Helsinki, Finland

4.–7.6.2024

BIOCOLOURS 2024

sustainable
value chain
for colour

UNIVERSITY OF HELSINKI

Biocolours2024

Sustainable Value Chain for Colour

Book of Abstracts

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Edited by Laura Dyster and Riikka Räisänen

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Foreword by Professor Riikka Räisänen

The Biocolours2024 conference was held from June 4-7, 2024, at the University of Helsinki, Finland. The conference theme was "Sustainable Value Chain for Colour," emphasizing the importance of every actor in the colourant production and application chain in transitioning towards biobased and more sustainable solutions. The event was held in person, providing time and space for networking and discussions among delegates from multidisciplinary backgrounds, including researchers, industry professionals, entrepreneurs, and designers. The event gathered a total of 155 delegates from 23 countries.

Biocolours2024 in Helsinki was the second Biocolours conference, the first being held in 2018 at Avans University of Applied Sciences in Breda, the Netherlands, with the next conference planned for Vienna, Austria, in 2028.

The conference featured 121 presentations, creating a dynamic program that covered a wide range of topics, from biocolour sources and processing methods to applications, colourant safety research, and biocolourant business cases. This Book of Abstracts provides an introduction to these presentations. The structure of this compilation follows the conference program according to the sessions and their topics:

- Agriculture, Sidestreams and Multifunctionality of Natural Compounds
- Biocolour Authenticity, Palettes and Potential
- Insight into the Chemical Structures of Natural Colourants
- Exploring Natural Colourants for Safer Dyes and Dyeing
- Biocolourant Production by Microbial Biotechnology
- Textile Colouration and Biodye Aesthetics
- Sustainable Solutions for Textile Dye Extraction and Application
- Designing with Biobased Colours
- Controlling Colour and Stability of Biocolourants
- Is Natural Better?
- Variety of Materials in Applications of Biocolourants
- Biocolour Spectrum: Exploring Cultural Perspectives of Dyes

The diverse talks demonstrated that natural and bio-based colourants are already being produced and used on an industrial scale, particularly in the food and cosmetics industries, with the textile field expected to follow. The conference highlighted the importance of collaboration among different research fields and value chain actors in designing and producing safe and sustainable colourants to meet the needs of a wide variety of product applications.

Why the Biocolours conference?

The Biocolours2024 conference was organized by the BioColour research consortium (https://biocolour.fi), which includes more than thirty researchers from eight research institutes in Finland, the USA, and Brazil. The BioColour research project aims to develop new methods for large-scale production, characterization, and application of biocolourants, while also studying consumer acceptance of new dyes and colours—essential when building sustainable products for consumer markets.

At BioColour, we believe that impact is vital, with both scientific and societal implications being equally important. Why? Firstly, colour is all around us, but we often don’t realize that it is a material substance, and there are sustainability issues related to colourant production and application processes. Furthermore, colour plays a key role in marketing, with trends and fashion driving consumer behavior and often leading to the disposal of items simply because their colour is old-fashioned and *passé*. In aiming for societal impact, the organizers seek to promote sustainability, diversity, and ethical practices in the societal and cultural environment, for example through education. One goal is to foster ethical discussions about colour, dye production, and the environmental impact of these processes. Through research, it is possible to develop new methods, technologies, and knowledge to support the wider use of biocolourants.

Building and supporting networks is of paramount importance. At the Biocolours2024 conference, the focus was on in-person participation, personal experiences, and active engagement. It seemed that delegates found fruitful discussions with each other during breaks and evening activities.

We would like to thank all participants for their contribution to the successful Biocolours2024 conference!

Keynote 1

Professor Harold S. Freeman, North Carolina State University

Color yes, cancer no – approaches to environmentally-friendly textile tdes

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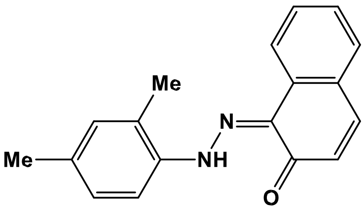
Abstract

This paper provides an overview of approaches to the selection of environmentally friendly textile dyes that are both safe to use and efficatious under end-use conditions. While the coloration of substrates such as textiles began with the use of natural colorants, the year 1856 marked the beginning of textile coloration using synthetic dyes. Over the century that followed, azo dyes (e.g. **1**) became the principal family of colorants used in this arena. However, once it was found that any of a group of 20 aromatic amines were cancer suspect agents, azo dyes derived from those precursors were banned from commerce. This discovery also led to studies aimed at establishing the relationships between azo dye structures and the observed genotoxicity. The goal was to generate information that could be used to design safe alternatives to azo dyes designated as cancer suspect agents.

Recognition of the potential for certain azo dyes to cause a risk to human health and the environment, the search for new textile dyes has come full circle – to the consideration of natural dyes in this arena. Their use in food, drug, and cosmetic products has led to the general belief that natural dyes merit wide investigation as synthetic dye alternatives for textiles. Unlike synthetic dyes, natural dyes lack the azo chromophore that has become the point of concern among synthetic dyes. Those currently under investigation as textile colorants often have anthraquinone (e.g. **2**) and flavonoid (e.g. **3**) structures, but little is reported about their genotoxicity. In this presentation, the story begins with the chemistry of synthetic dyes and the associated structure/property relationships regarding genotoxicity and moves to a summary of the corresponding information regarding anthraquinone and flavonoid natural dyes. It is anticipated that the results will facilitate the selection of preferred natural dyes for textile use.



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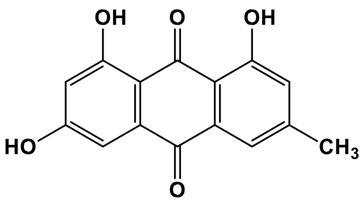

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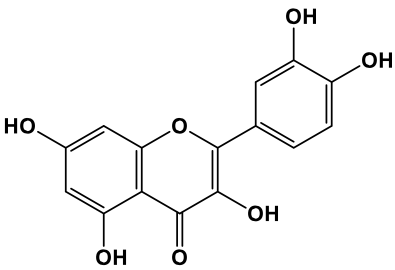

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Acknowledgments: BioColour project n◦ 327178, and 327213 and 352460

Keywords: Natural dyes, synthetic dyes, textiles, genotoxicity, azo dyes

This research was funded by the Strategic Research Council, which operates in connection with the Research Council of Finland, project No. 352468.

Agriculture, sidestreams and multifunctionality of natural compounds

<u>Session speaker:</u>

Professor Namrita Lall, University of Pretoria

Dyeing to find compounds with pharmacological activity

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Abstract

Plant pigments are one of the main groups of active principles, with the most crucial therapeutic role. Plant pigments have vital role in the maintenance of human health as well as in the environmental parameters. The major plant pigments are chlorophylls, carotenoids, flavonoids, anthocyanins and betalains. Each class of the pigments contain various chemical components with distinct subgroups. Plant pigments are acting as anti-cancer, anti-inflammatory, anti-diabetic, hepato-protective, anti-obesity, anti-allergic, cardiovascular protective, antimicrobial and antioxidant agent. Flavonoids are one of the largest subclasses of phenolic compounds, produced in different parts of the plant. They are playing a role as plant pigments, but also carry out many important functions in plants, such as pollination, regulation of cell growth, and combating environmental stresses like microbial infection. Plants also synthesized flavonoids in response to microbial infection. Thus, there is a growing interest in the antibacterial properties of flavonoids and their application in human therapy.

With about 25 000 known species, South Africa, is third only to Brazil and Indonesia as far as biodiversity is concerned. This constitutes about one tenth of all plant species in the world. Whether searching for ways to treat serious diseases like cancer and tuberculosis (TB), or formulating new acne creams and toothpastes that fight gum disease, one can be convinced that solutions can be found in South Africa’s indigenous plants. The vast traditional knowledge about SA’s plants is still untapped, and there is a huge amount of work needed to verify if and how traditional remedies and local plants actually work. The talk will focus on the application of plant pigments from South African plants with regard to their cosmetic and pharmaceutical applications.

Not just colour, from waste to beauty

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Abstract

Natural colorants are seeing a renewed interest from industry, and currently gaining traction across food and cosmetics to packaging.

In this context, the agri-food sector is also looking for solutions to reduce waste and adopt more sustainable practices. A considerable amount of fruit and vegetable peels, seeds, stones, grain husks, and shells are disposed either composted industrially, processed through anaerobic digestion, or, less ideally, landfilled or burnt. Valorising the highly valuable components of these residuals, specifically the non-edible by-products for diverse range of potential applications has been the focus of academics and industry these last decade. However, few examples are actually for production of colour ingredients.

This presentation will explore the natural colorants present in these residues and address the current challenges associated to their extraction and their stability. The study of the pigments at the molecular level enables to fine-tune their extraction and isolation, but also enhances understanding of their physicochemical properties for further formulation into products. This approach offers a holistic perspective on colour and performance, taking into account the natural environment of the pigment within the plant material and its potential interactions with various phytochemicals, which may synergistically enhance functionalities such as anti-oxidant, anti-microbial or anti-inflammatory and wound-healing properties associated with these colorants.

From these unique ingredients to products the journey takes us to the consumer. We are witnessing a shift of consumer perception and preferences with the rising awareness of the environmental impacts of petrochemical-based products. Looking for healthy lifestyle, the consumer is demanding multifunctional high-performance natural products without compromises.

This communication will outline how food waste can be utilised to create sustainable natural colorants, underscoring the challenges associated with seasonality, logistics, and processing complexities. ‘Turning waste into beauty’ encapsulates the shifts towards sustainability and the circular economy.

Keywords: Pigments, Polyphenols, Flavonoids, Carotenoids, Chlorophyll, Food waste.

On-farm methods for producing brown shades from plant species that enhance soil biodiversity

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Abstract

Crop diversification is needed to overcome the negative impacts of long-term monoculture practices on soil health and biodiversity. Also, the economic value should be shared much more equitable between the actors in the production chain.

Plant-based dyes are receiving increasing interest as the transition to a renewable economy progresses. The development of natural dye production can be an inspiring example of how to integrate raw material production into the value chain without repeating old, environmentally damaging mistakes. The production of dyes and other value compounds could be part of a decentralised biogas production on farms, also providing energy for extraction and dyeing processes.

As a part of BioColour project, legume-assisted cultivation system was developed with multipurpose plant species, in Natural Resources Institute Finland (Luke). Intercropped faba beans (Vicia faba, Fabaceae family) are used to improve the soil nitrogen status with their root-nodules symbiont, Rhizobacteria, and thus minimize the use of chemical fertilizers of dye plants. Faba beans also produce protein-rich food and feed, and about half of their above-ground biomass is left in the field after harvest as threshing waste. A perennial northern dock (Rumex longifolius) originates from Northern Europe, and its vigorous growth and significant aboveground and belowground biomass is largely underutilized e.g., for dye purposes (Pluenneke, 2017). It is potentially mycorrhizal fungi associated plant species which maintains this beneficial symbiont in the field soil.

Moreover, in Luke, modified extraction (Bouatay, 2019) and dyeing processes were developed for the studied plant species that contain tannins and phenolic compounds in their by-products. The results show that, crop diversity can generate new value chains. We present and discuss the results of these processes giving dark brown shades, and the methods applicability to farms or other small businesses.

References:

Bouatay F, Baak, N, Shahid A, Mhenni MF (2019). A novel natural source *Vicia faba* L. membranes as colourant: development and optimization of the extraction process using response surface methodology (RSM). *Natural Product Research* 33 (1): 59-65. https:/doi.org/10.1080/14786419.2018.1434632.

Pluenneke M (2017). Shift in Icelandic Plant Populations Due to Climate Change: Through the Lens of Natural Dyes. Independent Study Project (ISP) Collection. 2751. https://digitalcollections.sit.edu/isp_collection/2751.

Keywords: on-farm, *Vicia faba, Rumex sp.*, intercropping, extraction, dyeing, brown shades

The unique natural dye in the world certified with a protected designation of origin

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Abstract

Cochineal production was introduced to the Canary Islands in 1825. Though the climate and growing conditions were ideal, Canarian farmers were reluctant to grow this crop.

The Canary Islands then experienced a golden age and all social strata benefitted from the cochineal production. The working tools were normally rudimentary, Canarian women mainly took care of the gathering and cleaning of the product, while the men employed themselves in watching out for the plantations and the handcrafted techniques for drying the cochineal.

The quality of the cochineal produced was such that the Canaries soon achieved the leadership in exportations. Canarian cochineal was sent to the Spanish peninsula and abroad, mainly to England and France, through Marseille. In lesser amounts, some shipments were also sent to Morocco, Algeria, Holland, Gibraltar and the US.

With the coming of aniline dyes the price of Canarian cochineal decreased, the less-expensive aniline dyes displaced cochineal.

Canaturex Project:

Main Objetive : To Revive an historic European crop (Canary Islands Cochineal)

1. Stages of production:

a) Planting the cactus.
b) Cultivating the parasite.
c) Harvesting the parasite.
d) Drying the parasite.

2. Development of the EU Quality Standard for the cochineal.

a. What means a PDO?
b. Process to Obtain a PDO for agricultural product.

Main Results:

1. Increase Carminic Acid Concentration (quality)
2. Increase Local Rural Development (Environment)
3. Increase in demand by natural textile dyers.
4. Awards (2017, 2020, 2021)
5. Cooperation with The Royal Manufacture Tapestry of Spain.

Keywords: Cochineal, PDO,Organic EU label

Anthocyanin stabilization: experimental and theoretical strategies

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Abstract

Anthocyanins (ACN) are receiving intense attention for their potential health benefits. However, the high lability of anthocyanins limits their application in the food industry.

A novel model using the density functional theory (DFT) was established to screen small dietary compounds that could stabilize aqueous anthocyanins via secondary interactions (Lou & Jing, 2021). The change in binding free energy change (ΔG) was found to be an excellent indicator stabilization ability of dietary molecules on anthocyanins, which was demonstrated by comparing the calculated ΔG with the thermal half-time from the previous study (Qian, Wu, Lu, Xu, & Jing, 2017).

The hierarchical structures of protein nanofibrils, such as silk fibroin nanofibrils based on the heavy chain peptide (H-SNFs), and β-lactoglobulin fibrils (BLGF), one of β-lactoglobulin (BLG) thermal aggregates. The thermal degradation halftime (t1/2) of black rice anthocyanin (BAE) binding with H-SNFs increased of 4.04 folds (T=80°C). The halftime of the BLGF-BAE complex (T=90°C) is 1.70 times that of BLG-BAE. BLGF assembly is primarily driven by hydrogen bonding, hydrophobic interactions, and disulfide bonds.

In summary, ligands (small dietary molecules) could provide the rich π-electrons to ACN cations via secondary interactions to stabilize ACN. Macromolecules, i.e., protein in the form of fibrils, richer in surface hydrophobicity and stable parallel cross-β-sheet, showed more binding sites for anthocyanins, which also delaying fibril degradation and further improved the stability of ACN.

References:

1. Lou, YC; Jing, P*. 2021. DFT study of the stabilization effect on anthocyanins via secondary interactions. *Food Chemistry: Molecular Sciences*. 3:100057.
2. Qian, B.; Wu, C.; Lu, M.; Xu, W.; Jing, P.* 2017, Effect of complexes of cyanidin-3-diglucoside-5glucoside with rutin and metal ions on their antioxidant activities, *Food Chemistry*, 232: 545–551

Keywords: Screening; Nanofibrils; Stabilization; Silk fibroin; β-lactoglobulin

Biocolour authenticity, palettes and potential

Session speaker:

Professor Alain Trémeau, University Jean Monnet

Evaluation of colour differences and spectral reflectance differences

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Abstract

The authentication of a biocolourant can be investigated from its visual appearance, or just from its color appearance. But in some cases, it is not enough, hyperspectral analysis of pigments may be necessary. On the other hand, the richness of a color palette can be investigated from its color gamut.

