industrial application of these pigments is currently hindered by their limited colorimetric stability under varying thermal and acidic conditions compared to synthetic alternatives. Consequently, developing effective stabilization strategies requires a fundamental understanding of how specific betalain profiles behave during technological processing.
Our research group has characterized diverse betacyanin and betaxanthins profiles across various matrices, identifying betanin as the predominant betacyanin in red beet, prickly pear, and pitaya, phyllocactin in ulluco, and vulgaxanthin I or indicaxanthin in red beet and Opuntia spp., respectively [1, 2]. Furthermore, our studies about the effects of temperatures and pHs on color properties concluded that neutral to low-acid pHs and minimal thermal exposure are optimal for preservation. However, color stability remains highly dependent on the intrinsic betalain composition of the raw material [1, 2].
Advancing these stabilization strategies requires to have available individual betacyanins and betaxanthins in high purity and sufficient quantity. To this end, our research group has established robust extraction and purification protocols using Amberlite and C18 resins. Given the limited presence and low concentration of betaxanthins make isolation arduous, our research group has recently optimized a high-yielding semisynthesis methodology (above 70%) [3]. Analysis of these individual pigments revealed that color properties are strongly dependent on betaxanthin structure, identifying Pro-BX as the least susceptible to color degradation across a broad pH range, especially at pH 6 (ΔL^*=0.27, ΔC^*ab=0.44, Δhab=0.46; ΔE^*ab=0.64).
The evaluation of individual betacyanins and betaxanthins serves as starting point for developing natural colorant formulations with improved color stability based on the structure of betalains, offering significant potential for innovation in food technology.

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PT5.7. Black carrot anthocyanin stabilization via $W_1/O/W_2$ double emulsions for yogurt and 3D-printed food applications

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Anthocyanins (ANCs) are valued natural colorants, although their sensitivity to pH, light, and thermal processing limits commercial application [1]. Black carrot (*Daucus carota* L.) is an attractive source owing to its acylated cyanidin-based anthocyanins, which confer greater stability than non-acylated forms. Nevertheless, degradation still occurs during processing and storage, requiring protective strategies. The developed $W_1/O/W_2$ double emulsion (W_1/O (40/60 v/v), $(W_1/O)/W_2$ (50/50 v/v)) was prepared from corn oil, PGPR, gum arabic, and Tween 80 to stabilize black carrot ANCs [2], validated in stirred yogurt and 3D-printed systems with potential for dysphagia diets. Emulsions showed droplet sizes below 10 µm, a creaming index below 10% over 30 days, and stability up to 40 °C. When used as a carmine (E120) substitute in stirred yogurt, color remained stable ($\Delta E < 1.0$) over 15 days, unlike the free extract, which visibly degraded. The yogurt met FDA criteria for pH, acidity, and syneresis, and preserved antioxidant activity (IC_{50} = 13.07 mg/mL, DPPH). Sensory evaluation (n = 70) indicated acceptance close to carmine-colored yogurts, especially when sweetened. For structured foods, W_2 was gelled with gelatin (4–10 wt.%) to form printable emulgels. Rheological analysis confirmed shear-thinning behavior and an elastic, gel-like response ($G' > G''$), supporting 3D printing with good shape fidelity. Emulgels maintained encapsulation efficiency ≥ 99.97%, color stability, and structural integrity over 30 days. Texture analysis indicated a hardness ≤ 136 g, and the 3D-printed systems met the IDDSI Level 5 (minced and moist) criteria for dysphagia diets. Simulated *in vitro* digestion revealed a 3.7-fold increase in anthocyanin bioaccessibility in the emulgel (36.7%) compared with the free extract (9.9%). By modifying the continuous phase of the same $W_1/O/W_2$ system, the technology transitions from a liquid colorant for dairy products to a structured matrix for personalized diets, demonstrating versatility in anthocyanin stabilization and color preservation.

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POSTER

TOPIC 1. BIOSYNTHESIS AND GENETICS

PT1.4. Elucidation of a Blue Anthocyanin Biosynthetic Pathway and Its Reconstruction in Tomato Fruit

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Anthocyanins are widely used as natural food colorants. However, most commercially available anthocyanin-based colorants are derived from cyanidin-type pigments, which typically produce red to purple hues. In contrast, delphinidin-based anthocyanins can provide more purple-blue colors, making them attractive candidates for the development of natural alternatives to synthetic blue dyes. Such pigments, however, are often found mainly in flower petals, which are not suitable for large-scale biomass production. It would therefore be useful to produce delphinidin-based blue anthocyanins in high-biomass plant organs, such as tomato fruit. A further limitation is that the biosynthetic pathways leading to blue flower anthocyanins have been fully elucidated in only a limited number of species. Here, we report the cloning and functional characterization of all genes required for the biosynthesis of the blue anthocyanin from *Veronica persica*. The pathway was transferred to tomato, where stable engineering of fruit tissues resulted in accumulation of the complete anthocyanin. The pigment was extracted from engineered tomato fruit and tested in beverages and solid foods, displaying colors ranging from red under acidic conditions to purple-blue and blue at near-neutral pH. These results demonstrate that the *V. persica* pathway can be reconstructed in tomato fruit and used to produce an extractable anthocyanin colorant. Engineered tomato fruit therefore provides a promising plant-based production platform for natural purple-to-blue colorants.

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POSTER

TOPIC 3. HEALTH AND NUTRITION

PT3.4. Comparative study of selected anthocyanins on tau-induced toxicity in *Caenorhabditis elegans*

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Tauopathies constitute a heterogeneous group of neurodegenerative diseases characterized by structural and functional alterations in the tau protein. More than 20 forms have been described, including Pick's disease (PiD), progressive supranuclear palsy (PSP), and frontotemporal dementia, along with secondary tauopathies such as Alzheimer's disease [1]. This study evaluated the neuroprotective effects of cyanidin, malvidin, and pelargonidin glycosides against tau-induced chemotaxis deficits using the transgenic *Caenorhabditis elegans* strain BR5270, which expresses hyperphosphorylated human tau and exhibits progressive locomotor decline and chemotaxis impairment [2].
C. elegans strains BR5270 (tau-expressing) and BR5271 (non–tau-expressing) were maintained on *E. coli* OP50. BR5270 animals were either untreated (positive control) or exposed to anthocyanins (25–200 µM), while BR5271 served as the negative control. After 96 h at 20 °C, chemotaxis was evaluated (three independent biological replicates, with four plates per condition in each replicate) [3]. Data were analysed using Welch's ANOVA followed by Dunnett's T3 post hoc test. The chemotaxis index (CI) was significantly reduced in BR5270 compared to BR5271, confirming tau-induced impairment. Treatment with anthocyanins significantly improved CI values ($p < 0.001$) relative to untreated BR5270 nematodes, partially reversing this deficit. In general, the effect was comparable across compounds, with an approximate 50% recovery, although distinct dose–response profiles were observed. Cyanidin-3-glucoside was effective at intermediate concentrations (25–100 µM), while pelargonidin-3-glucoside and malvidin-3-glucoside showed significant effects at higher concentrations (50–200 µM). Overall, these findings indicate that anthocyanins partially counteract tau-induced chemotaxis deficits in *C. elegans*, highlighting their potential as modulators of tau-related neurodegeneration.

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PT3.5. Protective effects of anthocyanins against amyloid-β-induced toxicity in *Caenorhabditis elegans*

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Alzheimer's disease (AD) is a neurodegenerative disorder associated with aging, genetic predisposition, environmental factors and lifestyle. A primary pathological hallmark of AD is the extracellular accumulation of amyloid-β (Aβ) peptides, which aggregate into neurotoxic oligomers and form amyloid plaques [1]. Increasing evidence from intervention studies highlights the role of dietary polyphenols in neuroprotection. Specifically, anthocyanins exhibit potent antioxidant and anti-inflammatory properties, suggesting their potential to mitigate AD progression [2]. This study evaluated the protective effects of pelargonidin 3-*O*-glucoside (Pg3G), cyanidin 3-*O*-glucoside (Cy3G) and malvidin 3-*O*-glucoside (Mv3G)—the predominant anthocyanins found in strawberries, cherries, and grapes, respectively [3]—against Aβ-induced toxicity using *Caenorhabditis elegans* as an in vivo model. Two complementary strains were used: CL2355 (neuronal Aβ expression) to assess sensory deficits by chemotaxis, and CL4176 (muscle Aβ expression) to evaluate paralysis as a measure of Aβ proteotoxicity. Chemotaxis assays showed significant improvement in sensory behaviour in CL2355 nematodes treated with Pg3G, Cy3G and Mv3G, compared to the untreated control group. Furthermore, paralysis assays revealed a significant increase in the percentage of non-paralyzed CL4176 nematodes after treatment with these three anthocyanins, indicating a delay in Aβ-mediated proteotoxicity. Overall, these results suggest that Pg3G, Cy3G and Mv3G exert protective effects against Aβ-induced alterations in *C. elegans*, supporting their potential as neuroprotective agents.