In this presentation I will explain why it does not make sense to evaluate color differences in the CIELAB color space when color samples to compare are too different. I will also discuss how it is important to take care about lighting and viewing conditions, i.e. reference conditions, when evaluating color differences. Next, I will discuss how to evaluate color gamut (and color strength) and how to evaluate the influence of materiel properties (such as reflectance, transmittance, scattering properties) and environment on color gamut and color features.

In the second part of this talk, I will explain why in some study cases it is important to analyse and compare not only color data but also spectral data (not only in the visible domain but also in the infrared domain). I will discuss several spectral similarity measures. I will also discuss spectral bands selection vs spectral dimensions reduction, spectral mixing of pigments (e.g. for layered paints) vs individual pigments.

BCDB – BioColorant database

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Abstract

Use of biobased colorants suffers from lack of certification bodies, standards, and reference data. In BioColour-project, an open database and tools for characterization, authentication, quality analysis of biocolorant sources, biocolorants, and colored products is being developed. The database is multidisciplinary and currently combines taxonomy, botany, and chemistry to form chemo-taxonomical authenticity fingerprints of biocolourant compounds. The database collates numerical structured data from targeted analyses, such as HPLC-DAD-MS/MS from published peer-reviewed articles and books. The target audience, in addition to researchers, and expected use-cases of the database include biocolorant refining, companies using biocolorants, dye conservators, and archeologists. For craft enthusiasts and hobbyists, the database also records material and color information, both CIELAB-color coordinates as well as spectral reflectance, of textile samples dyed with various biocolourants.

The database is implemented as an online service with Django web framework and PostgreSQL relational database management system. This presentation shows the main features of the current prototype database system from users' perspective. The database will be published at the end of 2024.

References:

Primetta, A., Räisänen, R. 2023. New biocolourant database for the quality control of natural colourants and products containing them. Acta Horticulturae, 1361, 189-194.

https://doi.org/10.17660/ActaHortic.2023.1361.22

Keywords: biocolorant, database, chemo-taxonomy, fingerprint

Functionality and colours using lignin nanoparticles

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Abstract

There is a need to develop sustainable methods for both colouring and functionalization of textiles and other substrates. Lignin is a polyphenolic compound abundantly available as a side stream from the pulping industry. Lignin is in general coupled with varying shades of brown but herein we demonstrate ways of achieving coatings varying from transparent and totally colourless to bright purple or blue using lignin nanoparticles (LNPs) (Henn 2023). We also demonstrate multifunctional LNP-based coatings suitable for active wear textiles that combines water and stain repellence, breathability, UV blocking and antibacterial properties. The different functionalities and colours are achieved by control of the size and chemistry of the nanoparticles, as well as the layer thickness of the coating. The multifunctional textiles coatings were achieved by coating cellulosic fabrics with esterified LNPs. Since the LNPs were negatively charged, a layerby-layer approach using poly-L-lysine (PLL) as polycation and anchoring polymer was employed. Two bilayers were sufficient to achieve the functionalities, resulting in very thin and breathable coatings. These coatings were light yellow in colour.

Acetylation of the lignin prior to nanoparticle formation enabled formation of very small LNPs, below 50 nm. One monolayer of full acetylated particles produced an invisible superhydrophilic coating that could reduce fogging of glass. By applying several layers of partially acetylated particles using layer-by-layer approach and PLL, bright structural colours were achieved on solid surface. The colour depended on the thickness of the layers and the angle of observation, and hence could be controlled by controlling the number of layers applied. Bright red, purple, blue and yellow colours were demonstrated. The results demonstrate that both the colour and the functionality of lignin can be easily tuned and lignin could find new value in functional coatings.

References:

1. Henn, KA, Babaeipour, S., Forssell, S.,Nousiainen, P, Meinander, K., Oinas, P., Österberg, M. (2023)Transparent lignin nanoparticles for superhydrophilic antifogging coatings and photonic films, *Chemical Engineering Journal* **475**, 145965 https://doi.org/10.1016/j.cej.2023.145965.

Keywords: lignin, nanoparticles, hydrophobic, structural colours, functional coatings

The mushroom color atlas: dyes and pigments made from fungi

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Abstract

Humans love color. For millennia, we have been extracting a rainbow of colors from rocks, minerals, plants, insects, lichens, and mushrooms. The pigments and dyes created from these natural materials have been used to color everything from cave paintings to body art to clothing and adornments.

My project, the Mushroom Color Atlas (mushroomcoloratlas.com), is a living study of colors derived from the pigments of fungi. The colors presented in it are only a small slice of the countless hues that can be produced in collaboration with mushrooms. In an era when we are increasingly reliant on technology and corporate consumer goods, creating colorants from mushrooms feels like a radical act. Working hands-on with the fruits of the natural world is an opportunity to reorient ourselves toward biodegradable, renewable, ecologically sensitive sources and sustainable practices.

This presentation will focus on the dyes and pigments created from mushrooms and the quantity and quality of the colors produced. These hues appear to share a visual lineage as they transition smoothly, one to the next, in a sort of familial harmony. Each swatch in the Mushroom Color Atlas was assigned a numeric value in a 360-degree spectrum, creating a chromatic rainbow. Focusing on each color family—red, orange, yellow, green, blue, and purple—will provide the foundation for the presentation to evaluate the pigments found within mushrooms.

The audience will learn that while not all mushrooms produce colorful pigments, the ones that do can create a wide spectrum of hues. The subgenus of mushrooms *Cortinarius dermocybe* contains anthraquinones that produce shades of red. Some mushrooms host emodin, a widespread anthraquinone that creates shades of orange. White rot mushrooms contain yellow pigments. Tooth fungi, specifically the *Hydnellum* and *Sarcodon* genuses, are full of terphenylquinones (pigments found only in mushrooms!) that produce green, blue, and purple hues.

Keywords: mushroompigments, mushroomdyes, fungicolors, fungidyes, fungipigments

What colour can do? Functional potential of biocolourants

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Abstract

The recent interest in biobased colourants seems to be driven by two types of needs, both resonating with the UN Sustainability Development Goals (SDGs): First, the need for *less harmful* processes and applications, connected with SDGs advocating climate action, clean water, and responsible production. Together, these translate into demand for safer, non-toxic, biodegradable colourants produced from renewable sources by sustainable processes, possibly rising the production costs. Second, there is a need for *more beneficial* applications, encouraged by SDGs of good health and well-being, affordable and clean energy, economic growth, and innovations. They accumulate interest for functional applications with added-value, such as food colourants with health benefits, textile dyes with antibacterial properties, or biocolourants providing cosmetics with UV-protection. This attention is further motivated by a need to discover solutions to many of the complex problems currently facing the humankind, such as an augmented prevalence of chronic diseases and a severe need for renewable energy. We believe that gearing increased attention towards such functional benefits would propel the necessary investments for the Green Transition, too.

However, the published research reviews typically assume a perspective of a single industry, such as textiles, dye-sensitized solar cells (DSSCs), food, or pharmacology; or an outlook based on a particular chemical group (e.g., anthocyanins, proanthocyanins, or anthraquinones); a specific plant source (e.g. *Salix*, or *Camellia sinensis*), or a particular specialized metabolite (e.g., curcumin). There is a lack of systematic specification of relevant bioactivities of the colourants, and consequent functionalities in applications across disciplines and industries. To bridge this gap, we employ a transdisciplinary reading of previous review papers and recent original research for a meta-analysis of the functional benefits of biocolourants identified thus far. Furthermore, this analysis offers a scientific foundation for recognising new opportunities for biocolours in material innovations.

Keywords: Applications, biocolourants, material engineering, specialized metabolites.

Insight into the chemical structures of natural colourants

Session speaker:

Associate Professor Nelson Vinueza, North Carolina State University

Rapid analysis in situ of disperse dyes on polyester fibers and other dyes derivatives in soil: a mass spectrometry approach

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Abstract

Dyes are found in various applications nowadays, including but not limited to textiles, foods, and medicine. The analysis of these molecules is pertinent to several fields, such as forensics, environmental monitoring, and quality control, all of which require the sensitivity and selectivity of analysis provided by mass spectrometry. This work has characterized a collection of 31 commercially available textile dyes by developing a method to analyze dyed fibers in situ via Direct Analysis in Real-Time (DART) mass spectrometry. This methodology does not need sample preparation or extraction of disperse dyes from the fabrics for their fast characterization. Further, enhancing our dye characterization capabilities, we developed methods to understand better the degradation of reactive dyes in simulated landfills, which can be a new source of pollution that can potentially leach into the soil. A set of knitted cotton fabrics dyed with four common reactive dyes used in the textile industry, C.I. Reactive Orange 35, C.I. Reactive Blue 49, C.I. Reactive Black 5, and C.I. Reactive Blue 19, were studied by the ASTM D5988-03 method to determine potential degradation products in landfills. A modified QuEChERS method was used to extract all reactive dyes and derivatives from soil, and all extracts were characterized by high-resolution mass spectrometry by using a Quadrupole Time-of-Flight mass spectrometer for analytes with a degradation period of 45 and 90 days.

Structural elucidation of natural anthraquinones from cortnarius mushrooms using MS-MS fragmentation patterns and 2D-NMR spectroscopy

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Abstract

Natural anthraquinones are an established source of antioxidants due to phenolic functionalities in their molecular structures. The current study characterizes the chemical profiling of the mushroom species (from the Cortinariaceae family) collected from the wild inlands of Finland, leading to the identification of twentytwo natural anthraquinones. The preliminary extraction was facilitated in aqueous phosphate buffer of pH 5.0 (for acidic and water-soluble anthraquinones), which was subjected to retain in-situ beta-glycosidase enzymatic hydrolysis. Subsequently, the filtered and centrifuged solid residue retained from the aqueous buffer extract was extracted with acetone. We used tandem C_{18}-reverse phase high-performance liquid chromatography with mass spectrometry to identify the anthraquinones from these extracts. In our observation, the acetone extract contained non-carboxylic acid-containing anthraquinones, unlike the aqueous buffer extract, which has a prominent presence of COOH-anthraquinones. Using MS^1 -MS^2 profiles, twelve carboxylic-containing anthraquinones were identified in the aqueous buffer solution, while nine noncarboxylic-containing anthraquinones were identified from acetone extract. The abundant percentage of carboxylic anthraquinones that were confirmed using MS^1 -MS^2 fragmentation pattern profiles along with NMR spectroscopy were dermolutein, dermorubin, chlorodermorubin, endocrocin, physcion, chlorodermolutein, while non-carboxylic anthraquinones (majorly acetone extracted) dermoglaucin, emodin, and dermocybin. Besides focusing on identifying natural anthraquinones (derived from Cortnarius mushrooms) based on their fragmentation pattern, other metabolomic studies can take advantage of the reported experimental design and methodology to elucidate similar natural anthraquinone molecular structures.

References: [1] Räisänen, R. (2019). Fungal colorants in applications–focus on Cortinarius species. Coloration Technology, 135(1), 22-31.

[2] Räisänen, R. (2023). Natural Colorants from Lichens and Mushrooms. Handbook of Natural Colorants, 317-331.

[3] Räisänen, R., Primetta, A., Toukola, P., Fager, S., & Ylänen, J. (2023). Biocolourants from onion crop side streams and forest mushroom for regenerated cellulose fibres. Industrial Crops and Products, 198, 116748.

Keywords: Natural anthraquinones, LC-MS, Halogenated anthraquinones, NMR Spectroscopy, Cortinarius mushrooms

Chromatographic and spectrometric analyses of indigoids in some indigoproducing plants

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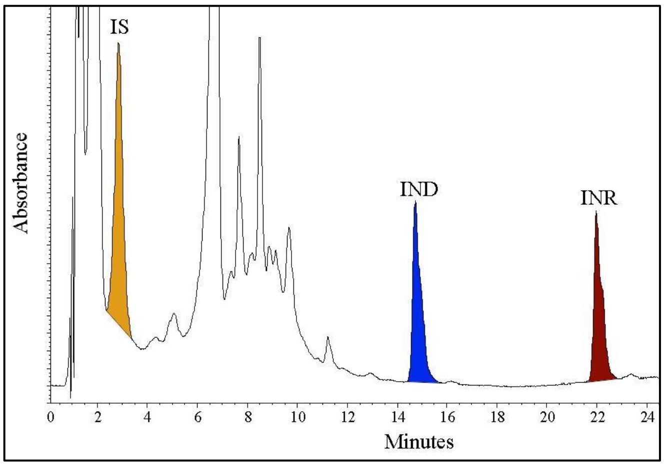
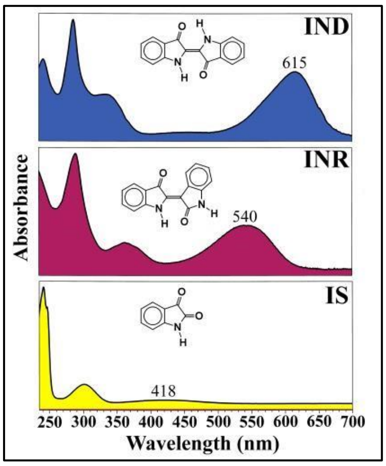
Abstract

In order to determine whether a bio-based colorant is sustainable, an important factor addressing that assessment is the determination of the relative content of the dye(s) within that plant. There needs to be a "critical mass" of dye material in order to make that dyestuff source economically worthy of being utilized on an industrial (or semi-industrial) scale for the production of that colorant. This is especially true when there are a number of bio-sources for the same dye, such as, in our project, the indigo pigment-dye. A classical case in point, highlighting this quandary, was the "Cactus Cochineal" (also known as "American cochineal") replacement of other old-world reddish-dye producing scale insect sources, such as Kermes and other cochineals, in that its dye content was significantly greater than all other insect dyestuffs.

The indigo pigment that we will be monitoring is produced from the precursors in the leaves of all indigoproducing plants. These precursors, are indoxyl derivatives with different attached sugar moieties (glycosylated forms of indoxyl). The range of these compounds can include indican, and the so-called isatans A, B, and C. Hydrolysis of these precursors will cleave the irrelevant sugar entities and leave the indoxyl derivatives to further react with oxygen and consequently to colored compounds. The main colorant product is typically, of course, the bluish indigo pigment (also known as "indigotin"), with usually some reddish indirubin and yellowish isatin also produced. Indirubin is an isomer of indigo, and the lessercolored isatin is an oxidized form of a "half-indigo" molecule.

This talk will briefly describe the mechanisms by which the three above-mentioned colorants are produced, and the main emphasis of the presentation will be a quantitative comparison of the results of HPLC and UV/Vis spectrometric analyses performed on the leaves from a number of indigo-producing plants.





Keywords: Indigo, indirubin, isatin, indoxyl precursors, HPLC, UV/Vis spectrometry

Tannin-based pigments in red wine and their mass spectrometric analysis

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Abstract

The manufacturing of red wine brings together two groups of specialized plant metabolites of grapes that have a vital role in the development of the sensorial properties of red wine. Namely, anthocyanins and proanthocyanidins (PAs). The former are pigmented flavonoids, while the latter are non-colored oligomers and polymers of flavan-3-ol monomers and the main group of tannins in red wine. During manufacturing of red wine, PAs and anthocyanins react together leading to formation of PA–anthocyanin adducts. Most of the formed sub-groups of PA–anthocyanin adducts are pigments because the anthocyanin moiety retains its structure, and the PA–anthocyanin adducts are important pigments for the color properties of red wine. However, PA–anthocyanin adducts are challenging to analyze in red wine. PAs themselves always exist in plants as mixtures consisting of dozens and even hundreds of individual compounds, which leads to the PA–anthocyanin adducts being complex mixtures as well. Laitila et al. (2019) developed a mass spectrometric method for the detection of PA–anthocyanin adducts in red wine that overcame several limitations of the past methods. The method detects PA–anthocyanin adducts via fragment ions specific for certain PA–anthocyanin adduct groups, which enables their efficient group-specific detection. This methodological advancement enabled to study the basic properties and composition of PA–anthocyanin adducts in red wine in greater detail than before (Laitila, 2021). Moreover, the analytical method could help in development of hemisynthesis strategies for PA–anthocyanin adducts in the future by accurately monitoring their formation and stability and, thereby, ease their use as biobased pigments in commercial applications.

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Laitila, J. E. (2021). Composition and evolution of oligomeric proanthocyanidin–malvidin glycoside adducts in commercial red wines. Food Chemistry, 340, 127905. https://doi.org/10.1016/j.foodchem.2020.127905

Keywords: Flavonoid, tannin, anthocyanin, polyphenol, mass spectrometry

Extraction and purification of melanin from insect production side streams

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Abstract

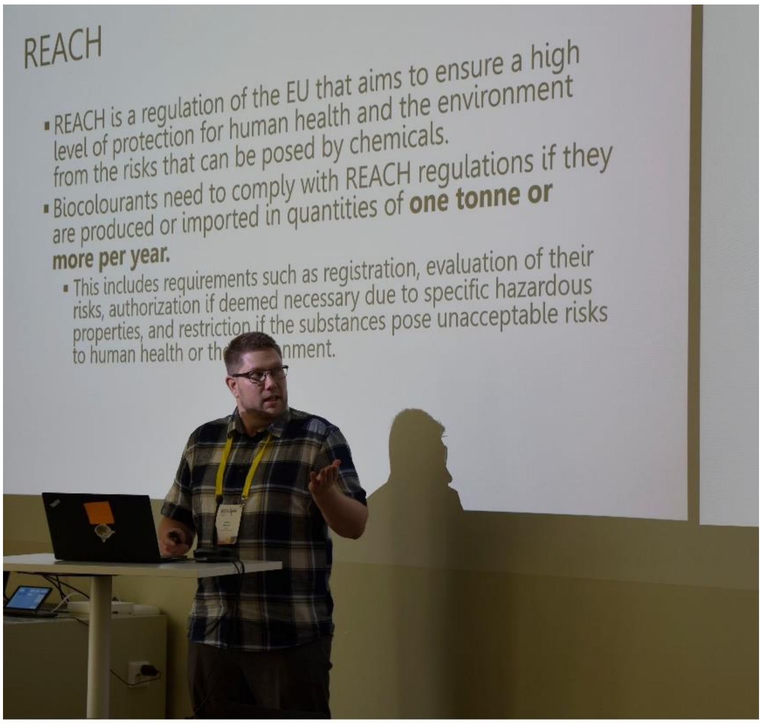
The FAO expect a high contribution of insects to food safety, livelihood and environment. The Black soldier fly (Hermetia illucens) is an interesting species which can feed bio waste and transform it into high quality protein and fat. Black adult flies and exuviae are side streams of this protein production process. The main focus of utilization of this side streams is the production of chitin and chitosan. The challenge for the production of white chitosan is the crosslinking of chitin with melanin, a brown to black pigment. Different opportunities are published for the "bleaching" of chitosan. All bleaching processes destroy the melanin. Melanin is an interesting biomolecule and can be used for technical applications, as UV radiation protection or as dye pigment. The presentation will discuss the results of extraction and purification of melanin from Black soldier flies as far as from chemical solutions used in the deacetylation process of Chitin into Chitosan. A purification of melanin and recycling of chemical solutions especially by using membrane separation processes with ceramic membranes will be presented.

Keywords: Black soldier fly, melanin, extraction, separation

Exploring natural colourants for safer dyes and dyeing

Session speaker:

Dr. Mikko Herrala, University of Eastern Finland



Safety of biocolourants – toxicity testing of natural anthraquinones

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Abstract

Synthetic dyes are massively used in textile industry, and they may pose health risks to workers, end-users and to the environment. There is an increasing interest in developing safer and more sustainable dyes and biocolourants provide novel possibilities for the textile dye industry. While developing new biocolourants it is essential to ensure the safety of these dyes. The toxicological assessment is needed as the natural origin alone does not ensure safety. The type of required toxicological studies depend on production volumes and legislation but it is recommended to consider safety aspects already in early phases of the process.

Good examples of promising biocolourants are natural anthraquinones dermocybin, dermorubin and emodin, extracted from bloodred webcap fungus (Cortinarius sanguineus). They are suitable for textile dyeing with different materials. However, anthraquinones are wide group of dyes which of some are harmful to humans or the environment.1 Furthermore, dermocybin and dermorubin were non-mutagenic and presented low cellular toxicity but both increased oxidative stress in studied cell lines. 2 Unlike emodin which is mutagenic, and it also exhibits cytotoxicity and induces oxidative stress. 3 These results motivated to study genotoxicity of these anthraquinones. None of them was genotoxic in alkaline comet assay or microflow in vitro. Interestingly, novel transcriptomics methods revealed that all three anthraquinones increased number of differently expressed genes in TempO-seq (Templated Oligo assay with Sequencing readout), but they were non-genotoxic in further biomarker analyses.

In conclusion, dermocybin and dermorubin may provide good alternatives for synthetic dyes as they are non-genotoxic in studied models. Emodin's potent mutagenicity in bacteria did not manifest as genotoxicity in human cells. However, anthraquinones affected to gene expression which needs to be analysed further to understand better the effects on molecular level. In addition to toxicological information, other information such as exposure assessment is needed for risk assessment of biocolourants.

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3. Brown, J.P.; Brown, R.J. Mutagenesis by 9,10-Anthraquinone Derivatives and Related Compounds in Salmonella Typhi-murium. Mutat. Res. Toxicol. 1976, 40, 203–224, doi:10.1016/0165-1218(76)90046-X.

Keywords: anthraquinones, biocolourants, C. sanguineus, genotoxicity, toxicity

Assessment of skin sensitization potential of *Cortinarius sanguineus* dyes

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Abstract

Biocolourants are an interesting, possibly ecofriendly option to be used in various applications. In the case of textiles, prolonged skin contact is expected, creating a possibility for the development of contact dermatitis, a type of allergic reaction. Following the safe by design principle, in vitro KeratinoSens™ assay, was used to study the skin sensitization potential of dermocybin (0.035–70 µg/ml), dermorubin (0.03–100 µg/ml), and emodin (0.01–30 µg/ml) obtained from Cortinarius sanguineus fungus. Also, the native dye mixture obtained from the fungus was tested (0.03–100 µg/ml). KeratinoSens assay is based on a luciferase Nrf2 gene activation in skin keratinocytes which is considered as the second key event in the skin sensitization Adverse Outcome Pathway (AOP)[1].

In the KeratinoSens assay, keratinocytes were exposed to dyes or dye mixture for 48 h. Then, cell viability was measured by adding 20 µl resazurin to each well, after which fluorescence was measured. Immediately after the viability test, 50 µl One-Glo™ reagent was added to the wells and luminescence was measured with an integration time of 1 s/well. The test result was considered positive if gene activation was increased by 50%.

Both emodin and C. sanguineus mixture exceeded the 50% induction level already at concentrations 2.02–2.4 µg/ml, respectively, and at concentrations above 10 µg/ml, viability started to decrease. Because the concentrations where the induction occurred are so similar, it is likely that emodin is responsible for the induction observed in the mixture. In our previously published results, it was seen that dermocybin and dermorubin did not induce the gene activation, suggesting that they would not cause skin sensitization2 .

According to these results, dermocybin and dermorubin are potential biocolourants while emodin and the mixture might cause dermal allergy – these results could be confirmed with another in vitro sensitization assay as recommended by OECD1 .

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Keywords: Cortinarius sanguineus, KeratinoSens, allergy, dermatitis, biocolourant, in vitro

Care-fully crafted colour: natural dyeing as care-full correspondence between people, plants and materials

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Abstract

If we are to cultivate pathways towards a more sustainable future, a cultural shift towards more care-full and balanced relationships with nature will be needed. In this paper I will highlight the contribution that crafts such as natural dyeing can make, by uncovering and reflecting on the care-full correspondence between people, plants and materials that it fosters.

For many natural dyers in the UK, cultivating their own dye plants in their gardens or allotments and/or foraging for dye stuffs in their local area is a fundamental element of their craft practice. Notions of care and attending to the more-than-human world (Puig de la Bellacasa, 2017), in this case through actions of cultivating and gathering, are woven into these correspondences. Creating colour naturally is also enjoyed as a form of self-care by many dyers and the opportunity to enact care for the natural world and develop a closer connection to plants is a key motivator for them choosing to practice the craft. These notions of care that are nurtured through direct contact with plants are then translated and extended into (the design, construction and maintenance of) naturally dyed garments, as demonstrated by the material care that they require and the crafted care that they embody.

The work presented in this paper forms part of my doctoral research into contemporary natural dyeing craft practice in the United Kingdom. My qualitative research (based on interviews and fieldwork visits to UK based dyers) aims to illuminate and reflect on the interactions between people, plants and materials that connect natural dyeing craft practitioners to their local surroundings and links them to other species and ecologies. Although framed as textile-crafts research, the project takes an interdisciplinary approach by drawing on literature/theory from human geography/ecology.

References: Puig de La Bellacasa, M. (2017). Matters of care: Speculative ethics in more than human worlds. U of Minnesota Press.

Keywords: care, craft, textiles, sustainability, human-nature relationships

Natural dyes are truly safe? Investigating the aquatic toxicity of the anthraquinones dermocybin, emodin and dermorubin

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Abstract

The concept of sustainability permeates our lives nowadays. Thus, the development of products that are less harmful to the environment has become an urgent matter, and synthetic dyes are a key component of this discussion. They are used by various industrial sectors providing a lot of market value; and their use on a large scale in the textile industry leads to large volumes of wastewater and makes them one of the main contributors to the toxicity/mutagenicity of water bodies. Natural dyes are re-emerging as potential alternatives to synthetic ones, as a solution to avoid those harmful environmental effects. With this in mind, the aim of the present work was to study the aquatic toxicity of three natural anthraquinones dermocybin, emodin and dermorubin extracted from the webcap *Cortinarius sanguineus* (all with >98% purity) and, compare their toxic effects to other anthraquinones. Dyes were evaluated in acute and chronic tests using organisms from different trophic levels, microalgae (*Raphidocelis subcapitata*), crustacea (*Daphnia similis*, *Ceriodaphnia dubia* and *Parhyale hawaiensis*) and fish (*Danio rerio*). Dermocybin was toxic to *D. similis* (EC_{50} = 0.51 mg L^{-1}), *C. dubia* (IC_{10} = 0.13 mg L^{-1}) and *D. rerio* embryos (LC_{50} = 2.44 mg L^{-1}) but not to *R. subcapitata* or *P. hawaiensis*. Although emodin was only toxic to *D. similis* (EC_{50} = 0.13 mg L^{-1}) and *D. rerio* embryos (LC_{50} = 0.025 mg L^{-1}), it presented the highest acute toxicity when compared to the 6 natural and 16 synthetic anthraquinones reported in the literature (Fig 1). Only dermorubin was not toxic to any of the organisms tested; therefore, it can be considered the most promising dye in this study. Our results also illustrate that natural does not necessarily mean benign in terms of aquatic toxicity. All dyes, natural or synthetic, should have their toxicological properties assessed before designating them as safe.

Acknowledgments: BioColour project n◦ 327178, 327213 and 352460, Capes financial code 001, Fapesp

ToxBiocolour Project n° 2020/04628-8. The fish embryos studies were approved by the ethics committee (UNICAMP) (protocol n◦ 5645- 1/2020).

Keywords: algae, crustacea, fish, sustainability, dye safety

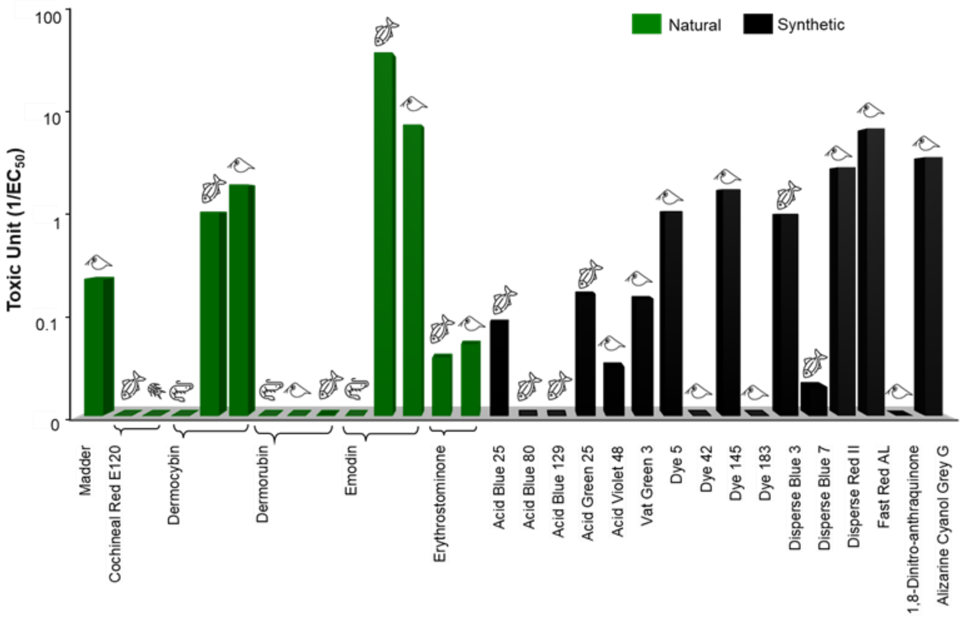

Fig. 1. Comparison of the acute toxicity of natural (green) and synthetic (black) anthraquinone dyes expressed in toxic units (1/EC_{50}). Organisms are represented as (*Daphnia*), (*Parhyale*) (artemia) and (fish).

From logwood to lasers: bio-digital textile dyeing and design

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Abstract

The textile industry is a global giant whose environmental consequences are severe. Textile processing accounts for 51% of a textile's overall carbon footprint (WRAP, 2022) and is estimated to contribute up to 20% of global industrial water pollution (Kant, 2012). The dyeing, printing, and finishing stages of textile production involve water and energy-intensive processes, leading to high water waste and chemical effluent. Synthetic petrochemical dyes are predominant in the industry. The very characteristics that make these dyes desirable for commercial applications, such as high stability to water, temperature, and light, also lead to their persistence in the environment, causing devastating consequences to aquatic ecosystems.

This paper presents a case-study of interdisciplinary design research that explored sustainable alternatives to conventional dyeing and finishing by innovatively integrating bio-dyeing with laser technology. Linen textiles were transformed by combining these processes, reimagining traditional textile coloration, and patterning. The developed processes addressed toxicity in textile coloration by replacing petrochemical dyes and metal salts. Experimental bio-mordants were used to enhance the dyeing process of natural and plant-based dyestuffs. Plant, fungi, and algae-based pre-treatments with advantageous properties as bioaccumulators, protein-binders, tannin-rich plant materials and natural nitrogen fixers were used in combination with natural dye extracts, using small scale commercial dye machines to show potential for industrial uptake of this process within the fashion system. Laser irradiation was creatively employed as a digital design process to introduce precision surface designs. Combining these approaches resulted in linen textiles that reimagined conventional coloration practices, aligning with sustainable, circular design principles.