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PT3.6. Anthocyanins as potential inhibitors of human salivary α-amylase

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Modulating carbohydrate digestion through the inhibition of human salivary α-amylase represents one of the most promising strategies for managing control glycemic directly from the oral cavity. It has been described that anthocyanin-rich extracts from different sources could act as potent natural inhibitors of salivary α-amylase [1, 2]. However, the relationship between the specific anthocyanin structure and the hypoglycemic effect has not been fully characterized, making essential to identify which molecular structures are able to optimize enzymatic inhibition and enhance the metabolic control of starch.
Here, a purified anthocyanin extract has been employed to slow down starch digestion by inhibiting human salivary α-amylase. The extract was obtained from red grape skins and purified by using a Sephadex LH-20 column. The presence of the five major anthocyanins found in red grape skins, *i.e.* malvidin-3-*O*-glucoside, delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside was confirmed by HPLC-DAD. The inhibitory effect of anthocyanins on the human α-amylase activity was determined by measuring the maltose liberated from starch through enzymatic activity. To characterize the molecular mechanisms underlying the inhibitory effect, interaction assays between anthocyanins and human α-amylase were carried out, and changes in the anthocyanin and enzyme profiles were determined by HPLC-DAD. Moreover, the driving forces involved in the formation of anthocyanin-enzyme complexes were determined by Isothermal Titration Calorimetry. The activity analysis showed an inhibition of α-amylase caused by the anthocyanins. After anthocyanin-enzyme interactions, a modification in the concentration of enzyme was detected, suggesting the formation of anthocyanin-enzyme aggregates that would modify the enzymatic activity. Moreover, not all anthocyanins showed changes in their soluble content in the same way, indicating the existence of different molecular mechanisms and forces involved in the formation of complexes, depending on the specific structure of the analyzed anthocyanin.

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PT3.7. Black rice as a functional source of bioaccessible anthocyanins: Chemical characterization and protective effect against oxidative stress in Caco2 cells

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Black rice is a functional food characterized by a high content of anthocyanins, primarily cyanidin-3-O-glucoside [1]. While thermal and milling processing often affects these pigments, their real biological impact depends on their bioaccessibility from the cooked grain matrix [2]. This study investigates the effect of microwave cooking and digestion using INFOGEST 2.0 on anthocyanin stability and antioxidant activity. These parameters were evaluated in five rice genotypes 'Onix', 'Quila 292008', 'Quila 299802', 'Ambar' and 'Zafiro' (Z4, C4, B4, D4 and E4). A high-resolution phytochemical profile of the 'first flour' fraction was performed using HPLC-QTOF-MS. The Z4 genotype was selected for whole grain digestion analysis. Biological activity was validated by scavenging of reactive oxygen (ROS) in Caco-2 cells subjected to stress induced by ethanol and t-BOOH. The black rice genotype Z4 and C4 had the highest concentrations of cyanidin-3-glucoside (62.06 and 49.82 mg 100 g, respectively) and peonidin-3-glucoside. In contrast, the E4 and B4 showed higher levels of taxifolin derivatives. In vitro digestion of cooked whole grain (Z4) showed that anthocyanins were highly stable under gastric conditions. Although cooking reduced the initial extractable levels to 56.9%, a significant release was observed in the gastric phase, reaching 161.8% for cyanidin and 114.6% for peonidin relative to raw flour. Final intestinal bioavailability remained at 44.1% and 52.1% for each anthocyanin. The first flour black rice extracts Z4 and C4 effectively mitigated lower ROS levels compared to the stress control (1mM t-BOOH) for 210 minutes, showing a protective effect comparable to that of pure cyanidin 3-O-glucoside standard (25-50 μg mL^{-1}). Black rice is a resilient source of bioaccessible anthocyanins. The whole grain matrix effectively preserves antioxidants capacity throughout digestion supporting its intake as part of the diet and potential for functional food development aimed at intestinal health.

Acknowledgments
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PT3.8. Sexual dimorphism in response to a whole-fruit *Opuntia stricta* var. *dillenii* extract rich in betalains and phenolic compounds in the prevention of diet-induced obesity

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Fruits of *Opuntia stricta* var. *dillenii* (OD) are rich in bioactive compounds, such as betalains and flavonoids, which have anti-obesity properties, among others [1]. Nevertheless, sexual dimorphism in response to bioactive compounds has not been widely analysed. This study aimed to evaluate the effects of a whole-fruit OD extract in male and female rats with diet-induced obesity. The main betalains and phenolic compounds of the extract were quantified using high-performance liquid chromatography (HPLC). Sixty Wistar rats (30 animals of each sex) were distributed in control groups fed a standard diet, obese groups fed a high-fat high-fructose (HFHF) diet, and groups receiving the HFHF diet and treated with a whole-fruit OD extract, at a dose of 75 mg/kg/day of body weight. The experiment length was eight weeks. Body weight and food intake were recorded daily. At the end of the experiment, fasting plasma glucose was measured by using a glucometer and serum and faecal samples were collected. Liver and adipose tissue (visceral and subcutaneous) were dissected. Triglycerides were quantified in serum and faecal samples using a commercial kit. Serum free fatty acids and transaminase levels were also measured. In addition, insulin levels were determined using an ELISA kit. High amounts of betanin, isobetanin and piscidic acid were found in the extracts, as well as phyllocactin, isorhamnetin and glycosides of quercetin. No significant differences were observed in final body weight or food intake between obese and supplemented groups. In females, liver weight was significantly reduced. In male rats, after OD supplementation perirenal and subcutaneous adipose tissues, and serum triglyceride levels were reduced, while increased faecal triglyceride content was observed. These findings suggest that the whole-fruit OD extract exerts sex-specific effects, thereby supporting the presence of sexual dimorphism. Further research is required to elucidate the underlying mechanisms of action.

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PT3.9. Anti-inflammatory effects of *Opuntia* extracts in rats fed an obesogenic diet

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Obesity is associated with chronic low-grade inflammation, which contributes to metabolic dysfunction and increases the risk of obesity-related comorbidities [1]. Health beneficial effects have been attributed to products derived from the *Opuntia* cactus due to their content of bioactive compounds such as betalains and phenolic compounds. This study aimed to analyse the effect of an *Opuntia stricta* var. *dillenii* (OD) peel extract and an *Opuntia ficus-indica* var. *colorada* (OF) pulp extract in the prevention of inflammation in adipose tissue of rats fed an obesogenic diet. Rats were distributed into 6 experimental groups: a group was fed a standard diet (C) and five groups a high-fat high-fructose diet (HFHF). Four of these groups were supplemented with OD or OF extracts at doses of 25 (L) or 100 (H) mg/kg/day, for 8 weeks. Protein expression of interleukin-1-beta (IL-1β), monocyte chemoattractant protein-1 (MCP1), and tumor necrosis factor-alpha (TNF-α) were measured by immunoblotting in both epididymal and subcutaneous adipose tissue. *Opuntia* extracts were not able to significantly prevent the body weight increase induced by the diet. Nevertheless, a significant reduction was observed in epididymal and subcutaneous fat pads weight in ODH and OFL groups, respectively. Therefore, regarding the *Opuntia*-supplemented groups, the pro-inflammatory marker profile was analysed only in these two experimental groups. Concerning epididymal tissue, ODH extract prevented MCP1 increased expression caused by the HFHF diet. However, no significant changes were observed with OFL extract supplementation. Concerning subcutaneous tissue, both extracts prevented HFHF diet induced IL-1β expression increase; moreover, OFL extract reduced significantly MCP1 expression, and showed a trend towards lower TNF-α values. These results suggest that although both extracts were effective in reducing the weight of certain fat depots (ODH in epididymal tissue, and OFL in subcutaneous tissue), OFL supplementation was more effective in preventing inflammatory markers expression increase caused by an obesogenic diet.

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anthocyanins and betalains

POSTER

TOPIC 4. PHYTOCHEMISTRY AND ANALYSIS

PT4.4. Analysis and determination of phenolic polymeric pigments present in experimental red wine by HPLC-DAD.

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Color is one of the most appreciated attributes of wines. In the case of red wines, the intensity of the color reveals details of the wine's structure and body and is taken as a standard of quality. Grapes contain 15 types of native monomeric anthocyanins (glycosilated, acetylated and coumaroylated) synthesized by the grape itself. Malvidin 3-glucoside is the major anthocyanin. During wine aging, the color of anthocyanin pigments changes from a deep red to a less intense brick-red hue due to the reactivity of these compounds and their ability to react with other phenolic compounds present in red wines, leading to the formation of more stable polymeric pigments. Especially in aged wines, more stable pigments are responsible for their color. Therefore, it is important to develop analytical methods for their qualitative and quantitative analysis. This research explores the development of a methodology for HPLC-DAD analysis of polymeric pigments in red wine aged for 15 years and enriched with phenolic fractions from Syrah grape pomace extracts. The elution was carried out through a HILIC column with acetonitrile: formic acid 95:05 v/v (mobile phase A) and water: formic acid 95:05 v/v (mobile phase B). The peak areas obtained at 280 and 520 nm were measured and recorded. Different enological color indexes were also calculated and discussed.