The presented research acts as an exemplar for a bio-digital methodology to enhance the precision, flexibility, and sustainability of working with biocolours in textile coloration and design.

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https://wrap.org.uk/resources/report/textiles-2030-annualprogress-report-202122

Keywords: Textile Design, Coloration, Pattern, Natural Dyes, Biomordants, Laser Processing

Keynote 2 & industry views

<u>Keynote speaker:</u>

Professor Laurent Dufossé, Reunion Island University

<u>Industry presentations:</u>

Ida Näslund, Mounid & Linda Magdalena Jonsson, Alder Olmai

Suvi-Elina Enqvist & Pauliina Varis, Marimekko, Elina Helenius, Lapuan Kankurit

Current and potential natural biocolours from microorganisms (bacteria, yeasts, fungi, and microalgae)

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Abstract

Pigment-producing microorganisms and microalgae are quite common in Nature. However, it is a long way from the Petri dish to the marketplace. Thirty years ago, scientists wondered if such productions would be a scientific oddity or an industrial reality. The answer is dual as processes using fungi, bacteria, or microalgae already provide carotenoids or phycocyanin at an industrial level (e.g. beta-carotene and lycopene with the fungus Blakeslea trispora; astaxanthin, adonixanthin and adonirubin with the bacteria Paracoccus carotinifaciens; blue phycocyanin with the cyanobacterium Arthrospira platensis or the extremophilic unicellular algae Galdieria sulphuraria). Another product is peculiar as Monascus red-colored food is consumed by more than one billion Asian people; however, still banned in many other countries (hepatotoxic citrinin issue). European and American consumers will follow as soon as toxin-free azaphilone-producing strains (e.g. Talaromyces fungal strains) have been developed. For other pigmented biomolecules, some laboratories, start-ups (Pili Biotech, Chromologics, Huue, Michroma, Colorifix, Phytolon) and companies (DSM, Chr Hansen) invest a lot of money as any combination of new source and/or new pigment requires a lot of experimental work, process optimization, toxicological studies, and regulatory approval. Time will tell whether investments in pigments such as azaphilones, niche carotenoids or anthraquinones (replacement of carmine from insect) were justified. Future trends involve combinatorial engineering, gene editing, gene knock-out, transfer of plant pigments in microbial chassis, extraction with green solvents (ionic liquids, deep eutectic systems) and the production of niche pigments not found in plants such as C50 carotenoids (e.g. bacterioruberin) or aryl carotenoids (e.g. isorenieratene and hydroxyl derivatives.

Multidimensional upgrade with natural colour

Linda Magdalena Jonsson[a)]Ida Näslund [b)]

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b) MOUNID AB, Gothenburg, Sweden

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Abstract

Linda Magdalena Jonsson is an entrepreneur and founder of Alder Olmai, developing (super) natural colours for textile, based on the Swedish forests. Her background is in Artdirection, brand building and herbal medicine. Ida Näslund is an entrepreneur with a background as a textile designer, working with natural methods made her start MOUNID, developing environmentally friendly textile colours by microalgae.

Going from synthetic textile dyes to biocolour will demand a system shift. We think that the role of colour can go beyond the function of aesthetics, and that we can open up to experience colour on multiple levels.

Our biocolours have made us connect the ocean and the forest and now when we come together we see a holistic viewpoint that we would like to highlight. We find it urgent to investigate how colours can connect us back to nature to shape the balance between living, seeing, feeling and creating, and here we find the multidimensional role of colours.

We want to open for the *multidimensional upgrade with natural colours*, to;

- Stimulate new perspectives, to make the shift practically possible
- Break old habits, create new patterns, relearn colours
- Create balance and health by colours

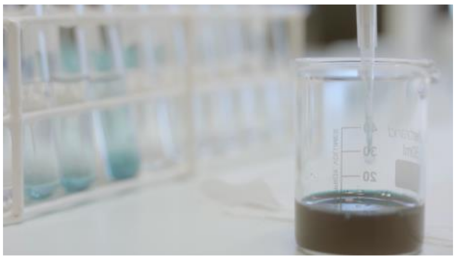
By working for several years developing our colours with the ocean and forest we are dedicated to be a part of this paradigm shift in textiles and to share our learnings and experiences – believing in the power of colour to regenerate and create thriving ecosystems and life. This talk will be a dive into colours as a fundamental ingredient for an enriching future.

We will share our experiences and insights to initiate innovative thinking in the crucial system shift and how to look at two natural colours coming together in our work.

References: max 3, APA syli

- *Bio-based colourants and materials*
- *Designing with biocolours*
- *Colour for the future*

Keywords: Biotech, Regenerate, Holistic, System shift, Innovation




Coloring outside the lines: the art of printmaking with natural dyes & bio-based alternatives

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Abstract

From its founding in 1951, bold, vibrant colors have been at the heart of Marimekko's mission. Emerging out of a monochromatic, post-war world, Marimekko's abstract patterns and architectonic shapes, offered in a range of unexpected colorways, were an ideal fit for a new generation looking to experiment with new forms of creative expression that, literally and figuratively, allowed them to color outside the lines. Over the next 70 years, Marimekko's designers have created over 3,500 print designs, which then have been re-created in numerous imaginative color palettes and on thousands of different products, from dresses and bags to ceramics, fabrics, and more. Given this artistic and experimental legacy, it is no surprise that color, in all its facets, remains a signature element in Marimekko's art of printmaking and a central focus of its innovation work.

Over the last few years, Marimekko's innovative approach to color has taken on yet another facet, as the discussions surrounding the color space have expanded to include sustainability and circularity initiatives. In an effort to create a more sustainable value chain for color, Marimekko has launched a range of innovation projects that explore the rapidly developing world of natural dyeing techniques, including plant-based woad and willow dyes, recycled pigments extracted from industrial by-products, mineral-based dyes, as well as bio-based auxiliaries used in the fabric printing process. These projects are part of Marimekko's broader efforts to improve the functional properties of natural dyes, explore synthetic biology in fabric printing and developing pigments from industrial waste streams.

This presentation will share some of our findings from this ongoing work, which is being carried out at Marimekko's printing mill –our very own color testing laboratory – as well as in multidisciplinary collaborations with start-ups and growth companies, and in research partnerships with academic institutions from around the world.

Weavers of good life – Lapuan Kankurit

Elina Helenius

Lapuan kankurit

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Abstract

LAPUAN KANKURIT, www.lapuankankurit.fi is an ambitious future orientated design company with own production of sustainable textiles in Finland.

Our principles of sustainable design are:

MATERIAL - We use natural, sertified, eco friendly materials, linen and wool, and purchase yarn only from the best spinning mills in Europe.

LONGEVITY - We prefer to design our colour palette according to the interior decor of our clients instead of going along fast fashion

MULTIFUNCTIONALITY - Our aim is to create products and patterns that are as versatile and multifunctional as possible.

WEAVING ACCORDING TO EXACT MEASUREMENT - Most of our products are woven using exact measurements in order to avoid producing cutting waste.

FINISHING - Almost all of our linen fabrics undergo a finishing treatment.

This ensures that the high quality product maintains its shape also after it has been washed at home. Whenever possible, in terms of structure and composition of fabric, we use water and heat to finish our products.

The most important things for us are a QUALITY and a LONG PRODUCT LIFESPAN.

We call ourselves WEAVERS OF GOOD LIFE.

This means lot to us:

- we only manufacture products that are good and stand the test of life
- we use only certified natural materials
- being a domestic employer we bring work to Finland
- we take good care of our employees
- we keep our ecological footprint as small as possible
- we are constantly looking better and more sustainable new technologies, innovations and ways of production
- we co operate with high schools and universities and spread our knowledge of textiles for future generations.

As a stakeholder of BioColour project our target so far has been on natural colours such as woad from Natural Indigo Finland https://www.lapuankankurit.fi/en/kaamos-blanket-white-woad

and leftover coffee from a Finnish coffee roasting factory https://www.lapuankankurit.fi/en/juhannus-blanket-white-coffee-dyed-orange which we have been using in our Finn sheep wool products.

Biocolourant production by microbial biotechnology

<u>Session speaker:</u>

Professor Thomas Larssen, Technical University of Denmark

Biotechnological production of basidiomycetous colorants

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Abstract

Since a global transformation towards circular bioeconomy relies on sustainable processes and environmentally friendly products, alternatives to currently used colorants and dyes are explored. One of the proposed solutions are biotechnologically produced pigments from fungi.

Two different species belonging to the phylum Basidiomycota and their potential to produce colorants were investigated: the sulphur shelf (*Laetiporus sulphureus*) and shaggy bracket (*Inonotus hispidus*). Both species are known to synthesize colorful molecules in their fruiting bodies and in the mycelium grown in liquid culture. *L. sulphureus* synthesizes several orange laetiporic acids and *I. hispidus* produces the yellow hispidin, which is a known antioxidant with several other bioactivities.

The cultivation of both species was scaled up from shake flask to a 4 and 7 L bioreactor and nutrition medium was optimized. By comparing four different *L. sulphureus* strains, the most potent pigment producer was identified and achieved yields of around 1 g/L laetiporic acid. Different physical and chemical influencing factors for improved hispidin yield were tested for the cultivation of *I. hispidus*. Irradiation and oxidative stress stimulated pigment synthesis, as well as supplemented precursors. The effects were combined in a comparison of two different types of bioreactors and a yield of 5.5 g/L hispidin was achieved. Different product recovery strategies were investigated, from conventional extraction to a biphasic system. The prior poor stability of laetiporic acid extracts was improved by addition of nitrogen and storage at low temperatures. The potential for application in textiles, cosmetics and food was demonstrated.

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Keywords: Basidiomycota; Bioprocess; Laetiporic acid; Hispidin; Bioeconomy

Fermentative production of betalain-type natural food colors

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Abstract

Colour is an essential characteristic of food, associated with quality, freshness, and taste perception. Natural and synthetic food dyes are added to processed foods to enhance or correct variations and give an expected colour to colourless foods, such as soft drinks or candies. There is a strong consumer-driven conversion of the food colour market towards natural colours, where conversion in Europe region is already above 60%. As the natural sources of colours are limited, we aimed to develop biotechnological process to produce betalain-type food colours by fermentation. Betalains are natural red-purple-yellow pigments found in plants of the order Caryophyllales and fungi of the Amanita genus. While nearly a hundred natural betalains have been identified, only red pigment betanin is produced commercially, by extraction from red beets. The betanin content in red beets is very low, ~0.2% wet weight, and the downstream process is challenged with impurities, such as geosmin, nitrates, and sugars, co-extracted with the pigment. We developed a yeast-based fermentation process to produce betanin and several other betalain pigments. The work comprised biosynthetic pathway discovery, optimization of pathway expression and precursor supply, and prevention of product degradation. Due to the simple visual detection of betalains, parts of the strain improvement program could be carried out using high-throughput genome engineering and screening. Engineered oleaginous yeast Yarrowia lipolytica made ~1.2 g/L betanin in 48-h fed-batch fermentation. According to the life cycle assessment, the fermentation process would have a significantly lower impact on resources and ecosystem quality than the traditional extraction of betanin from beets. We also demonstrate production of other betalains by fermentation, such as the pigments from the red dragon fruit or amaranth.

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Keywords: biotechnology, food colours, betalains, yeast fermentation.

Heterologous production of cortinarius-derived anthraquinone pigments in an industrially feasible host

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Abstract

The use of natural bio-based colorants to replace synthetic, petroleum-derived colorants has gained significant interests in recent years, owing to the harmful environmental and health impacts posed by the latter [1].

One such compound group of interest is the anthraquinone pigments derived from *Cortinarius semisanguineus*. Traditionally used for dyeing purposes, they are known for their stability, color fastness and compatibility with various dyeing methods, making them ideal candidates for many commercial applications. The chemical production of such compounds is complex, involving multiple steps and uses metal catalysts, making this approach not feasible commercially [2].

Using the synthetic biology approach to unravel the biosynthetic pathway that yield the Cortinarius derived anthraquinones, we aimed to construct platform strains for emodin and endocrocin production by heterologous expression of the Cortinarius- sp. KIS3 polyketide synthase in Saccharomyces cerevisiae. Furthermore, we discovered two O-methyl transferases from Cortinarius, which can modify these anthraquinone precursors to yield physcion and dermolutein respectively.

Using this approach, these compounds could be produced all year round as compared to the seasonal collection of the mushrooms which requires expertise in distinguishing them from other related species, making it a laborious process [2]. This work will further aid in discovering suitable oxygenases towards the production of the red coloured anthraquinones, dermorubin and dermocybin.

Generating efficient production strain for these anthraquinones would open the way to modify these scaffolds in vivo or in vitro to tune their chemistry for the textile, plastics, paints, food, electronics, and cosmetic industries [3].

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Keywords: Anthraquinones, Metabolic engineering, Synthetic biology

Vienna Textile Lab – rethinking colour

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Abstract

Vienna Textile Lab (VTL) is a startup biotech company established in 2021 and located in the heart of Vienna. The mission and vision of VTL is to bring biobased dyes and pigments to the market with a primary focus on textile applications. VTL owns an exclusive collection of microbial cultures collected directly from nature and with selective fermentation methods is able to achieve production of different colours. However, the production of biobased pigments does not end at fermentation stage. We believe that extracted and purified pigments have the biggest potential to compete against well-established synthetic dyes. Vienna Textile Lab took a holistic approach and pays a specific attention to every step of the process including sourcing the raw material, chemistry of pigments, safety and regulations, life cycle analysis as well as designing with biocolours.

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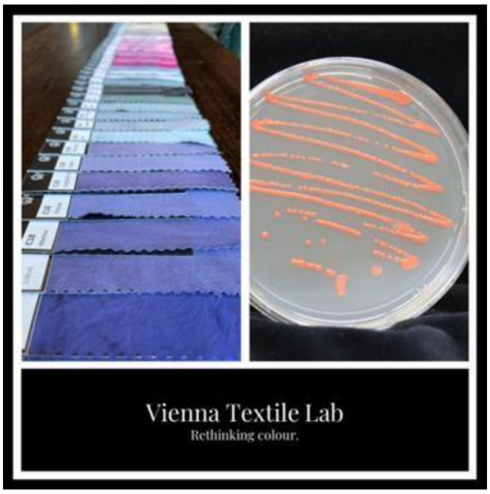
Keywords: start up, microbial colours, pigments,



Textile colouration and biodye aesthetics

Enzymatic indigo and indican for low-impact blue denim dyeing

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Abstract

Most of the environmental impact from textile dyeing in the world can be attributed to the production and usage of indigo, the dye responsible for the iconic blue denim look. Indigo denim dyeing emits 4 million tonnes of CO2 equivalents yearly from the dyeing process alone and gives rise to significant pollution of waterways and soil(Mia et al., 2019; Paul et al., 2023). Indigo, the only known molecule yielding the unique hue of blue denim, is a vat dye requiring harsh reducing agents and alkaline conditions. Indican, the O-glycoside of the indigo precursor indoxyl, has been suggested as an environmentally mild blue denim dyeing agent, since indican can be transformed to indigo directly on the yarn by enzymatic treatment (Hsu et al., 2018). For both indigo and indican, non-fossil, sustainable sources are needed.

We employ enzyme and process engineering to develop low-impact biotechnologies for the production and application of indigo and indican. Notably, we can produce both indigo and indican with enzymes, and have demonstrated indican dyeing with enzymes, as well as with artificial and natural light. These biotechnologies rely on enzyme discovery and stabilization through engineering. We have performed techno-economic and life-cycle analyses, and found that these technologies have the potential for at least a 50% reduction in the environmental impact of blue denim dyeing. The project is ongoing, and the current status will be presented.

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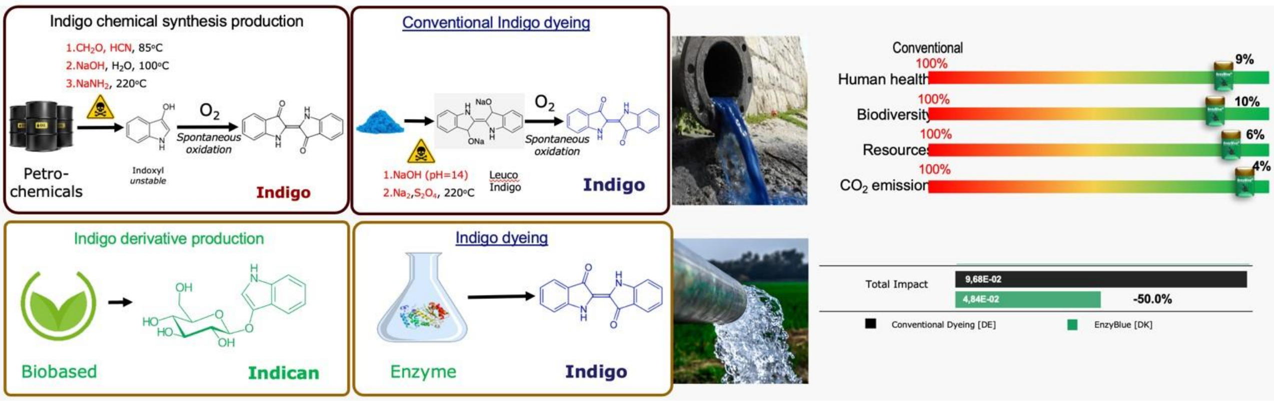
Keywords: indigo, indican, biocatalysis, sustainability, denim



Lignin as a colorant for screen-printing on textiles

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Abstract

Currently, most colorants used by the textile industry are synthetic. Due to sustainability concerns regarding synthetic colorants, the industrial scale application of colorants from natural sources has raised interest in the textile field. However, challenges in fastness properties and production costs of bio-based colorants in comparison to synthetic colorants have limited their application. Industrial side streams originating from plant sources have been recognized as a possibility for producing bio-based colorants with lower cost1. Lignin as an abundant by-product from the forest industry could therefore be of interest as a bio-based colorant.

Pigments are commonly used as colorants in textile printing processes. In this work, bio-based printing pastes containing softwood Kraft lignin and chitosan were used for screen-printing on cotton fabric. Lignin was used in particle form as a pigment colorant in the pastes. Chitosan in dissolved form was added to the pastes to function as a bio-based binder. Fabrics with colors ranging from light beige to brown were achieved (Figure 1). Wash, rub and light fastness properties and surface morphology of printed fabrics were determined and based on the results, advantages, and challenges of applying lignin as a pigment colorant on textiles are discussed. The adsorption of lignin particles on cellulose nanofibril thin films with chitosan as binder was also studied to demonstrate the effect of chitosan in binding lignin to cellulosic surfaces.

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Figure 1. Paste prepared using Kraft lignin as colorant (left) and cotton fabric screen-printed with lignin containing pastes (right).

Stabilization of natural and synthetic indigo on nanocellulose network - towards bioactive materials and facile dyeing processes

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Abstract

Synthetic dyes, widely employed for coloring various materials, pose well-acknowledged environmental risks.(Kant, 2012) Improving existing dyeing technologies for efficiency and cleanliness is crucial, and valorizing natural dyes can contribute to sustainable developments in the dyeing industry. Natural indigo, extracted from Isatis tinctoria, offers a bio-based alternative to chemically synthesized indigo.(Maugard et al., 2001) However, the traditional vat technique for converting indigo into a soluble leucoindigo form involves harsh chemicals, and maintaining the leucoindigo during dyeing necessitates additional reducing agents.(Kulandainathan et al., 2007)

This study explores stabilizing leucoindigo on a nanocellulose matrix carrier using spectroscopic and photophysical methods. Results indicate successful stabilization on the nanocellulose suspension, likely due to limited oxygen diffusion. Natural indigo exhibits better stability compared to synthetic indigo, attributed to radical scavenging properties absent in the synthetic counterpart. The nanocellulose stabilized leucoindigo proves applicable for creating patterns on cotton via screen printing, eliminating the need for re-reduction before dyeing and reducing harmful chemical usage. This research challenges perceptions of natural dyes as disadvantageous, showcasing that their inherent characteristics, such as co-products in the mixture, can enhance material functionality.

In summary, the study demonstrates the potential of natural indigo stabilized on nanocellulose, offering a cleaner and more efficient alternative to traditional dyeing methods. The enhanced stability and reduced reliance on harmful chemicals present a promising avenue for sustainable development in the dyeing industry, highlighting the value of natural dyes with additional functionalities in comparison to their synthetic counterparts.

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Keywords: Fluorescence spectroscopy, Nanocellulose, Indigo, Leucoindigo, *Isatis tinctoria*

Textile dyeing with *Indigofera tinctoria*-derived dye: exploring eco-friendly processes and colour spectrum

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Abstract

The increasing emphasis on sustainable practices in the fashion industry has underscored the significance of natural dyes due to their connection to reduced environmental impact and improved health considerations (Sabyrkhanova et al., 2023). Despite this positive trend, the search for alternative dyeing processes and auxiliary products aligning with eco-friendly principles while ensuring chromatic qualities remains challenging (Brudzyńska et al., 2023). An example is the natural dye indigo, widely recognized as a blue vat dye. Traditional industrial reduction processes of indigo dyes, often involving the use of conventional agents like sodium dithionite, have been associated with adverse consequences and environmental pollution (Maulik et al., 2022). In response to this challenge, this study explored the application of fructose as a sustainable reducing agent in the dyeing of cotton fabrics with a dye derived from the *Indigofera tinctoria* plant. Additionally, an adaptation of an artisanal dyeing process to industrial equipment was proposed. Dyeing tests were conducted, involving the combination of indigo with various non-toxic auxiliary products. Colour strength and colour fastness of dyed fabrics were evaluated. The integration of diverse auxiliary agents in the dyeing process explored the potential for unique shades and tones. The results indicated that the use of fructose emerges as an effective and environmentally friendly alternative in an industrial context compared to commonly used toxic reducing agents. The dyeing process developed introduced a straightforward and more sustainable approach than traditional industrial processes. This study achieved a colour spectrum extending beyond the conventional blue typically associated with indigo dye. Moreover, this study allowed the recovery of traditional knowledge related to indigo dyeing, incorporating more environmentally friendly products and adapting them to the industrial environment, while exploring a diverse chromatic palette. Such innovation significantly expands the horizon of sustainability in colour options for fashion design.

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Keywords: Indigo, natural dyes, colour palette, dyeing processes, textile and fashion design

Evolving patterns - utilizing plant derived dyes for designed changeability in textiles

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Abstract

The increasing awareness of sustainability issues in textile sourcing, manufacturing, use, and disposal has sparked a reassessment of natural colorants as a viable alternative to synthetic dyes. This shift is driven by the historical use, natural origin, biodegradability, and limited toxicity of natural dyes. Recent research has focused on optimizing the extraction and reproducibility of natural dyes, refining the dyeing process, and enhancing characteristics like UV, rub, sweat, and wash fastness. While synthetic dyes predated the advent of man-made fibres, evidence of natural dye usage on regenerated cellulose or petrochemical-based fibres is relatively recent. Conversely, historical recipes and pattern books of textile dyers and printers showcase the extensive historical use of natural dyes on cellulose and protein fibres, with recent studies and industrial trials reinforcing this knowledge. Current investigations explore the interdependencies of natural dyestuff application and pre- and post-treatments, involving conventional metal-salt and bio-mordants, as well as sonic- and plasma-treatments (Li et al., 2023), and their impact on the properties of dyed/printed textiles. Sourcing natural colorants is a focal point of study, considering issues related to food security (land use for non-food production) and the expanding bio-manufacturing sector. Natural dyes pose challenges in colour mixing due to their inability to replicate the traditional trichromy (yellow, red, blue) of synthetic dyes. The non-additive character of natural dyes results in a non-trivial colour palette, where different mordants cause one dye to develop various shades, potentially affecting all colours in one pattern. Additionally, natural dyes respond to environmental changes, altering shades, fading, or darkening, influencing the complex aesthetics and the pattern design process. Designing with natural dyes requires a comprehensive approach, acknowledging and embracing their inherent changeability (Talman, 2022) and imperfect aesthetics (Niinimäki&Hidalgo, 2023) as quality to strive for. Presented practice-based design projects have experimented with plant and food-waste derived dyestuffs, pigments, and bio-mordants (protein and tannin) to create evolving patterns on cellulose, exemplifying the intricate nature of designing for and with natural dyes, considering their unique aesthetics, usage behaviour, and signs of wear.

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Keywords: natural dyes, bio-mordants, evolving textile prints, changeability as quality in textiles, aesthetics of imperfection

Color changeability as a quality to extend lifespan furnishing textiles

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Abstract

In the western textile industry, the founding principles of aesthetic and technical objects quality is largely related to the unchangeable nature of the product surface over time of use. In this way, material change — like sun faded furnishing textiles — is commonly perceived as damage or degradation. Known as 'cosmetic obsolescence', this phenomenon contributes to premature disposal and unsustainably short product lifetimes (Lilley & al.,2016).

Futhermore, the industry is focusing its efforts on improving recycling and reusing processes without challenging the economic model paradigm through product replacement.

However, research demonstrates that a carefully orchestrated change on the surface of a material has the potential to be leveraged to increase the lifespan of products by creating and maintaining physical and emotional durability (Chapman, 2005), as well as to globally reduce the environmental impact of a product (Kumar & al., 2023).

From this perspective, for and through a design research approach, this article examines how taking into account the reactions of natural dyes to long-term exposure to natural light upstream in the design of furnishing textiles can propose a new definition of color resistance and open a new path for design with biocolours.

Thus, a range of natural dyes are submitted to industrial tests for accelerated aging to sunlight in order to understand their chromatic evolution. These empirical data, characterized by levels of changeability (changes in hue, brightness, and saturation) and by temporalities of evolution (from the shortest to the longest), allow to build a predictive system based on color changes parameters. As a creative material for textile designers, evolving colors can be associated according to their contrast levels in order to design furnishing textiles capable of negotiate our perception of wear and tear by making patterns appear or disappear over the time of use.

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Keywords: evolving biocolours; graceful ageing; material change; cosmetic obsolescence; product lifetime

Sustainable solutions for textile dye extraction and application

Session speaker:

Dr. Gerardo Montero, Consultant Engineer for NCS

Supercritical fluid dyeing technology: an overview

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Abstract

Supercritical dyeing process has been considered as a viable alternative to water-based dyeing, which leads to environmental pollution. Supercritical fluid dyeing technology has the potential for many commercial textile applications at the present and in the future around the world. Supercritical fluids have special properties (such as densities and solvating powers similar to liquid solvents, but have extremely rapid diffusion characteristics and viscosity similar to those of gas) above their critical temperature and pressure. Supercritical carbon dioxide (SC-CO_2) is one of the most environmentally acceptable solvents in use today and textile processes using this solvent have many advantages when compared to conventional aqueous processes.

Increased interest in this alternative technology has made fundamental understanding of thermodynamics properties (equilibrium solubility) and transport (kinetics) properties of such fluids and fluid mixtures. Although several consortia exit for the purpose of developing processes and equipment for SC-CO_2 processing, their priorities do not include developing fundamental understanding of SC-CO_2 processing principles. Supercritical fluid dyeing is known to consist of the following steps processes: 1) dissolution of the dye in SC-CO_2, 2) transfer of the SC-CO_2 solution (dye in solution) to a substrate interface, 3) adsorption of the dye onto the substrate surface, and 4) diffusion of the dye into the substrate that is generally considered to be the rate-determining step during the SC-CO_2 dyeing processing.

Supercritical fluid dyeing technology has several positive environmental effects that range from drastically reduce water consumption to eliminating hazardous industrial effluent. Therefore, economic benefits increased productivity by reducing processing times, avoiding the generation of wastewater and the use of other chemicals substances (such as dispersants and surfactants), energy saving, as no additional drying process is required, and generated lower greenhouse gas emissions than conventional textile aqueous process. Successfully commercializing SC-SCO_2 processing will improve the economics of dyeing and other chemical processes. As a result, SC-CO_2 dyeing processing will more rapid, more economical, and less polluting.

Application of anthraquinone dyes from madder (*Rubia Tinctorum L*) to hydrophobic fibers using $ScCO_2$ versus water: a comparative study

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Abstract

The textile industry is one of the most water-intensive sectors, primarily attributed to the substantial amount of water required for dyeing [1]. During dyeing, wastewater containing synthetic dyes and auxiliary chemicals is produced, underscoring the need for more sustainable dyeing methods to address environmental concerns such as the release of pollutants [2]. Supercritical carbon dioxide ($scCO_2$) dyeing is an attractive sustainable method, as it is zero-water and zero-effluent [3]. Coupled with an interest in utilizing natural dyes as renewable sources of color for textile coloration, this study investigated the feasibility of applying anthraquinone dyes from the madder plant (*Rubia tinctorum L*) including alizarin (an aglycone), and ruberythric acid/RA and lucidin primeveroside/LP (glycosides) using $scCO_2$. Thus, the dyes (0.5%, 1% owf) were applied to polyester/PET (including recycled PET), nylon, and/or cellulose acetate at 120C, 34.45 MPa for 60 min, and the uptake of the dyes on the fibers were compared using water. Additionally, the uptake of the dyes was compared to a commercial synthetic dye known to display excellent uptake on PET using $scCO_2$, C.I. Disperse Yellow 54/DY54. Furthermore, the light, wash, and crock fastness of dyed samples were evaluated. Alizarin exhibited affinity toward all fibers, with uptake ≥65%, notably the greatest for recycled PET (95.7±4.3%). In contrast, RA/LP exhibited low uptake (27.1±2.7%) on PET due to their hydrophilicity and poor affinity for hydrophobic fibers. The uptake of alizarin on the fibers was slightly greater using $scCO_2$ compared to water, and the color of dyed samples resembled that of fibers dyed with DY54. Regarding fastness, PET fibers dyed using $scCO_2$ displayed the best properties across the board. Taken together, these findings underscore the potential of using $scCO_2$ to achieve sufficient uptake of alizarin on hydrophobic fibers without the need for water or auxiliary chemicals all while achieving good fastness properties.

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Keywords: Alizarin, Natural dye, Anthraquinone, Textile dyeing, Supercritical carbon dioxide

Inspirations from the past for eco-innovative natural dyeing and mordanting solutions for the 21st textile dyeing industry

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Abstract

Before the advent of synthetic colours, natural dyes were widely used to dye natural textile fibers. The use of natural products is currently of great interest due to increase awareness concerning environmental and health-related issues. Traditional knowledge is a unique source of inspiration and, although most people are convinced that the use of natural dyes may lead to poor to moderate light/colour-fastness, the truth is that we still see the brightness and colour hue of some of these natural colours in artworks, which have lasted for centuries. This resilience is related to the colour formulations, i.e., the recipes developed in the past.

Project REVIVE aims at studying these natural colour formulations by reproducing dyed textiles following historical recipes (Santo, Cardon, Teixeira, & Nabais, 2023), optimizing them for the modern textile industry.