Acknowledgments

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PT4.5. 50 Shades of pink: An in-depth analysis of the anthocyanins present in the Princettia series (Euphorbia *hybrida*)

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Princettia® is a special series of the classic poinsettia (*Euphorbia pulcherrima)* that is commercialized by Suntory Flowers in Japan. The interspecific hybrid (*E. pulcherrima* × *E. cornastra*) shows a particularly compact growth, smaller bracts, and a high number of flowers. But most striking are their intense colours in various shades of pink which differ significantly from the red hues of the common poinsettias.
Red colouration of poinsettia is mediated by anthocyanins, mostly cyanidin derivatives with increasing portions of pelargonidins if the hues turn towards orange-red. The main pigments identified so far are cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside and cyanidin-3-*O*-galactoside with similar glycosylation patterns for the pelargonidin pigments. Traces of delphindins can be present as well, but it remains unclear, whether this is a result of oxidation processes during anthocyanidin extraction.
We assumed, that the pink shades could be a result of a special anthocyanidin composition either in the cyanidin:pelargonidin:delphinidin ratios or with respect to the decoration pattern (glycosylation, methylation, acylation) of the main anthocyanidin structures. We therefore investigated six princettia varieties with a colour spectrum from crimson to white by HPLC and LC-MS for their anthocyanin composition.
In contrast to our expectations, pink colour seems to be based on the same anthocyanins as common red colour. With the exception of the white bracted cultivar, all varieties showed four main anthocyanin pigments, two major and two minor peaks, which could be identified as cyanidin 3-*O*-glucoside, cyanidin 3-*O*-galactoside, cyanidin 3-*O*-rutinoside, and pelargonidin 3-*O*-glucoside. Concentrations, however, varied from 70 µg (pink) to 2 mg (crimson) cyanidin equivalent/g fresh weight and correlated with flower colour. LC-MS did not indicate that there is a different decoration pattern for different colours. We assume that variations in the pH or in the copigmentation could be vital for the colour expression in princettia flowers.

Acknowledgments

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PT4.6. Effects of Foliar Applications of Methyl Jasmonate and Methyl Jasmonate + Urea on Anthocyanins Content in Tempranillo Grapes and Wines

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Anthocyanins are key phenolic compounds determining red grape and wine colour. Their accumulation in berry skins can be stimulated by elicitors, although environmental conditions strongly modulate this response. This study evaluated the effect over two consecutive vintages of foliar applications of methyl jasmonate (MeJ) and methyl jasmonate plus urea (MeJ+Ur) on anthocyanins composition in Tempranillo grapes and wines. Treatments and control (water) were applied at veraison and one week later, and anthocyanins in grapes and wines were quantified by HPLC-DAD.

In 2019, both treatments significantly enhanced grape anthocyanins concentration compared to the control. MeJ increased several individual anthocyanins as well as total non-acylated, total acylated, and total anthocyanins. The combined MeJ+Ur treatment produced an even stronger response, increasing a wider range of non-acylated and acylated forms and surpassing MeJ alone. These results suggest a synergistic interaction between MeJ, which activates enzymes of the phenylpropanoid pathway, and urea, which may stimulate nitrogen-related metabolic processes involved in anthocyanins biosynthesis. In 2020, treatment effects were weaker. MeJ and MeJ+Ur increased only specific anthocyanins, and no significant differences were observed in total anthocyanins concentration. MeJ+Ur even reduced total acylated anthocyanins relative to the control. These seasonal differences were likely associated with higher pre-harvest rainfall in 2020, which increased berry weight and may have diluted skins phenolics, highlighting the climatic dependence of treatment efficacy. In wines, the impact of treatments was less pronounced than in grapes. In 2019, MeJ wines showed increases in several anthocyanins and in total acylated forms, which are associated with greater colour stability. However, in 2020 the effect of the treatments on the wines was minor.

Overall, MeJ, especially combined with urea, can enhance grape anthocyanins accumulation under favourable seasonal conditions but differences were attenuated during winemaking, possibly due to extraction limitations and treatment-induced changes in skin cell wall composition.

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PT4.7. Studies on Conjugating Betalain Pigments with Sulfhydryl Radical Scavengers

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Due to their labile nature, betacyanin pigments can undergo dehydrogenation reactions when exposed to metal ions, oxidizing agents, and high temperatures [1]. To investigate the intermediate products formed during the reaction, a method was developed to 'freeze' the reaction system by coupling the unstable quinone forms generated by the reaction with compounds containing -SH groups [2]. The aim of the study was to couple thiols with betacyanins and betaxanthins in the presence of an oxidizing agent, identify the products, and propose possible reaction pathways. The pigments used in the study were extracted from plant sources and purified by chromatographic techniques. The reactions were carried out on a micro scale, and their progress was monitored by liquid chromatography coupled with mass spectrometry (LC-DAD-ESI-MS). High-resolution mass spectrometry (LC-Q-Orbitrap-MS) was used for further identification of the new compounds. For the first time, a sulfhydryl conjugate was synthesized from a betaxanthin pigment. This suggests that quinone forms may also be produced during betaxanthin oxidation. Given that conjugates of natural compounds with thiols can be formed *in vivo* [3], future research will focus on isolating the obtained derivatives on a preparative scale and studies on their properties.

Acknowledgments

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PT4.8. Effect of drying temperature on berry press residue anthocyanin stability and profile

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Berry press residues represent a significant underutilised by-product of the juice industry, rich in bioactive anthocyanins and polyphenols. This study systematically evaluated the impact of conventional hot air, vacuum, and freeze-drying at temperatures between 30 and 90 °C on the stability of anthocyanins across 10 different berry press residues. Results indicate that freeze drying provides the highest bioactive retention, while vacuum drying at 30–60 °C serves as a comparable, energy-efficient alternative. A critical thermal degradation threshold was identified above 75 °C, with substantial losses occurring at 90 °C. Chokeberry press residues yielded the highest total anthocyanin content at 3,826 mg/100 g DW (vacuum, 45 °C), yet displayed high thermal sensitivity with total polyphenolic content reductions exceeding 46% at 90 °C. Conversely, strawberries and raspberries exhibited superior stability under vacuum, with TPC losses as low as 2.1-4.3%. Chromatographic analysis of 24 anthocyanins revealed clear structure-stability relationships: rutinosides demonstrated resilience (e.g., cyanidin 3-O-rutinoside, 16-19.8% reduction), whereas sambubiosides were highly labile (e.g., cyanidin 3-O-sambubioside, 54.2% mean reduction). Radical scavenging activity (DPPH) correlated more strongly with TPC ($r = 0.891$) than TAC ($r = 0.662$), identifying total phenolics as the primary driver of antioxidant potential. These findings provide a roadmap for the industrial valorisation of berry waste by-products, suggesting that vacuum drying at 45–60 °C optimises the balance between phytochemical reservation and process efficiency.

Acknowledgments

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PT4.9. Studies on identification of decarboxylation and dehydrogenation reaction products of betacyanins from *Hylocereus polyrhizus* fruits

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Betacyanins, belonging to betalains, are nitrogen-containing water-soluble plant pigments derived from the amino acid L-tyrosine as secondary metabolites, mainly in plants of the order *Caryophyllales*. They exhibit increased stability under mildly acidic conditions (pH 4–6) [1]. Under certain environmental factors, such as temperature or the presence of metal ions, betacyanin degradation may occur, including oxidation processes. *Hylocereus polyrhizus* (Weber) Britton & Rose is a valuable source of betalain pigments, in which betanin and its acylated derivatives – phyllocactin and hylocerenin have been identified [2].
The fruit pulp of *Hylocereus polyrhizus* was extracted and purified using solid-phase extraction techniques. The eluates were lyophilized and subsequently used for degradation studies conducted under various conditions, including elevated temperature, different buffer systems (acetate, citrate, phosphate; pH 3–8), ethanolic media, and in the presence of ABTS•+. Reaction products were analyzed by UV–Vis spectroscopy and LC–DAD–ESI–MS based on retention times, mass spectra, and absorption maxima. The data enabled tentative identification of transformation products and the proposal of possible pathways for betacyanin-derived product formation under the applied conditions.
The results indicate a significant influence of the reaction environment on oxidation product formation. In ethanolic solutions, degradation favors decarboxylation, likely due to reduced chromophore stabilization and increased thermal susceptibility. In contrast, in buffered aqueous systems, particularly under acidic conditions, betacyanins exhibit relatively higher stability; however, oxidation and dehydrogenation still occur depending on pH and buffer composition. Elevated temperature accelerates all degradation pathways, including decarboxylation, dehydrogenation, and structural rearrangements. Based on the data, possible betacyanin transformation pathways are proposed. Furthermore, the purification strategy affects degradation profiles, indicating that matrix components and co-eluting compounds may influence pigment stability.