Most natural dyes have little to no affinity for the natural fibers requiring the use of metal salts as mordants (Al, Sn, Fe) to bind to the textile, which can also lead to ecological distress. Previous works on the use of biomordants have shown promising results (Shahid & Mohammad, 2017). If these are obtained from discarded agro-food byproducts/wastes, symbiosis between circular economy and environmentally responsible practices is achieved. However, the lack of well-documented research, and their influence on colour stability downplays their role in natural textile dyeing. Eco-friendly biomordants recovered from agro-food byproducts/wastes such as oak galls, pomegranate peels and nut shells, have been selected for wool broadcloth dyeing.

This project is the first approach to the development of new sustainable dyeing and mordanting methods for the 21st-century textile industry, inspired by the ancient practice of natural dyeing. Following these recipes, we will prove that antient knowledge can be the answer for natural resilient dyeing.

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Keywords: Natural colours, Dyed Textiles, Master Dyers, Biomordants

The impact of supercritical carbon dioxide on natural dyes extraction

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Abstract

The dye compounds are widely present in various sectors (textile, cosmetics, food) and consumers are increasingly seeking for natural dyes. The development of synthetic dyes since the early 20th century has led to a loss of skills in dye plants and in extraction of dye compounds. There is, therefore, a need to rediscover plants and enhance the extraction performances to obtain those dye compounds.

Natural dyes are commonly extracted using green solvents such as hydroalcoholic mixtures. Depending on the raw material used and the extraction conditions, yields can greatly vary (from 1 to more than 40%). The aim of the present work is to study the impact of supercritical carbon dioxide (sc-CO_2) on the extraction of natural dyes in term of yield, colour and extract composition. Sc-CO_2 being a non-polar solvent, hydroalcoholic solutions as cosolvents will be added to modulate the polarity of the extraction fluid and its selectivity. For sc-CO_2 extraction and classical maceration, the raw material was extracted at the same temperature and with the same solvent/co-solvent to solid ratio for comparison purposes. In case of scCO_2, co-solvent composition was the same as the hydroalcoholic solvent used for maceration The plant extracts have been characterized by several techniques: spectrophotometry for colour intensity, HPLC-UV/DAD for dye molecules identification and quantification, and textile application on silk for color assessement.

Two plants have been studied, considering their local cultivation and the different chemical nature of their coloring molecules: Madder (*Rubia tincorum L.*) and Weld (*Reseda luteola L.*). Madder roots contain anthraquinones (alizarin, purpurin and glucosides) with a pink-red-orange colour (Derksen, 2001) whereas flavonoids (luteolin, apigenin and glucosides) are the yellow dying compounds present in aerial part of Weld (Botirov et al., 1979). This study shows there is no impact of the sc-CO_2 process on the extraction yield for Madder and for Weld (about 32 and 15 % respectively). Nonetheless, significant differences have been observed in the extract composition and in the colour.

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Keywords: Supercritical CO_2 Extraction / Maceration / / madder / weld / Textile dying

Enzyme-based textile coloration

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Abstract

Textile coloration is typically characterised as a resource intensive production process that requires the use of large amounts of water, high concentrations of processing chemicals, high temperatures and long processing times, commonly resulting in high energy consumption and effluent waste. Consequently, this has led to rethinking approaches to textile coloration. The research presented gives an overview of current studies that investigated the use of two specific oxidoreductase enzymes, laccase (EC 1.10.3.2) and peroxidase (EC1.11.1.7) to develop a one-step coloration process. Both enzymes are capable of polymerising simple aromatic compounds to form colorants with the potential for textile coloration through the formation of conjugated chromophores via their distinctive catalytic oxidation and coupling/polymerization mechanism. A diverse gamut of hues were achieved on a range of different fibre types (flax, wool and nylon) through enzymatic catalysis of various aromatic compounds as laccase or peroxidase substrates or precursors and alteration of processing parameters such as buffer systems, pH values and reaction times. Enzymatically dyed fabrics were tested against commercial standards, resulting in reasonably good colour fastness to wash. The research demonstrates the potential offered by laccase and peroxidase as transformative tools to replace conventional industrial coloration and surface pattern design processes with biological systems, which offer important advantages of simpler processing using milder conditions that eliminate additional chemical use and reduce energy consumption. The adoption of enzyme-based biotechnologies could help the textile coloration industry transition towards a sustainable future.

Keywords: Coloration, Enzymes, Laccase, Peroxidase, Wool, Nylon, Flax

Designing with biobased colours

<u>**Session speaker:**</u>

Karin Altmann, University of Applied Arts Vienna

A world of blue – dialogues in indigo

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Abstract

Indigo is the only natural dye that can produce a permanent blue on textile materials, a colour extremely rare in nature but associated with the sky and the sea; probably the reason why it has always awakened the longing for the pure, infinite and transcendent in human civilization. Indigo extraction and indigo vat dyeing, which have been used for over 6,000 years, are among the earliest cultural biotechnological processes. Indigo's distinctive blue has fascinated and inspired artists around the world over time, while also leading to horrific exploitations of people and the environment. Transferring blue to textiles is nowadays related to the use of synthetic compounds made from fossil fuel-derived substances on an industrial scale. Natural indigo makes up less than one per cent of the indigo dye currently produced worldwide, and today's dyeing practices, which are driven by economic interests, hardly allow environmental compatibility to be taken into account. As both an artist and researcher, Karin Altmann explores the question of how a return to natural indigo dyeing practises can be a solution to create a space for cultural, social and ecological responsibility and provide a solid basis for contemporary, sustainable processes in art and design. In her session keynote, Karin Altmann presents examples of her arts-based research projects, artistic dialogues, intercultural and interdisciplinary collaboration and sustainable design processes that explore the history and potential of natural indigo and open up spaces for joint thinking and acting in which indigo is not simply understood as a colour, but as a conceptual space with historical, social, political, philosophical, and spiritual aspects.

Chromoculture: cultivating colour through art and design in Limousin (France)

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Abstract

Chromoculture is a research-creation project that investigates how, in Limousin (France), artists and designers collaborate with farmers, chemists, craftspeople, and engineers to engage on ecological alternatives to polluting petrochemical colours. By turning away from urbanity as an artistic horizon and a realm of progress, the project identifies rurality as a physical and cultural space for creating new imaginaries that extend the aesthetic domain into a renewed "environmental ethic" (Rolston III, 2002) of colour creation.

Building on a relationship between art, design, agroecology and green chemistry, the project started with the creation of a garden-laboratory conceived as a place for artistic making and scientific knowing about bio-colours. In Limousin, the survival of preindustrial colouration practices intertwined with modern synthetic colour industries: from medieval enamel to 18th-century porcelain glaze and 16th-century dyed tapestries, up to contemporary dyeing workshops and enamel factory. This long history of the colour industry in this region makes it an ideal site for exploring transformative bio-colour practices through art and design.

A central aspect of the research involves critically examining the synthetic dye chemistry, tracing its origins in the 19th century to its contemporary environmental impacts. The project emphasizes on green chemistry and developing a "friendly" relationship with plants (Haudricourt, 1969), providing an alternative to polluting azo dyes used in the textile and graphic industries. This contemporary choice is also grounded in reflective considerations of historical relationships between humans and plants, addressing issues of predation and consumption associated with colour and colonization.

Chromoculture aims to empirically test the concept of colouration as a "negative commons" (Mies and Bennholdt-Thomsen, 2001). It prompts artists and designers to take responsibility for their chromatic waste, offering potential solutions to colour-producing communities while reshaping social and symbolic representations associated with colouration in contemporary societies.

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Keywords: colouration, art and design, rurality, environmental ethic, negative commons

Dyeing as an encounter of lifelines: crafting a colour library through foraging practices in southern Finland

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Abstract

The emergency of the environmental impact in textile dyeing practices highlights the necessity to explore alternative approaches to colour application. A significant part of research that involves biocolourants has concentrated on achieving existing colour standards in controlled settings, neglecting the complex ecological dynamics where bio-based dyeing materials grow. Employing foraged materials provides an opportunity to comprehend dyeing from a place-based standpoint, where colours emerge from the interplay between dyers and their surroundings. This article delves into foraging and dyeing practices using practice-led and autoethnographic methodologies, examining the author's experiences in the forest areas of Vanhankaupunginlahti and Kalkkiranta in southern Finland. The study documented 21 walks through diary and collage methods and assembled a colour library with 76 swatches of wool yarns utilising 14 different dyestuffs. Crafting a colour library with biomaterials from a specific ecosystem allows dyers to learn about their environments through movement and sensory engagement, shaping their understanding of textile dyeing processes and colour. The article argues that dyers can connect with the landscape by recognising and corresponding with the lifelines of dyestuffs through walking and observation. During foraging, the dyer's agency and intentions are constrained by environmental limits, provoking a reconsideration of colour in design practices that often ignore dyestuff ecologies when creating palettes. Working with foraged materials for dyeing textiles will not solve the current environmental crisis of dyeing practices. But it can trigger imagination to find ways to coordinate colour-making processes with the rhythms of the environment by teaching us about waiting, following, improvising and corresponding with landscapes, organisms and dyestuffs.

Keywords: Biocolours, foraging, correspondence, human-environment relations, walking

Harmonic color palettes from bio-based colorants

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Abstract

Development of harmonic color palettes for textile printing, using locally cultivated natural dyes as a tool for designers aiming at industrial niche production.

Scaling up the use of natural dyes for smaller industrial niche production requires designers to have access to an alternative natural color palette that can compete to some extent with synthetic dyes in various shades and colors. Since 1850, synthetic dyes have gradually pushed natural dyes into obscurity. However, current aims toward biobased products and sustainable processes have changed the picture.

How can we broaden the color palettes of natural dyes to make them a realistic alternative to synthetic dyes in terms of diversity?

This study focuses on the idea of developing harmonic color palettes for natural textile printing using locally cultivated dyes as a tool for designers working on printed textile designs for smaller industrial production. Various techniques are employed to expand the color palette, including overprinting techniques, mordants and working with different pH values in the recipes. Additionally, two printing methods are used: direct printing and "dye style" technique.

Using established color theories, the printed color samples are analyzed individually and in combination to uncover the breadth and limitations of the potential color palette available to the designer. Concrete examples of color palettes in this study prove to be a useful way to establish an understanding of the possibilities and aesthetic solutions available to designers working with natural printed colors on textiles. Simultaneously, the achievable solutions within the color palettes also highlight potential constraints and complications in the smaller industrial production of printed textiles.

The premise of limiting the selection of natural colors is grounded in the desire to work with locally cultivated colors and the requirements of dyes fastness to eventually be realized in smaller industrial production. Based on the experiential knowledge gained, this paper will also discuss the developed method, process used in the investigative craft practice that forms the basis for the developed color experiments.

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Keywords: Natural colors, sustainability, resources, craftsmanship, colorants

Trend forecasting of bio-based colourants in the fashion and textile industry

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Abstract

A deep understanding of the alignment of the design process with company strategy is important and has been calculated to add 50% to the whole value chain for the fashion and textiles sector (Cooper et al., 2017) Colour, the very first decision made in the fashion design process, is influenced by the designer's creative response to the zeitgeist, trend forecasting, and factory capabilities (Hidefi, 2017). In the history of textile and fashion industries, the colour technology revolution in synthetic dyes transformed the visual culture of fashion in the 19th century (Blaszczyk, 2023) and changed the significance of forecasting fashion trends (including colours, silhouettes, and fabrics). The colour forecasting currently requires the textile industry to predict colour trends and specify dye recipes. However, with the rise of bio colourant utilisation among textile and fashion designers recently, questions have been raised: What are the implications of introducing bio-based colourants into the design brief – does it influence the process, the decision making, time, and production volume? What are the implications for trend forecasting? This paper will present the results from a structured literature review of trend forecasting in the textile and fashion industry and the implications of incorporating bio-based colourant practices by textile dyers into colour forecasting. The literature review will inform a set of in-depth interview questions to be conducted with fashion designers, textile designers, and textile dyers as part of an ongoing study for the Colour4CRAFTS (C4C) project. The C4C project aims to cultivate craft skills in textile colouration, transform traditional processes, and shape cultural practices into sustainable, cutting-edge solutions for the future of bio-based practices in creative industries and industrial-scale textile production.

Controlling color and stability of biocolourants

<u>Session speaker:</u>

Associate Professor Heidrun Halbwirth, Technical University of Vienna

The real green chemists: how plants paint leaves, fruits and flowers

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Abstract

Plants produce a broad spectrum of pigments for many purposes, of which light harvest, protection against UV light and visual signals are the most important. Plant pigmentation covers all the colours of the rainbow, from red to yellow, green, blue and violet, including also white and black colouration. Particularly flowers can show every imaginable hue of red, orange and magenta. Fine nuances are frequently achieved by small modifications of basic structures, which themselves can be remarkably complex. Pigments are soluble in natural solvents, biodegradable and, considering the limited lifespan of respective tissues and organs, sufficiently long-lasting. While plant pigmentation seems to be lavish and abundant, the production of pigments strictly follows the rules of economic efficiency: production occurs locally, on demand, just-in-time, and exploits common, cheap intermediates and green energy. Colour effects are ultimately achieved by low concentrations of pigments and their sparing distribution at a cellular level.

In this talk, an overview of the colour chart of plant pigments as well as selected aspects of their production, cellular distribution, and the main drawbacks in their exploitation for dyeing are discussed.

Pyranoanthocyanin production from byproducts of the agroindustry

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Abstract

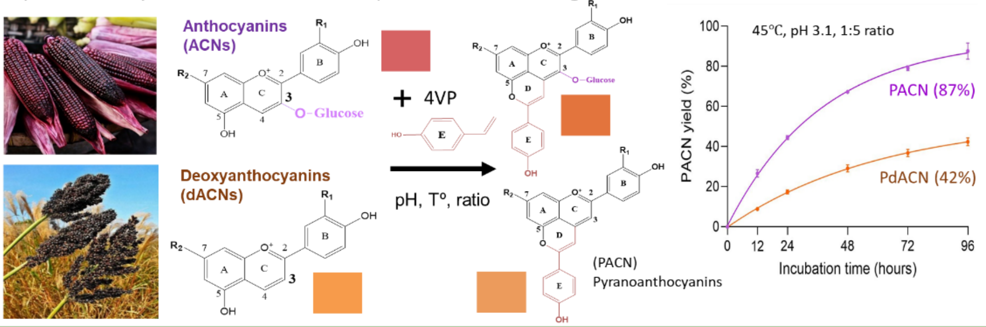
Today's consumers demand foods with cleaner labels and made with naturally derived ingredients, preferably plant based. Anthocyanins, polyphenols widely distributed in plants, offer a spectrum of colors from orange to red to blue across different pH levels and are associated with health benefits. However, they exhibit limited stability, hindering their application. Pyranoanthocyanins are anthocyanin-derived compounds naturally formed in red wines during fermentation and maceration [1] and exhibit enhanced stability. However, pyranoanthocyanin are scarce in nature and form slowly and inefficiently select foods [2]. Inspired by wine, we have investigated different approaches to increase PACN formation efficiency by controlling the choice and ratio of the reactants, temperature, pH and agitation, reducing the formation time to just days [3]. In this study, we focused on the potential production of pyranoanthocyanins from byproducts of the agroindustry to facilitate their production as sustainable food colorants.

Anthocyanin-rich materials included grape pomace, black sorghum bran, purple corn cob, and other residues from food colorant production. Alkaline hydrolyses and lactic acid fermentation were used to release phenolics bound to the insoluble residues and to induce decarboxylation of hydroxycinnamic acid. Different ratios of starting phenolics, pH and temperatures were compared to determine the optimal conditions for PACN formation.

Alkaline hydrolysis released hydroxycinnamic acids and anthocyanins from grape pomace and other plant residues. Incubation with select strains of lactic acid bacteria induced decarboxylation of hydroxycinnamic acids to form the more reactive 4-vinylphenols. Incubation at temperatures between 40 and 45 Celsius at pH between 2.7 and 4 induced PACN formation with yields of ~ 90% of the original anthocyanin content in 96 hours. Deoxyanthocyanins from sorghum produced PACN less efficiently under the conditions evaluated, with yields of ~40% in 96hr.

Efficient PACN production from pigment-rich and agro-industrial food byproducts could make PACNs into promising food colorant alternatives.

Pyranoanthocyanin Formation from Purple Corn Cob and Sorghum Bran



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Keywords: Pyranoanthocyanins, food colorants, upcycling, agro-industrial residues, sustainable

"Fifty shades" of black: melanin pigments as a unique source of inspiration for the implementation of materials endowed with tunable chromophoric and functional properties

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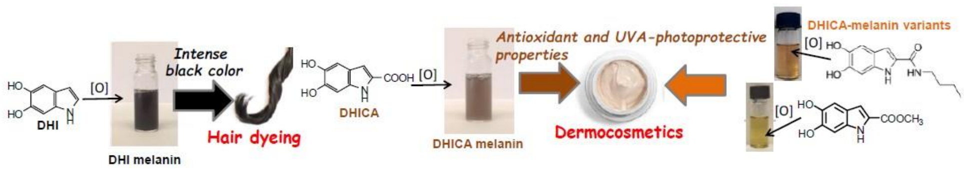
Abstract

Melanins are the key determinants of skin and hair pigmentation in humans and mammals. The most widespread class of these pigments are the dark brown-to-black eumelanins, originating in melanocytes by tyrosinase-catalyzed oxidation of tyrosine via 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Due to their peculiar chemical-physical properties, eumelanins have stimulated considerable interest as potential biocompatible functional materials. However, the occurrence in very low amounts in natural tissues together with the chemical heterogeneity make the actual and rational use of these pigments rather impossible. Biomimetic pigments obtained by the oxidative polymerization of the eumelanin precursors DHI and DHICA have therefore emerged as the most valuable alternative for the exploitation of the multifaceted application opportunities of melanins. Herein we will report the results of a several studies showing how it is possible to tune the chromophoric and functional properties of bioinspired melanin pigments by proper selection of the indole precursor and of the oxidizing conditions. In particular the key role of the carboxyl group in determining the color, the solubility and the antioxidant properties of melanin pigments will be presented. Indeed, melanins from the carboxylated precursor DHICA exhibit lower visible light absorption and hence appear lighter in color relative to the intense black colored DHI-based melanins, but are endowed with markedly enhanced antioxidant properties (Panzella et al., 2013). The possibility to exploit DHI oxidation giving rise to black pigments to develop hair dyeing systems as well as as the potential of variants of DHICA melanin with good solubility and marked antioxidant properties as ingredient for cosmetic formulations (Argenziano et al., 2023; Panzella et al., 2018) will be also presented. Finally, the novel oxidation pathways of DHI under solid-state, more sustainable reaction conditions will be reported.



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Keywords: melanins, dihydroxyindole, UV-Vis absorption, antioxidant, cosmetics

From red to green: oxidative coupling of chlorogenic acid with amino acids as a facile route to colorants with remarkable thermal stability for food applications

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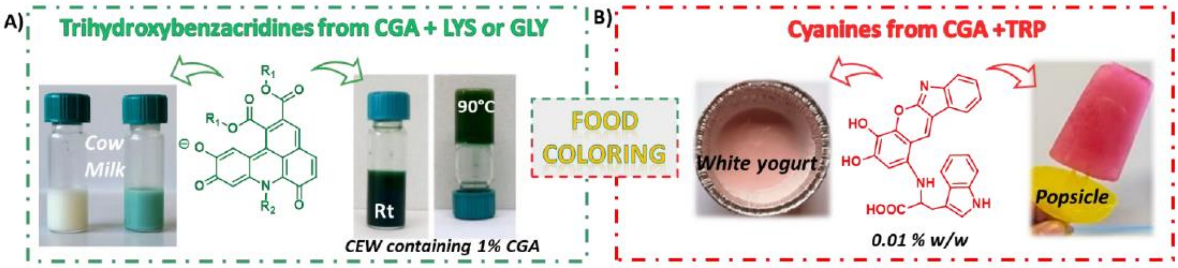
Abstract

Chemical interactions between proteins and phenols are the focus of great deal of research in the food sector, though in most cases these are regarded as unfavourable processes. A remarkable example is the greening of defatted sunflower proteins, ascribed to the reaction of the amino acid residues with oxidized chlorogenic acid (CGA), preventing the use of sunflower meal as protein source. Only recently the possible exploitation of this pigment-forming reaction for the preparation of food-grade green coloring agents has become increasingly attractive. We report herein the optimized biomimetic preparation of the green trihydroxybenzacridine pigments from CGA and amino acids like glycine (GLY) and lysine (LYS) in air at pH 9, their purification by gel filtration chromatography, and characterization of the pH-dependent chromophore (Panel A). Similar green pigments were obtained by analogous reaction of CGA with bovine serum albumin, or by simply adding CGA to chicken egg white (CEW). (Iacomino et al., 2017) Differently from all other aminoacids, tryptophan (TRP) leads to the development of an intense red coloron reaction with CGA. The red pigment prepared under the above conditions (37% yield) was evaluated as food dye against commonly used natural red pigments. The main pigment component was formulated as an unusual benzochromeno[2,3-b]indole linked to a TRP unit, featuring a cyanine type chromophore (panel B) (Moccia et al., 2021). Addition of the trihydroxybenzacridine pigments or pigmented CEW to different food matrices imparted intense green hues, whose thermal stability proved satisfactory up to 90 °C. An intense, thermally stable (90°C) coloring of both hydrophilic and lipophilic food matrices was obtained with the red pigment. No significant toxicity against human hepatic and colonic cell lines was exhibited by both the green and the red pigments at doses compatible with common use in food. The pH-dependent trihydroxybenzacridine chromophore was exploited for fish deterioration sensing.



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Keywords: food colorants, trihydroxybenzacridines, chlorogenic acid, cyanines, thermostability

Lightfastness of natural colorants in polylactic acid matrix

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Abstract

In modern society, most dyes and pigments come from petroleum-based sources or mining. Obtaining those dyes creates immense stress for the environment, nudging researchers towards more eco-friendly solutions, such as colorants from natural sources or industrial side streams. Even though natural colorants and their stability properties are well known in textile coloration, their potential in plastic coloration remains relatively unexplored. Thus, our study presents lightfastness properties of naturally colored bio-based polylactic acid (PLA) matrix. Five natural colorants are examined, namely wood-based biochar and lignin, purified natural indigo from *Isatis tinctoria*, alizarin from *Rubia tinctorium*, and luteolin from *Reseda luteola*. Indigo, alizarin and luteolin are plant-based colorants mainly used in natural coloration of textile fibers, whereas biochar and lignin are more known as fillers than as colorants due to their high availability. Among these colorants, only natural indigo had previous studies about its lightfastness in thermoplastic matrix. Stemming from promising results with natural indigo in the previous studies, this study broadens the scope with mechanical analysis methods and new colorants.

In this study, the colorants and PLA granules were injection molded into sample rods, which were exposed to a lightfastness test (ISO 105-B02). Appearance and lightfastness of the samples were assessed with visual inspection, reflectance spectrophotometry and Fourier-transform infrared spectroscopy (FTIR) before and after the Xenon arc lamp test. Furthermore, effect of the light exposure for mechanical properties of the samples were assessed with tensile testing, differential scanning calorimetry (DSC) and dynamic mechanical analysis (DMA). Most of the colorants produced an even and stable color with good lightfastness without weakening mechanical properties of the thermoplastic. Based on the results, further study of especially biochar and lignin as colorants is recommended due to their good stability and high availability.

Keywords: Color stability, lightfastness, mechanical properties, natural colorants, thermoplastics

Is natural better?

Session speaker:

Lorenzo Li Greci

Herbarium cyanotype as a form of preservation of memory

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Abstract

An herbarium is a collection of dried, pressed, and cataloged plant samples along with associated information about where, when, and by whom they were collected. Herbaria contain the information about biodiversity, geographical distribution and morphological characteristics relating to the flora of a given region in a precise time frame. The creation and the preservation of an herbarium are two practices that combine the scientific and the artistic knowledge. Herbaria can also be seen as tangible examples of the interaction between humans and nature, inviting us to consider plants as entities, beyond their simple biological aspect.

Many herbaria that are not maintained in specialized institutions will undergo deterioration due to physical, chemical or biological external forces leading to the complete destruction of the dried plants collection. To overcome this inevitable loss the tested botanical cyanotyping with old plant speciments as a method to preserve the memory of Marianna Tidström's work from 1935 when she has collected and cataloged plants from Finnish landscapes for a school assignment. Cyanotype is an alternative photographic approach that relies on the reduction of ferric ions to create the Prussian Blue pigmentation after UV exposure. Botanical cyanotypes use plant shadows to block the chemical reaction mediated by UV light, capturing the shape of the plants usually on paper. In the talk I will elucidate about: the chemistry and the physics involved in the process, as well as, toning with lignin and alternative processes in the field of contemporary art.

The bio-colour trajectories for textile practices

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Abstract

This paper aims to decipher a chronological overview of the bio colourants progression from prehistoric times to the present for textile practices using the context of time. Human beings have always been interested in bio-colours; the art of dyeing has a long past and many dyes go back into the prehistory. It's interesting to question, "What came first, what came next, where, and why?" recalling the significant bio colorants history (Sarkar et al., 2022). Varied bio-colourants are available in India (Gulrajani, 2001) and around the world (Kay-Williams, 2013) for textile applications. With time, bio-colour sources, regional distribution, technologies, practices as well as perspectives have changed for creating textiles.

This paper traces the evolutionary shift of the popular bio-colorant gamut and practices from past to present through methods like literature review, archival records, and museum visits investigating them on a historical time scale. The findings reveal that modern bio-colorants for textile practices can be uniquely derived from plants, insects, invertebrates, minerals, and microorganisms creating an understanding of Bio-colour practices from various elements of nature. A detailed qualitative and comparative analysis delineates the intriguing colour stories, experiences, and preferences of various ages. Moreover, the science behind biocolours has changed the historical trajectories providing insights for the civilization and cultural know-how to address today's challenges. The paper reflects on how the evolution of Bio-colourants helps individuals and organizations to make informed decisions for future biocolour practices and sustainable textile and design applications. The biocolour practices act as a bridge between ages, civilizations, and textiles stimulating interdisciplinary design thinking. Additionally, the biocolour trajectories studied over various generations also construct a deeper understanding of the evolving relationship of fabric and natural colour on the planet and how socio-cultural, environmental, and economic conditions shaped them.

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Keywords: Textile Biocolours, Historical evolution, colour stories, sustainable practices

Exploring the dyeing properties of plant extracts for sustainable and efficient hair coloring

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Abstract

Traditional hair coloring products, often containing synthetic dyes, have been associated with potential long-term damage to hair structure and the risk of irritant and allergic reactions. In response to these concerns, there is a growing interest in the utilization of natural dyes derived from plants. These herbal dyes are recognized for their biodegradability, sustainability, eco-friendliness, non-toxicity, cost-effectiveness, and widespread applicability in various industries. This work aimed to investigate the dyeing properties of twenty powder plant extracts under different conditions, with a focus on developing alternative, efficient, and long-lasting hair-dyeing methods, utilizing tannin structures.

The methods involved diluting each plant extract in water at 40-45°C in different ratios to create fine pastes. Testing was conducted at both acidic and basic pH levels, using different hair types (yak, salt and pepper, and bleached human hair) for treatments lasting 45 and 120 minutes. Some plant extracts were also tested in synergy, and the obtained colors were stabilized through the addition of tea or coffee containing tannins to the pastes.

Results demonstrated that ten plant extracts produced diverse colors at acidic pH, including red, yellow, brown, and neutral shades. The addition of an alkaline agent influenced the dyeing properties, especially when combining certain plants and applying them to previously bleached hair. Time of application also impacted results. Furthermore, the addition of tea or coffee powder enhanced color intensity and brightness, likely due to their tannin content.

The discussion highlights the role of tannins in enhancing color fixation on hair fibers through complex compound formation. The study suggests that previous hair bleaching enhances the effectiveness of tannin complexes, promoting stronger cross-links between keratinous fibers and coloring complexes. The study concludes that the color strength of dyed hair depends on various factors, emphasizing the potential of plant extracts for sustainable and efficient hair coloring.

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Keywords: natural hair coloration, hair care, plant hair dyes, plant tannins, tannin complex

Derivatization of natural dye emodin for utility for fiber coloration

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Abstract

There is growing interest in utilizing natural dyes as renewable resources in industrial processes such as textile dyeing. Anthraquinone dyes such as emodin (6-methyl-1,3,8-trihydroxyanthraquinone) show promise as natural dyes to employ in waterless processes such as supercritical CO2 and atmospheric plasma. However, their mutagenicity, dependent on exogenous metabolic activation (CYP 450 from rat liver S9) limits their commercial application. Therefore, a study was dedicated to the synthesis of acryloyl ester and allyl ether derivatives of emodin, in an attempt to reduce the mutagenic potential of the parent dye and to introduce a functional group needed for bonding to textiles using atmospheric plasma.

Emodin was derivatized by reacting the parent dye with 3-ethoxy-acryloyl chloride or allyl bromide in the presence of triethylamine. The structure of each of the derivatives was established using high resolution mass spectrophotometry and proton and carbon nuclear magnetic resonance spectroscopy. In addition, the derivatives were compared to emodin using UV-Vis spectroscopy, whereupon absorption maxima in the yellow region were obtained (409-434 nm). Metabolism studies involving incubation with S9 enzymes suggested the cleavage of acryloyl ester groups, regenerating emodin. In contrast, allyl ether groups showed resistance to cleavage by these enzymes. Furthermore, textile coloration studies were conducted to compare the depth of shade of the derivatives to emodin. All derivatives resembled emodin regarding their dyeability of textile fibers using supercritical CO2.

Plasma dyeing studies using the derivatives are currently in progress. Although finalizing the comparison on the mutagenicity of emodin with different derivatives is also underway, preliminary results indicate the allyl ether of emodin as a promising alternative to the parent dye.

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Producing safe natural dyes: elimination of the mutagenicity and aquatic toxicity of emodin using derivatization

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Abstract

Traditional textile dyeing processes use large volumes of water and can generate toxic/mutagenic effluents. Waterless methods provide alternative media for the dyeing process and consume less energy, and one example is the supercritical carbon dioxide (sc-CO_2) method (Montero et al 2000). The natural anthraquinone emodin showed promising results for dyeing different fibers in this process. However, emodin was mutagenic to bacteria (TA1537) with metabolic activation (S9) in the Salmonella microsome assay, perhaps because of its planar chemical structure, and caused chromosomal damage (increase in micronuclei) in the crustacean *Parhyale hawaiensis* (Magalhães et al., 2023). This study aimed to develop a viable emodin derivative by adding a substituent group that removes its planarity and consequently its mutagenicity. Two different derivatives were produced using ethoxy acryloyl chloride (Em-DR1 and Em-DR2) and one using allyl bromide (Em-allyl). Em-DR1 and Em-DR2 were as mutagenic as emodin (Magalhaes et al. 2023) but Em-allyl was not when tested in the bacteria assay using TA1537 (Fig 1) and with another 4 strains in the presence and absence of metabolic activation (rat liver S9) as recommended by OECD (TA1535, TA100, TA98 and TA102) (OECD, 2020). Tests *in vivo* with *P. hawaiensis* to verify chromosome damage are being conducted. Because emodin was highly toxic to the microcrustacean *D. similis* (EC50 =0.13 mg L-1) and to fish embryos (LC50 = 0.025 mg L-1), category 1 for acute aquatic toxicity in the GHS, we also tested Em-allyl with the same organisms for comparison. Em-allyl was not acutely toxic to *D. similis* and a test with fish embryos is underway. These results show that derivatization techniques can be a viable way to remove/reduce the mutagenicity and aquatic toxicity of natural dyes such as emodin, enabling their safe use.