Acknowledgments

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PT4.10. Oxidation Pathways of Decarboxylated Gomphrenins Isolated from *Basella alba* L. var. 'Rubra' Fruit Extracts

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Gomphrenins, the major betacyanin pigments in *Basella alba* L. var. 'Rubra' fruits, are valued for their high extinction coefficients and antioxidant properties [1]. Their decarboxylated derivatives, which retain different coloring properties, can be generated during thermal processing, yet their oxidation behavior remains poorly characterized [2]. This study investigated the oxidation pathways of three decarboxylated gomphrenins: 17-decarboxy-gomphrenin (17-dGp), 2-decarboxy-gomphrenin (2-dGp), and 2,17-bidecarboxy-gomphrenin (2,17-dGp).
Gomphrenins were extracted from *B. alba* 'Rubra' fruits with water, purified on cation exchange resin and C18 silica gel as well as isolated by preparative HPLC. The decarboxylated derivatives were obtained by controlled thermal decarboxylation in the presence of citric acid. Oxidation experiments were performed by reaction with ABTS cation radicals (200 μM) in acetate-phosphate buffers (pH 3-8) at 22 °C. Reaction mixtures were monitored by UV-Vis spectrophotometry and analyzed by LC-DAD-ESI-MS. Comparative oxidation of the three decarboxylated gomphrenins revealed a convergent pathway with 2,17-bidecarboxy-xangomphrenin (*m/z* 461, λ_{max} 469 nm) as the central reaction product. This derivative was generated most efficiently from 17-dGp, which possesses the carboxyl moiety at C-2, enabling concurrent rearrangement of the dopachromic intermediate and irreversible decarboxylation [3]. Subsequent oxidation led to a formation of 2,17-bidecarboxy-xanneogomphrenin (*m/z* 459, λ_{max} 413 nm) and further decarboxylated products. Notably, no hydrolysis to betanidin was observed for any of the decarboxylated substrates during oxidation, in contrast to native gomphrenin [3].
The elucidated pathways demonstrate that all the three decarboxylated gomphrenins converge to a common intermediate, providing a comprehensive framework for understanding their oxidative transformations during food processing and storage.

Acknowledgments

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PT4.11. Expanding Betalain Diversity: The First Modification of the Chromophoric Ring to Produce Methylated Pigments

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The structural diversity of betalains has been traditionally explored by modifying the moiety that condenses with betalamic acid. However, the discovery of dopamine-derived decarboxylated betalains in nature has recently expanded the chemical landscape of these pigments [1]. In this work, we introduce a paradigm shift in betalain chemistry by modifying the chromophoric core itself for the first time [2]. Using the substrate promiscuity of the 4,5-DOPA-extradiol-dioxygenase (4,5-DODA) enzyme from *Gluconacetobacter diazotrophicus*, we developed a synthetic pathway starting from α-methyl-DOPA to produce 6-methyl-betalamic acid. Kinetic characterization revealed a substrate inhibition model with a V_{max} of 56.8 mol min^{-1}, where the presence of the methyl group at the C6 position was found to hinder the formation of certain secondary metabolites. This novel structural unit was successfully used as a building block for the biotechnological synthesis of ten previously undescribed methylated betalains, including eight betaxanthins (yellow) and two betacyanins (violet). The pigments were purified by preparative HPLC and characterized using ESI-Q-TOF mass spectrometry, spectrophotometry, and spectrofluorometry. All compounds exhibited high molar extinction coefficients (45,000 to 68,000 M^{-1} cm^{-1}) and maintained the characteristic fluorescence of the family, with histidine-6-methyl-betaxanthin showing an increase in intensity compared to its traditional analog. Furthermore, ORAC assays demonstrated significant antioxidant capacity against peroxyl radicals, with indoline-6-methyl-betacyanin being the most potent. These findings mark the beginning of "betalain core chemistry," offering new strategies for the targeted design of pigments with customized spectroscopic and bioactive properties for food and pharmaceutical applications.

Acknowledgments

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PT4.12. Nanocellulose matrices from invasive plant biomass for anthocyanin stabilisation

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The limited stability of anthocyanins remains a key challenge, driving the development of effective carrier systems for their stabilisation. In this study, cellulose nanofibers (CNF) were investigated as potential sorptive matrices for anthocyanins, with particular emphasis with particular emphasis on adsorption capacity. CNF were produced from biomass of three invasive plant species widespread in Europe – *Heracleum sosnowskyi*, *Reynoutria japonica*, and *Solidago canadensis* – using combined chemical and mechanical treatment, resulting in highly fibrillated structures with a large specific surface area. Berry extracts containing anthocyanins, along with other co-extracted polyphenols from berry processing residues (aronia, blueberry, blackcurrant, and honeysuckle), were used in sorption experiments to evaluate the performance of the CNF materials. The results showed that adsorption depended on both the CNF source and extract composition. The highest experimental sorption capacity was observed for CNF derived from *H. sosnowskyi* (16.18 ± 0.61 mg g^{-1}), followed by *S. canadensis* (12.02 ± 2.48 mg g^{-1}) and *R. japonica* (10.45 ± 1.85 mg g^{-1}). Sorption from different berry extracts varied significantly, with the highest values observed for blueberry extract on *H. sosnowskyi* CNF (6.07 ± 0.34 mg g^{-1}) and aronia extract on *S. canadensis* CNF (6.5 ± 0.22 mg g^{-1}). The Langmuir adsorption model provided a good fit for *H. sosnowskyi* CNF and an acceptable fit for *R. japonica* CNF, whereas *S. canadensis* CNF did not show a clear saturation within the studied range. These findings demonstrate that CNF can serve as carriers for anthocyanin immobilisation. In addition, the use of invasive plant biomass as a raw material highlights a sustainable approach to developing functional materials for natural pigment applications. Further research is needed to evaluate the stability of anthocyanins within CNF-based systems under different environmental conditions (e.g., temperature, light, and pH).

Acknowledgments

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PT4.13. Polysaccharide influence on anthocyanin and phenolic composition in young red wines

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Polysaccharides constitute one of the major groups of compounds found in wines, primarily originating from the cell walls of grapes and yeast. Among them, mannoproteins and glucans can be released by yeasts during alcoholic fermentation and throughout the aging-on-lees process. Depending on their composition and structure, these polysaccharides can have both beneficial or detrimental effects on red wine characteristics. Several commercial products enriched in yeast-derived polysaccharides, particularly mannoproteins, are currently available on the market; however, their impact on wine composition is not always clearly demonstrated.
The aim of this study was to characterize several commercial yeast products and to evaluate their effect on the anthocyanin and phenolic composition of a young red wine. The experiences were carried out in duplicate using a *Tempranillo* red wine and six different commercial products. The treatments were applied for eight weeks before the onset of malolactic fermentation. Subsequently, the wines underwent malolactic fermentation. Phenolic compounds were evaluated using spectrophotometric methods, while individual anthocyanins and their derivatives were determined by injecting filtered wine samples directly into a liquid chromatograph equipped with a diode array detector (DAD) [1].
The commercial yeast products presented different purity and composition, and different effects were observed on the red wine composition. In general, the concentration of monomeric anthocyanins was lower in the treated wines, while an increase in new polymeric pigments was observed. Some of the treated wines had higher concentrations of total phenols and tannins than the control wines, suggesting that these products could prevent the precipitation and loss of these compounds.
The use of some commercial yeast products in red wines before malolactic fermentation could help to stabilize the color due to the formation of new polymeric pigments that are more stable and resistant to pH changes and oxidation reactions.

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PT4.14. Anthocyanin stabilisation in berry residues using plant co-pigments

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Berry processing generates large amounts of press residues that are rich in anthocyanins, but their future use as natural colourants is limited by poor stability. This study investigated co-pigmentation using extracts of Japanese knotweed (*Reynoutria japonica* Houtt.***)*** and Canadian goldenrod (*Solidago canadensis* L.), both invasive plant species with potential for valorisation, as co-pigments to assess effects on anthocyanin stability in press residues from six berries: honeysuckle (*Lonicera caerulea* L.), blackcurrant (*Ribes nigrum* L.), highbush blueberry (*Vaccinium corymbosum* L.), chokeberry (*Aronia melanocarpa* (Michx.) Elliott), bog cranberry (*Vaccinium oxycoccos* L.), and bilberry (*Vaccinium myrtillus* L.). Berry press residue extracts (2.5 mg/mL) were combined with co-pigment extracts at 0.1, 0.5, and 1 mg/mL. The samples were stored at three different temperatures: 4 °C, 21 °C, and 40 °C. Colour intensity was monitored over 30 days using a UV-Vis spectrophotometer. Co-pigmentation affected anthocyanin stability depending on berry type, co-pigment, and its concentration. At 40 °C, the highest temperature condition, co-pigmentation generally reduced colour loss. For example, in blackcurrant, knotweed reduced colour loss to 49.8% and 39.2% at 0.1 and 1 mg/mL, respectively, compared to 64.8% loss in the control. Goldenrod showed a weaker and less consistent effect. Similar trends were observed in bilberry, where knotweed at 1 mg/mL reduced degradation to 56.7%, relative to 80.9% loss in the control sample. These results indicate that Japanese knotweed extract can enhance anthocyanin stability in the studied berry extracts at elevated temperatures, whereas the effect of Canadian goldenrod is more variable and concentration dependent. Co-pigmentation with plant-derived extracts may offer a practical approach for improving the stability of anthocyanin-rich by-products intended for use as natural colourants, although the effect depends on co-pigment type and conditions. Further research is needed to understand the underlying anthocyanin–co-pigment interaction mechanisms.

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PT4.15. *Sambucus* anthocyanins as natural pigments for advanced dermocosmetic formulations

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The growing demand for natural and safe ingredients in cosmetic products is driving innovation in the sector, particularly in the replacement of synthetic colorants. Anthocyanins have emerged as promising candidates due to their intense coloration and well-recognized bioactive properties. Among natural sources, *Sambucus* spp., widely found in Portugal, represents a valuable and underexplored source of anthocyanins. In this context, this study aims to evaluate *Sambucus* anthocyanins as natural pigments for advanced dermocosmetic formulations, combining aesthetic and protective functions. To assess the influence of extraction efficiency, anthocyanins were recovered using different extraction techniques, including conventional maceration (MAC), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE), employing an acidified hydroethanolic system (80% EtOH, pH 3) under controlled conditions (absence of light and low temperature) to preserve compound stability. Results demonstrated that extraction yield was influenced by the extraction method, with UAE and MAE generally enhancing efficiency compared to MAC. Differences were also observed in total anthocyanin content (TAC) and monomeric anthocyanin content (MAC), highlighting the impact of extraction conditions on pigment recovery. The obtained extracts exhibited intense color parameters, supporting their suitability as natural pigments. Regarding bioactivity, the extracts demonstrated strong antioxidant activity, indicating their potential to mitigate oxidative damage in skin under environmentally relevant stress conditions. Furthermore, anthocyanins showed cytocompatibility with keratinocytes and fibroblasts, highlighting their safety for dermocosmetic applications. In summary, *Sambucus* anthocyanins showed strong potential as dual-function ingredients, combining colorant properties with bioactivity, supporting their use in the development of advanced dermocosmetic formulations.

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PT4.16. Valorizing Winery Waste: Nutritional, Phytochemical, and Bioactive Potential of Anthocyanins from Portuguese Grape By-Products

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Grape by-products, generated as solid residues during winemaking, are rich sources of anthocyanins, as well as insoluble dietary fiber, making them promising candidates for sustainable valorization [1,2]. Their fiber-rich composition, high antioxidant capacity and anthocyanin content support their application as functional ingredients and natural colorants. The valorization of these residues aligns with circular economy principles by transforming agro-industrial waste into high-value products and improving resource efficiency within the wine sector [3]. In response to the increasing demand for sustainable, biodegradable, and clean-label ingredients, the NOVAPACK project investigates the potential of grape by-products capable of replacing synthetic additives in food applications. The present study aimed to valorize winemaking by-products from three Portuguese grape cultivars: Vinhão, Touriga Franca (TF), and Touriga Nacional (TN), through a comprehensive characterization of their nutritional composition, phytochemical profile, anthocyanin content, and antioxidant potential. Nutritional analyses were performed according to AOAC official methods, anthocyanins were quantified by HPLC-DAD, and antioxidant activity was evaluated using the ORAC assay. Based on its superior pigmentation, dietary fiber and phenolic content, Vinhão by-products were selected for anthocyanin extraction using food-grade acidified aqueous solvents [ethanol:water (50:50, v/v) containing 1% citric acid] combined with microwave-assisted extraction (MAE) or ultrasound-assisted extraction (UAE). All grape by-products exhibited low moisture (3.70–4.20%), ash (4.50–5.10%), and lipid contents (9.65–11.90%), moderate protein levels (10.10–15.50%). A high dietary fiber content was observed, ranging from 44.00 to 68.30%. Anthocyanin analysis of the Vinhão by-products extracts revealed malvidin-3-O-glucoside as the predominant compound (4.27–17.64 mg/g DW), followed by delphinidin-3-O-glucoside (1.46–1.63 mg/g DW) and petunidin-3-O-glucoside (0.85–0.97 mg/g DW), with promising antioxidant capacity, ranging from 3.30 to 7.37 µmol Trolox equivalents/g DW. These findings highlight the potential of grape by-products as a readily available, low-cost source of anthocyanin compounds, supporting their conversion into bioactive extracts for a range of industrial applications.

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anthocyanins and betalains

POSTER

TOPIC 5. APPLICATIONS IN FOOD AND INDUSTRY

PT5.8. Bio-hybrid material for improved anthocyanin stability

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Anthocyanins are natural pigments relevant to the pharmaceutical, food, and cosmetic industries due to safety concerns associated with synthetic colorants. Despite their attractive coloration and health-related benefits, anthocyanins are unstable when exposed to light, metallic ions, and temperature factors. Degradation also occurs through structural transformations that depend on the pH of the aqueous solution. Their stabilization has been accomplished by lipophilization, co-pigmentation, and encapsulation. A historical example of pigment stabilization is Maya Blue, where the indigo is trapped within clay channels. Emulating this process, in this work, a stabilization of hibiscus anthocyanins has been achieved by Van der Waals interactions with sepiolite clay. Bio-hybrids (BH) were prepared by mixing sepiolite and hibiscus extract, or hibiscus-rich anthocyanin extract for 1 h at 100°C. After that, hibiscus extract produced two BH, elaborated with 100 (BHH-100) and 200 (BHH-200) mg of anthocyanins per gram of sepiolite. While hibiscus-rich anthocyanin extract produced a BH (BHA) elaborated with 100 mg of anthocyanins per gram of sepiolite. The resulting BH powders shifted repeatedly between flavylium ion and quinoidal base forms while alternating between acidic (HCl) and alkaline (NH_4OH) media. The higher hygroscopicity of BHH-200 allowed only one atmosphere shift, while BHH-100 achieved six cycles, with similar absorbance intensities before and after the trial. Nevertheless, BHH-200 showed higher absorbance than BHH-100 and BHA. Moreover, powder samples showed positive results in stability testing under 24 h of UV radiation exposure (360-400 nm). Finally, color measurements after radiation indicated smaller variations in BHs compared with sepiolite. Eye perception of these changes was minimal. Overall, hibiscus anthocyanins can be stabilized through sepiolite interaction to produce BHs capable of maintaining the quinoidal base without fast degradation seen in aqueous media. These BHs exhibit UV protection characteristics and are derived from natural resources.

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PT5.9. Influence of Zein Purification, Mixing Rate, and Temperature on Anthocyanin Encapsulation Efficiency and Stability in Zein/Gum Arabic Nanoparticles.

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Anthocyanins are bioactive compounds with recognized antioxidant properties. In addition, these compounds exert a wide range of bioactive effects, including hypolipidemic, bacteriostatic, chemoprotective, anticarcinogenic, and anti-inflammatory actions [1]. However, their application in food systems is limited by their low stability and low bioavailability. Encapsulation using biopolymeric systems such as zein/gum arabic (Z/GA) nanoparticles has emerged as an effective strategy to enhance their stability and functionality. The stabilizing effect of gum arabic and pH on anthocyanin-rich extracts from purple corn cob has been previously reported [2]. This study evaluates the influence of key processing and material variables on encapsulation performance. Z/GA nanoparticles were produced by antisolvent precipitation, incorporating three main variables, the mixture rate of the continuous and disperse phases, the processing temperature (20–50 °C), and the type of zein (commercial vs. purified). Encapsulation efficiency, particle size, polydispersity index, ζ-potential, thermal stability, and in vitro gastrointestinal release were analyzed.

Results showed that lower processing temperatures significantly enhanced anthocyanin encapsulation efficiency, likely due to reduced molecular mobility and improved biopolymer interactions during particle formation. Additionally, purified zein exhibited higher encapsulation efficiency compared to commercial zein, suggesting that the removal of hydrophobic impurities improves the availability of interaction sites for anthocyanins [3]. Particle size was moderately affected, with slightly larger nanoparticles observed in systems prepared with purified zein, while no significant differences were detected in polydispersity index or ζ-potential, indicating comparable colloidal stability across treatments. Encapsulated anthocyanins demonstrated improved thermal resistance in the range of 100–180 °C and enhanced release in simulated small intestinal conditions (INFOGEST), confirming the protective and delivery capabilities of the Z/GA system. These findings highlight the importance of processing conditions and protein purity in optimizing zein-based nanoencapsulation systems and support their potential application in thermally processed foods and functional delivery systems.

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PT5.10. Influence of grape seed peptide fractions with different molecular weights on colour stability and antioxidant capacity of malvidin-3-*O*-glucoside model solution

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The chemical stability of anthocyanins, mainly malvidin-3-*O*-glucoside, poses significant challenges to the preservation of colour red wines from warm climates [1, 3]. In this context, grape seed peptides may act as colour stabilisers of malvidin-3-*O*-glucoside [1-3]. The objective of this study was to determine the effect of the molecular weight of peptide fractions (Fr<5 kDa and Fr<10 kDa) on the colour evolution and antioxidant capacity of a wine-like model solution containing malvidin-3-*O*-glucoside, over 20-days storage. A synthetic wine solution containing 120 mg/L of malvidin-3-*O*-glucoside was prepared at pH 3.5, and considered as control. Peptide fractions with molecular weights below 10 and 5 kDa were added at 1 g/L. The samples were analysed over 20 days in order to determine colour parameters, antioxidant capacity, and turbidity. After 20 days, lightness (L^*) decreased about control (L^*=76), only in the presence of Fr<10 kDa (L^*=71). The addition of Fr<10 kDa and Fr<5 kDa induced a significant (p<0.05) increase of hue (h_{ab}=15˚ and 3˚, respectively) compared to control (h_{ab}=-0.5˚). Regardless of the size, the addition of peptide fractions exhibited not significant (p>0.05) differences on chroma (C^*_{ab}). Furthermore, while control samples exhibited a gradual increase in colour differences (ΔE^*_{ab}) over time, the wine-like solutions treated with peptides showed a minor fluctuations of ΔE^*_{ab}, indicating a more stable colour. Peptide fractions also increased the antioxidant capacity regardless of the molecular weight. No significant (p>0.05) differences in turbidity were observed because of peptide fraction addition. Once demonstrating colour stability and antioxidant capacity, both peptide fractions appear suitable for addition to red wines from warm climates. However, the <10 kDa grape seed peptide fraction would be the most industrially feasible option; its ease of production facilitates the optimisation of both processing time and operational costs.

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PT5.11. Light-driven spatial regulation of anthocyanin accumulation in strawberry (*Fragaria* × *ananassa*) fruit tissues

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Anthocyanins are key determinants of strawberry fruit colour and nutritional value, yet their spatial distribution within the fruit and its underlying regulation remain insufficiently understood. Previous studies have indicated a heterogeneous distribution of phenolic compounds within strawberry fruit tissues [1]. The objective of this study was to investigate tissue-specific accumulation of anthocyanins in relation to light exposure in strawberry (*Fragaria* × *ananassa*) fruit.

Fruits of selected cultivars were separated into outer (epidermal and subepidermal tissues) and inner tissues and analysed using LC-MS to quantify individual anthocyanins, including pelargonidin and cyanidin derivatives. The results revealed pronounced spatial heterogeneity in anthocyanin accumulation, with concentrations in outer tissues being 2.1–4.8-fold higher compared to inner tissues ($p<0.001$). This pattern was consistent across cultivars, although the magnitude of differences was genotype-dependent, with the highest ratios observed in ‘Frederica’ and the lowest in ‘Clery’.

Outer tissues, directly exposed to light, showed enhanced accumulation of anthocyanins, with pelargonidin derivatives dominating the profile, while cyanidin derivatives were predominantly localized in epidermal layers. In contrast, inner tissues exhibited substantially lower anthocyanin levels and a distinct metabolic profile.

These findings indicate that light exposure is a major driver of localized anthocyanin biosynthesis in strawberry fruit. The observed spatial gradients highlight the importance of tissue-specific metabolic activity and surface-to-volume ratio in determining overall anthocyanin content. This has important implications for fruit quality evaluation and postharvest research, where homogenized samples may obscure biologically relevant differences. Our results demonstrate that anthocyanin biosynthesis in strawberry fruit is spatially regulated by light at the tissue level and should be considered in future studies of fruit metabolism and quality.

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PT5.12. Comparison of kinetic, thermodynamic, and color properties of blueberry, blackberry and red grape colorants

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Anthocyanin colorants are plant-based additives whose color stability and shades result from strong pH dependent equilibria among their structural forms [1]. Studying the influence of the pigment matrix on the structural evolution of anthocyanins provides information to understand industrial applications in which they can be efficiently applied depending on source. This study compares the hydration process and color expression of anthocyanin extracts from blueberry (*V. corymbosum*, cv. Cupla), blackberry (*R. fruticosus*, cv. Loch Ness), and red grape (*V. vinifera*, cvs. Tempranillo and Syrah). Anthocyanin stock solutions were diluted to 40 µM in 0.1 M citrate buffers and hydration kinetics were monitored at 517 nm to determine the rate constants from pH 2 to 5 at 25 °C [2]. After 24 h equilibration, absorbance spectra were recorded to estimate the pK'_a and pK'_h constants and the CIELAB parameters. Anthocyanin composition was assessed by HPLC-DAD-MS. The rate of hydration differed among the colorant extracts. It was slowest for the blueberry extract (k_h = 4.5×10^{-2} s^{-1}) and fastest for the blackberry extract (7.6×10^{-2} s^{-1}), while two grape extracts showed intermediate values (5.7 to 6.1×10^{-2} s^{-1}). The thermodynamic descriptors were similar across pigment matrices (pK'_a = 3.13-3.22; pK'_h = 3.16-3.26). Color loss (%) revealed differentiation between extracts as pH increased. From pH 3.6 to 4.6, blueberry extracts showed lower losses (42.9 and 71.2 %) than Tempranillo grape or blackberry (52.6 and 83.1 %). This variability may reflect source-dependent anthocyanin profiles: blueberry is richer in delphinidin, petunidin and malvidin glycosides, blackberry in cyanidin glycosides, and red grape in malvidin derivatives. The greatest color differences were found between blueberry and blackberry extracts at pH 3 and 3.6 (ΔE^*_{ab} = 10.7 and 7.4), while differences decreased at pH 4.6. Overall, source-dependent behavior supports blueberry for moderately acidic applications, blackberry for high-chroma products, and red grape for intermediate stability requirements.

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PT5.13. Color improvement of spray dried powders from copigmented fruit extracts for beverage applications

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Recently, novel microstructured anthocyanin colorants have been developed by combining copigmentation and encapsulation, improving stability and expanding their application range [1-3]. In this approach, anthocyanin matrices are blended with phenolic copigments to form non-covalent complexes that enhance color intensity and pH stability. Then, pigment-copigment complexes are encapsulated with maltodextrin to protect against environmental degradation. In this study, the phenolic enrichment and color-related functionality of blueberry (*V. corymbosum*, cv. Star), blackberry (*R. fruticosus*, cv. Loch Ness), and red grape (*V. vinifera*, cv. Tempranillo) anthocyanin extracts copigmented with ferulic acid (ratio 1:5) and spray-dried (2.5 % maltodextrin) were compared with anthocyanin matrices encapsulated without copigment. The coloring power of the powders was evaluated in an alcoholic model beverage (10 % sucrose, 1 % citric acid, 15 % ethanol, final pH=2.5). Phenolic and color stability were assessed for 30 days at room temperature. Encapsulating anthocyanin matrices copigmented with ferulic acid increased total phenolic content (TP) and antioxidant capacity (DPPH) compared with non-copigmented ones. RG and BK copigmented powders showed the highest TP content and antioxidant capacity, increasing by 4-fold and 1.5-fold over non-copigmented ones. BB copigmented powders showed the highest total monomeric anthocyanin content (TMA) and encapsulation efficiency (2.1 mg/g and 87 % vs. 82 % and 79 % for BK and RG, respectively). In all cases, ferulic acid reduced encapsulation efficiency, likely due to matrix effects. After reconstitution, copigmented powders showed higher chroma than non-copigmented ones, intensifying darker-bluish hues. During storage, beverages colored with copigmented powders showed greater TMA losses than those colored with non-copigmented powders (BB: 16.6 vs. 7.8 %; BK: 22.3 vs. 14.8 %; RG: 20.6 vs. 8.9 %), but also higher TP contents and polymeric color; chroma was matrix-dependent, being higher for copigmented powders only in BK beverages (38.64 vs. 33.85), whereas BB and RG showed similar values.

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PT5.14. Synergistic Effects of Whey and Plant Proteins on the Stability and Bioaccessibility of Encapsulated Jaboticaba Anthocyanins

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Jabuticaba (*Plinia cauliflora*) peel is a rich source of anthocyanins and phenolic compounds with recognized antioxidant properties. However, its industrial application is limited due to the low stability of these bioactives under processing and gastrointestinal conditions. In this context, this study aimed to evaluate the effectiveness of hybrid protein systems in the microencapsulation of jabuticaba peel extract by spray drying, focusing on anthocyanin stability, encapsulation efficiency, and in vitro bioaccessibility. The extract was obtained by ultrasound-assisted extraction using acidified ethanol and subsequently encapsulated using three formulations: whey protein isolate (WPI), WPI combined with pea protein (PP), and WPI combined with soy protein (SP). The microparticles were characterized in terms of total anthocyanin content (TAC), total phenolic content (TPC), antioxidant activity (ABTS and FRAP), encapsulation efficiency, color parameters, particle size, zeta potential, and morphology. Stability under light exposure and simulated gastrointestinal digestion were also evaluated. Results demonstrated that the incorporation of plant proteins improved the functional properties of the microparticles. The WPI+PP formulation showed the highest encapsulation efficiency (64.75%) and superior retention of anthocyanins and phenolic compounds. Although FRAP values did not differ significantly among treatments, ABTS results indicated variations depending on the protein matrix. Structural analyses confirmed the formation of stable protein–phenolic interactions, contributing to enhanced protection of the bioactive compounds. During storage, all encapsulated systems exhibited greater anthocyanin stability compared to non-encapsulated extract, particularly under dark conditions. Furthermore, microencapsulation significantly improved anthocyanin bioaccessibility, with WPI+PP showing the best performance. Overall, the results highlight the potential of hybrid protein systems, especially whey and pea protein combinations, as effective encapsulating agents. This approach not only enhances the stability and bioaccessibility of anthocyanins but also promotes the valorization of agro-industrial by-products, contributing to the development of functional ingredients with added value.

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PT5.15. Tracing Wine Identity through Anthocyanin Signatures: From Grapes to Authenticity

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Authenticating wine requires markers that reflect both biological and technological influences. Among them, anthocyanins exhibit remarkable structural diversity shaped by grape variety, terroir, and winemaking practices. Their quantitative and qualitative patterns provide valuable chemical fingerprints capable of linking grape identity, environmental adaptation, and oenological history, offering an objective basis for varietal authentication and quality control.
This study characterized the anthocyanin composition of monovarietal red wines from the Canary Islands and evaluated the potential of colored phenolics as molecular fingerprints for wine authentication, varietal discrimination, and aging assessment. A total of 205 red wines from ten grape cultivars and six islands were analyzed using high-performance liquid chromatography with diode array detectio. Eleven individual anthocyanins were quantified, and statistical analyses including correlation analysis, Principal Component Analysis (PCA), and Linear Discriminant Analysis (LDA) were applied to explore interrelationships among pigments and classify wines according to cultivar, Denomination of Origin, geographical origin, and aging stage.
Malvidin-3-glucoside (oenin) was the predominant anthocyanin in all samples, followed by peonidin- and petunidin-glucosides. Wines from international cultivars such as Syrah, Merlot, and Rubí Cabernet showed higher anthocyanin concentrations than autochthonous varieties like Listán Negro and Negramoll. Aging exerted the strongest influence: glucoside anthocyanins decreased while coumaroylated derivatives increased, reflecting polymerization and pigment stabilization. Strong positive correlations were found among non-acylated anthocyanins ($r > 0.88$), while coumaroyl derivatives correlated inversely ($r = -0.924$), suggesting divergent transformation pathways. PCA explained 78% of total variance, and LDA achieved correct classifications of 76% by cultivar and 86% by aging group, demonstrating robust discriminant capacity.
Anthocyanin profiles thus constitute reliable molecular fingerprints linking grape variety, origin, and wine aging. This chemometric approach provides a rapid, cost-effective, and scientifically grounded method for authenticity verification and quality control, offering a transferable framework for tracing the identity of other phenolic-rich plant-derived products.

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PT5.16. Spectrally selected white grape pomace cell walls used as fining agents: impact on anthocyanins and colour

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White grape pomace is one of the most abundant by-products of the wine industry and represents a promising resource for sustainable oenological applications. This study presents a detailed molecular and macromolecular characterization of cell wall material (CWM) isolated from fifteen spectroscopically selected pomace samples of Moscatel, Zalema, and Pedro Ximénez grapes [1]. The fining capacity of each CWM was evaluated in Syrah wine, revealing a strong ability to reduce anthocyanin content, with decreases of approximately 50% in most cases.

Statistical analysis using ANOVA and Tukey's test identified two distinct groups: samples producing anthocyanin reductions greater than 50% and others showing reductions below 40%. Although all CWMs significantly removed anthocyanins, CWMs from white grape pomace caused reductions approximately 20–40% higher than those previously reported for red grape skins [2]. Given the close relationship between anthocyanins and red wine colour, this effect must be carefully considered when selecting fining agents.

Chromatic properties were evaluated using CIELAB parameters. Compared to the untreated control, fined wines showed slight decreases in lightness (L^*) and increases in chroma (C^*_{ab}) and hue angle (h_{ab}), indicating darker wines with more intense and less bluish colours. Colour differences (ΔE^*_{ab}) ranged from 3.47 to 13.40, and ANOVA and Tukey's test revealed two statistically distinct groups consistent with anthocyanin reduction patterns. These findings highlight the key role of anthocyanins in wine colour and the importance of selecting fining agents that align with the desired sensory profile.

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PT5.17. Comparative Study of Maqui Berry Anthocyanin Dynamics During Fermentation with Water-Kefir and Kombucha Microbial Consortia

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Nowadays, fermented beverages have gained great importance due to their nutritional characteristics and health benefits, largely attributed to their probiotic nature. Within these beverages Kombucha and Water-Kefir are two of the most popular fermented beverages consumed, yet they differ fundamentally in their microbial consortia. Kombucha is fermented by a SCOBY dominated by acetic acid bacteria and yeasts, whereas Water-Kefir uses gelatinous grains primarily composed of lactic acid bacteria and fermentative yeasts. Despite their popularity, how these distinct microbial environments affect the stability of specific bioactive compounds, such as anthocyanins, remains poorly understood. Following our research line on alternative matrices for functional enrichment, this study aimed to compare both fermentation processes using maqui berry (*Aristotelia chilensis* (Mol.) Stuntz) as a potent source of anthocyanins. Maqui-based Kombucha and Water-Kefir were prepared at the same concentration to evaluate anthocyanin dynamics at the time of addition, after fermentation, and during 21 days of shelf life. Results revealed the complete profile of the eight characteristic anthocyanins of maqui, based on delphinidin and cyanidin derivates. At the initial time, anthocyanin concentrations were 18.4 mg/100mL for kombucha and 17.5 mg/100mL for kefir, with no significant differences between them. Following fermentation, this concentration was reduced by 15.8% and 5.7%, respectively. It is hypothesized that this decrease could be due to microbial biotransformation into other metabolites. Furthermore, these concentrations were maintained during the 21-day shelf life, demonstrating high anthocyanin stability throughout the storage period. In conclusion, no significant differences were found between the analyzed beverages, making both excellent candidates as healthy drinks due to their high anthocyanin concentration. These findings enhance the functional value of the formulations and suggest positive biological effects, such as analgesic capacity, previously demonstrated by our group.

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PT5.18. Gelatin-based intelligent films incorporating anthocyanin-loaded double emulsions for food freshness monitoring

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Food packaging is crucial in safeguarding food from environmental exposure and contamination, ensuring product longevity and reducing food waste. Yet the dominance of non-biodegradable plastics contributes to long-lasting environmental pollution, highlighting the need for sustainable packaging solutions that minimize waste and enhance food quality assessment [1]. In this study, gelatin films were produced using black carrot anthocyanins, comparing free incorporation (GAE) with an encapsulated double (W/O/W) emulsion system (GDE) designed to protect the pigment and improve its stability and performance as a freshness indicator. The double emulsion was prepared using corn oil, PGPR, gum Arabic, and Tween 80, and then integrated into a gelatin-glycerol film-forming solution. The films were characterized by color, transparency, thickness, mechanical behavior, antioxidant capacity, water vapor permeability, water contact angle, and pH responsiveness. Application tests were performed using yogurt and fresh chicken breast. Encapsulation improved anthocyanin retention and film performance. Compared with the film containing free extract, the emulsion-loaded film exhibited higher opacity, increased thickness, and superior antioxidant activity (48.1 % DPPH inhibition). FTIR analysis confirmed interactions between anthocyanins and the gelatin matrix. Both anthocyanin-containing films displayed clear chromatic transitions from red (pH 1−3) to purple (pH 4−6), blue (pH 7−8), and brownish tones (pH 9−10). However, the free-extract film showed visible pigment diffusion, whereas the emulsion-based film maintained color homogeneity and reduced migration. In yogurt, color variation remained low for the encapsulated system ($\Delta E < 5$), whereas the free extract caused noticeable changes ($\Delta E \approx 7$). In the chicken, pH increased from 5.82 to 6.8, with color shifts from purple to bluish and greyish tones. These were more pronounced in the GAE film, while the GDE film showed greater stability and reduced pigment release. Overall, double emulsions enhanced anthocyanin stability and improved the performance of gelatin-based intelligent films as visual freshness indicators for perishable foods.

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PT 5.19. Impact of blending minority grape varieties from Castilla y León with Tempranillo on the anthocyanin profile and color of wines

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Climate change is altering grape composition, creating a gap between technological and phenolic maturity that compromises red wine quality. Rising temperatures and water stress accelerate sugar accumulation and lower acidity, while phenolic ripeness lags behind, affecting color, astringency and bitterness [1]. Tinta de Toro (*Vitis vinifera* L. cv. Tempranillo), widely grown in Castilla y León, is highly sensitive to climate, making it an ideal model for studying adaptation strategies [2]. Phenolic compounds play a key role in the organoleptic properties and ageing potential of wines. Among them, anthocyanins determine color, whilst flavanols and flavonols influence astringency and bitterness, as well as contributing to the stability of the wine's color over time. The use of autochthonous minority varieties of grapes is a possible strategy for the wine sector to adapt to climate change. These varieties, traditionally sidelined due to production criteria, are being reconsidered for their potential to adapt to changing climatic conditions, as well as for their unique phenolic profiles (2). Some have longer ripening cycles, greater stability against heat stress or distinct phenolic compositions, which could help to improve the quality of wines when used in combination with Tinta de Toro [3].

This study aims to determine the changes in Tinta de Toro wines due to the blending with three different minority varieties of grapes (Gajo Arroba, Bruñal and Mandón) cultivated in the same region (Toro). The wine blends were prepared at three different proportions (5 %, 10 % and 15 %) to comply with the requirements of the Regulatory Council of the Protected Designation of Origin. The resulting wines were analyzed by HPLC-DAD-MS to determine the anthocyanin composition, as well as the other phenolic families that could impact on wine color. Moreover, color parameters (CIELAB) and copigmentation were spectrophotometrically measured and the basic oenological parameters (acidity, pH and alcohol) were also assessed.

The results point out to a different impact of the assayed varieties on both the pH and the phenolic composition, which clearly impacts on wine color. These effects also depended on the percentage at which these varieties were blended. Overall, this work will provide scientific evidence on the potential of minority varieties to mitigate the effects of climate change on wine composition and quality, while contributing to the recovery of local biodiversity.

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PT5.20. Impact of yeast mannoproteins on the formation and stability of pyranoanthocyanins under simulated ageing conditions

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Mannoproteins (MPs) are glycoproteins from the yeast cell wall that are authorized as enological additives to improve some sensorial and technological aspects of wine. Among these beneficial effects, some studies have reported that MPs could enhance color stability, above all from a colloidal point of view [1]. In addition, recent studies point to the possible influence of MPs on the formation and stability of some anthocyanin-derived pigments, such as pyranoanthocyanins (PAs) [2]. Formation of PAs in wine is of enological relevance, as these pigments have distinct color characteristics and greater chemical stability than monomeric anthocyanins. PAs formation can be influenced by several factors, like the presence of oak-derived compounds [3], which, in turn, might be affected by the presence of MPs. In addition, the effects of MPs in wine seem to be strongly conditioned by their structure and composition.

The objective of this study was to evaluate the influence of different MP extracts on the formation and stability of A- and B-type vitisins from the main wine anthocyanin monoglucosides in a model wine system containing an oak wood extract. These conditions aim to simulate the oxidative environment that occur in the barrel but avoiding the risk of adsorption of pigments onto the surface of the wood. One of the MP extracts was obtained from wine lees following autoclave treatment and three, from *Saccharomyces cerevisiae*, *Pichia kluyveri* and *Torulaspora delbrueckii* cultures by enzymatic extraction (Zymolyase). MP characterization by Lowry method, HRSEC-RID and HPLC-DAD-MS revealed important differences among them. The evolution over time of color (CIELAB) and pigment composition (HPLC-DAD-MS) of the model systems was influenced by MP addition. In general, greater levels of monoglucosides and lower of PAs were observed in the MP-containing model systems, which also presented a less evolved color (lower h_{ab}* and greater C_{ab}*).

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PT5.21. Obtaining soluble polysaccharides from grape pomace cell wall material and their role in anthocyanin and color stability

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Color is a key attribute of red wine and that is closely related to its quality and consumer acceptance. Climate change is significantly affecting grape composition and wine quality, leading to unbalanced musts and wines characterized, among other effects, by color instability, due to a decrease in anthocyanin content. One of the possible strategies to solve this problem is the addition of polysaccharides obtained from winemaking by-products, which, in turn, could reduce the environmental impact of wine production [1].

The aim of this study was: i) to obtain and characterize the soluble polysaccharide fraction (SPF) from the insoluble cell wall material of white grape pomace, and ii) to evaluate the effect of these polysaccharides on the stability of anthocyanins.
Insoluble polysaccharides from white grape pomace from *Vitis vinifera* L. cv. Verdejo were obtained from the residue remaining after extraction of cell wall material with boiling distilled water, followed by precipitation of polysaccharides with 30% ethanol [2]. The insoluble polysaccharides fraction was subjected to acid hydrolysis (HCl) at different pH values (pH 1-3) at 100ºC during 1, 2, 3 and 4 hours, in order to promote the deconstruction of grape pomace cell wall material and facilitate the partial solubilization of the polysaccharides that comprise its structure. The SPF obtained were submitted to a dialysis process for purification and then were analyzed by HRSEC-RID in order to characterize their molecular weight. Anthocyanin 3-*O*-glucosides were obtained by purification of a grape skin extract (*Vitis vinifera* L. cv. Tempranillo). The obtained SPF was added to a model solution containing the main anthocyanin 3-*O*-glucoside in presence and absence of flavonols, in order to evaluate the role of SPF on the anthocyanin and color stability, which was evaluated using HPLC-DAD-MS and colorimetry analysis. Acid hydrolysis at pH 1 for 3 hour promoted cell wall transformation, resulting in the release of SPF with an approximate molecular weight of 400 kDa. Moreover, the results showed that the addition of this SPF contributed to a stabilization of anthocyanins and may enhanced copigmentation of the anthocyanins, suggesting a potential positive impact on wine color.

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PT5.22. Grape pomaces from Spanish minority *Vitis vinifera* L. varieties as valuables sources of anthocyanins and antioxidant compounds

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It has been estimated that 30% of the total quantity of vinified grapes corresponds to wine by-products [1]. Grape pomace, which is the main by-product, can represent up to 25% of the initial grape weight and can retain 60-70% of the phenolic compounds of grape [2] and among them, anthocyanins. The recovery of these bioactive pigments from pomace can be a strategy to reduce the environmental impact of wine production. In the current context of climate change, minority and autochthonous *Vitis vinifera* L. varieties have gained significant enological interest due to their resilience. Consequently, the characterization of their by-products is essential for achieving efficient valorization. The objective of this work was to characterize [3], by HPLC-DAD-MS^n, the detailed anthocyanin composition of grape pomaces from three Spanish minority varieties (Piñonera, Estaladiña, and Mandón). Furthermore, the chromatic potential (CIELAB parameters) and antioxidant capacity (FRAP and ABTS assays) were evaluated and correlated to the anthocyanin composition and to total phenolic composition (Folin–Ciocalteu and total polyphenol index (TPI)). Grape pomaces still contained important amounts of anthocyanins, with Piñonera showing the greatest amounts (6.88 mg/g of freeze-dried pomace). In relation to fresh grapes, all the pomaces were richer in *p*-coumaroyl derivatives and in malvidin-derivatives. Pomaces additionally showed anthocyanin-derived pigments (pyranoanthocyanins or flavanol-anthocyanin condensation products). Varietal differences were observed: Piñonera showed the most different anthocyanin profile, with *p*-coumaroyl derivatives accounting for nearly 45% of the total content and malvidin derivatives representing 90%. This variety also recorded the highest antioxidant activity, despite not having the highest total phenolic content, which points to its distinct anthocyanin composition.

In conclusion, grape pomaces from Spanish minority varieties emerge as valuable sources of natural colorants and antioxidant additives. However, considering varietal differences is crucial for optimizing their specific industrial applications and valorization strategies.

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PT5.23. Heat-Induced Beta-lactoglobulin Aggregates: Stabilizing Anthocyanins through Hydrophobic Interactions and Dynamic Structural Remodeling

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Anthocyanins are natural colorants with significant health benefits, yet their thermal instability limits industrial applications. Heat-induced protein aggregation can enhance the efficacy of proteins for stabilizing bioactive components. β-Lactoglobulin (BLG) is a promising carrier for stabilizing anthocyanins, yet the relationship between its heat-induced aggregate structures and their protective efficacy remains unexplored. This study aimed to systematically compare the binding affinity and protective effects of three structurally distinct BLG aggregates—fibrils (BLGF), nanoparticles (BLGNP), and worm-like chains (BLGWC)—on anthocyanins, and to elucidate the dynamic structural changes occurring in BLGF-anthocyanin complexes during extended heating. Aggregates were prepared under controlled pH and heating conditions. Surface hydrophobicity, secondary structure, and binding forces were characterized using fluorescence spectroscopy, circular dichroism (CD), and chemical bond dissociation assays. Thermal stability of anthocyanins was evaluated at 90 °C, and morphological and structural transitions were monitored via electron microscopy, X-ray diffraction, and Thioflavin T binding assays. Results showed that BLGF exhibited the highest surface hydrophobicity and demonstrated the strongest protective effect, extending anthocyanin half-life from 28.5 min (native BLG) to 57.2 min (BLGF) at 90 °C, representing a two-fold improvement. However, after 80 min of heating, the protective effect diminished, with the degradation rate constant increasing from 0.0216 min^{-1} to 0.0374 min^{-1} by 120 min. Mechanistic investigation revealed that anthocyanins bind competitively to hydrophobic grooves of BLGF. During prolonged heating, hydrogen bonds within cross-β-sheets weakened, parallel β-sheets transitioned to antiparallel conformations, and fibrils fragmented into amorphous aggregates, correlating with accelerated anthocyanin degradation. These findings demonstrate that while BLGF offers superior initial protection via hydrophobic interactions, anthocyanin-mediated structural remodeling under prolonged heating compromises stability, providing critical insights for designing thermally stable protein fibril carriers in food applications.

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