Acknowledgements: BioColour project n◦ 327178, 327213 and 352460, Capes financial code 001, Fapesp ToxBiocolour Project n° 2020/04628-8, and the fellowships FAPESP n°2021/063969-2 and FAPESP n° 2022/04482-9. The fish studies were approved by the ethics committee (UNICAMP) (protocol n° 5645-1/2020)

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Keywords: Ames test, Daphnia, micronuclei, fish embryos, emodin, waterless dyeing

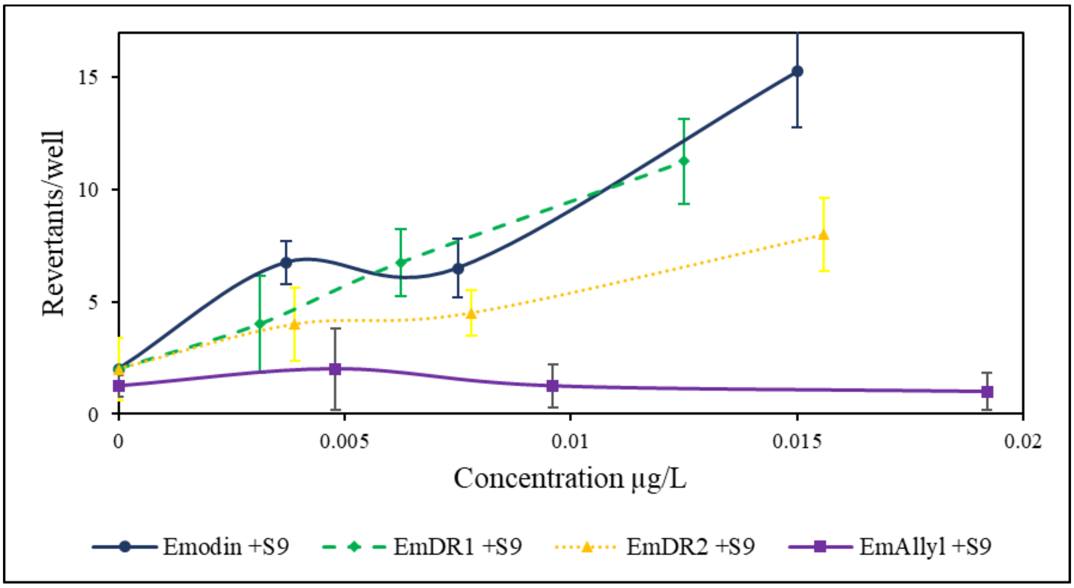

Figure 1 – Comparison of the bacterial mutagenicity of emodin with the acryloyl esters (Em DR1,Em DR2) and allyl ether (Em allyl) derivatives with TA1537 in the presence of metabolic activation (S9).

Variety of materials in applications of biocolourants

Session speaker:

Emeritus Professor Kumi Yoshida, Nagoya University

Anthocyanins in colored seed-coat of beans and their potential

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Abstract

Most of colored seed-coat of beans contained anthocyanins. Their structures are relatively simple compared with those of flower petals. In beans mono- and di-glycoside of anthocyanidins are major, contrasting to this, in flower petals acylated anthocyanins and complicated metal-complex pigments are found.

We have been interested in anthocyanins of colored seed-coat of beans. We isolated anthocyanins from seed-coat and determined the structure and quantified the contents [1]. In black soybean (Glycine max) cyanidin 3-O-glucoside (Cy3G, 1) is contained and its contents is more than 15 mg/g dried seed-coat. In red kidney bean (Phaseolus vulgaris cv. Kintoki) pelargonidin 3-O-glucoside (Pg3G, 2, 3.6 mg), in scarlet bean (Phaseolus coccineus) delphinidin 3-O-glucoside (Dp3G, 3, 2.2 mg). However, in small red bean (Vigna angularis) very low amount of cyanin (< 0.0003 mg) is contained and instead of cyanin, purple colored pigment, catechinopyranocyanidin A, B (cpcA 4, B 5) was found [2].

The biosynthetic pathway of Cy3G (1) has been well studied, and almost all genes and enzymes involved have been identified. However, we have found a new precursor of Cy3G, namely 5,7,3′,4′-tetrahydroxyflav-2-en-3-ol 3-O-glucoside (2F3G, 6). Through quantitative analysis, it was clarified that during natural maturation and the rapid color change, an increase in the Cy3G content was observed along with the corresponding decrease in the 2F3G content. Our findings allowed us to propose a new biosynthetic pathway of Cy3G via a colorless glucosylated compound, 2F3G, which was oxidized to give Cy3G [3].

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Bio-based camouflage fiber foams from cellulose and biocolorants

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Abstract

Foams are versatile materials utilized in a wide range of applications including cosmetics, firefighting, packaging, and thermal insulation. (Hill et al., 2017) Owing to the insulating properties, they provide also thermal stealth properties that are useful for instance in camouflage applications (Lohtander et al., 2022). When combined with colorants derived from Nature, the foam can provide stealth in full spectral range in a boreal forest environment (Forsman et al., 2021).

Here in, we produced bio-based, lightweight wet foams from cellulosic fibers and two biocolorants; green microalgae Chlorella vulgaris and brown kraft lignin. The fiber foam provided stealth in the infrared light region while the biocolorants camouflaged the foam in visible light wavelengths. The foaming and stability of the wet foam were optimized using an experimental design, which indicated the key parameter in the thermal performance of foams was the cellulose content. The green and brown biocolorants gave similar spectral fingerprints in the visible light range compared to natural reference materials from the boreal forest.

Overall, these results demonstrate that wet cellulose-based foams colored and stabilized using microalgae and lignin are environmentally friendly options for short-term camouflage since the biocolorants and chemically unmodified cellulosic materials are readily biodegradable. The findings of this study also give insights into how stability impacts the thermal and spectral properties of wet fiber foams.

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Keywords: Nanocellulose, Foam, Biocolorants, Lignin, Microalgae

Production of violacein from *J. lividum* for dyeing wool

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Abstract

Today the textile industry is largely reliant on synthetic dyes because of their low cost and high colour fastness. However, the use of synthetic dyes has a substantial impact on the environment. Textile processing is responsible for large amounts of wastewater, in which recalcitrant synthetic dyes can cause significant harm to aquatic environments (Lara, L., Cabral, I., & Cunha, J., 2022). One relatively new and emerging alternative to synthetic dyes is the use of microbially produced pigments. These can be produced sustainably in large amounts and often at a relatively low cost (Kramar, A., & Kostic, M. M., 2022). A wide palette of different colours can be produced by microorganisms, but in this study the focus was put on the purple pigment violacein, produced by the bacterium *Janthinobacterium lividum*.

In the realm of natural dyes, the colour purple has always been considered unique and rare; however, violacein is not only interesting because of its colour. Violacein has a number of bioactivities, including broad-spectrum antimicrobial activities, which encompass antibacterial, antiviral, and antifungal properties.

In this project, cultivation conditions of *J. lividum* were optimized with the aim of maximizing violacein production, and furthermore to investigate the possible use of violacein for dyeing Icelandic wool.

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Keywords: Violacein, Microbial, Dye, Cultivation, Textile

Extraction of marine-based colourants for sustainable materials

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Abstract

The negative impact regarding ecological and health issues of synthetic dyes makes it necessary to find for more eco-friendly alternatives, placing back natural products as main solution. In this context, microorganism pigments gain prominence, since their production at industrial scale has economic advantages when compared to the obtention of conventional natural dyes, being faster to produce [1]. The colouring potential of microalgae pigments, together with their reported environmental benefits [2], make the exploration of these natural materials as colourants an appealing path to follow.

This project aims to study different extraction processes with more sustainable techniques and materials to obtain natural and functional marine-based colourants and incorporated them into fibres, and woven and knitted fabrics. Thus, the colour of aqueous and hydroethanol extracts obtained from different microalgae species were evaluated. Different tones and intensities were achieved for different solvent proportions (Figure 1).Since pigments obtained from microalgae also depend on the extraction methodologies, the impact of diverse extraction processes and conditions was assessed.Several coloured extracts from microalgae were obtained using conventional maceration, ultrasound assisted extraction, and pressurized liquid extraction. Despite the similarity of tones the coloured extracts, the intensity reached through the ultrasound assisted extraction stood out from the other methodologies.

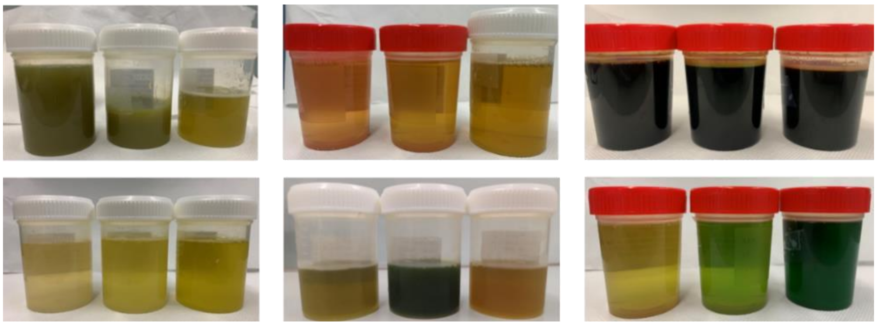

Figure 1. Examples of coloured extracts obtained from microalgae.

Resulting data are valuable for the optimization of assisted extraction techniques to adjust the colour tone and intensity given by microalgae extracts. This is particularly useful for the textile industry, interested in finding valid alternatives to the wide range of colours that can be achieved using synthetic dyes, but also for other sectors.

Lastly, to improve the ecological footprint of final products, the incorporation of these colourants into textiles is performed with more sustainable technologies. These marine-based colourants will give rise to value-added textile and footwear products.

Acknowledgement: This work was developed in the scope of the Project “Blue Bioeconomy Innovation Pact”, co-financed by the Recovery and Resilience Plan (RRP) and by the European funds NextGeneration EU (https://recuperarportugal.gov.pt/), through the Incentive System “Agendas for Business Innovation”.

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Keywords: marine colourants, extraction, sustainable materials

Acknowledgement: This work was developed in the scope of the Project "Blue Bioeconomy Innovation Pact", co-financed by the Recovery and Resilience Plan (RRP) and by the European funds NextGeneration EU (https://recuperarportugal.gov.pt/), through the Incentive System "Agendas for Business Innovation".

Vouwo mud dye: a material, microbiological and cultural approach to researching sustainable dyes

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Abstract

Colouration using soil-based dyes is a fast-growing subject of enquiry in fibres and textiles surface design, where the pigment oxides, fungi and bacteria-producing pigments contained in soils are extensively researched in industrial and high-end bio-fashion (Lin & Mammel, 2012). Nevertheless, dyeing techniques using this rich natural resource have been integral to long-standing textile traditions in parts of Asia and Africa (Jabasingh, 2019). Grounded in the anthropological field study of the dyeing practice of Marka-Dafing hunters in Burkina Faso, West Africa (Douny et al., 2023), this paper presents a descriptive analysis of the colouration mechanism operative in vouwo, or mud dye, a dye that shows as shades of dark brown on fabrics and which is generally produced for domestic use. The paper highlights aspects of the complex biochemical and microbiological interactions occurring in the dye and curated by dyers throughout the year-long alkaline fermentation process, interactions that help to make the colour more durable. The authors propose an interdisciplinary approach to the study of vouwo that combines craft practice with a materials science–based elemental analysis of iron (via energy-dispersive X-ray (EDX) microanalysis) and a microbiological description of bacterial iron metabolism and of complex interactions with plants such as Acacia nilotica (added in the dyeing procedure) and the bacteria contained in mud or termite mound soil (Cubitermes). A detailed examination of the consecutive steps of the collection of materials in the savanna, the preparation of the bath, followed by the dyeing and maintenance of mud-dyed fabrics allows us to underline the material, biochemical, microbiological and cultural properties of vouwo dye, while noticing aspects of environmental and social sustainability in the craft value chain. Dyeing with soil as a natural colourant for the future calls attention to the role of traditional craft knowledge in the production of sustainable dyes in dialogue with modern science.

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Keywords: Soil, iron oxides, colouration mechanism, interdisciplinary descriptive analysis, Burkina Faso.

Biocolour spectrum: exploring cultural perspectives of dyes

Session speaker:

Professor Richard Blackburn, University of Leeds

Extraction of anthocyanins from food industry waste and their application for fibres, textiles and cosmetics

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Abstract

The environmental impact toxicological effects of colorants used in the cosmetics, textiles and food industries can no longer be ignored and sustainable alternatives are in demand for all these industries. Anthocyanins are water-soluble polyphenolic pigments that occur in a wide variety of fruits, vegetables and flowers – waste materials from food processing industries offer a significant opportunity to valorise pigments from this waste for coloration applications. Anthocyanins were extracted from waste epicarp from the fruit pressing industry using and extraction and purification process; chemical constituents were characterised and quantified. In application of anthocyanin-rich extracts on hair, intense blue-coloured dyeings could be achieved which displayed good stability to multiple washes. These colorants could also be used to provide blue 'toning' products to counteract yellowing in bleached hair, to offer sustainable and safe alternatives to 'purple shampoos' that are commonplace in the beauty market. In textile application, regenerated protein fibres were dyed with anthocyanins; under optimal conditions, dyeing is achieved with medium depths of colour with good wash fastness. Highest sorption of anthocyanins is observed at pH 4, where anthocyanins are a mixture of 60% neutral purple quinonoidal base form and 40% flavylium cation form. Presence of alum in the dyebath enhances sorption of anthocyanins onto fibre at pH 4 due to formation of Al–anthocyanin complexes. Wash fastness of the dyeings is better as pH increases and as temperature increases. In related work a new, novel method for in situ coloration of regenerated protein fibers was developed using an anthocyanin-based natural dye in a wet-spinning process, to reduce the environmental impact of the dyeing process. Coloured fibres were achieved that required half the water consumption and minimal energy compared to the conventional dyeing system. In widening the application of anthocyanins, enzymatic acylation increased stability without compromising bioactivity and chromatic features.

Colour for the 21st century

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Abstract

Since the industrial revolution, the fashion industry has evolved to use synthetic dyes. This is very much a manifestation of what McDonough and Braungart highlight in their book, Cradle to Cradle, when addressing our still 19th century production practices. When it comes to colour the textile industry is a major contributor to this linear model, 'putting billions of pounds of toxic material into the air, water and soil every year.' (McDonough, 2009). A possible alternative, natural dyeing that uses plant-based sources to create colour, has on the whole been relegated to craft use. Reviving techniques and adapting the use of natural colour for contemporary textile production is the subject of the research. It has particular relevance at this time, contributing to the critical discourse on how we make things (McDonough, 2002) and the impact on the environment. In order to develop a strategy that fosters both environmental concerns and works with industry, we have explored some key techniques that develop this process of dyeing. Alongside our industry partner, ao textiles have been able to investigate and determine whether plant based dyes can be part of 21st century circular production. This paper will discuss the challenges as well as the more tangible successes of the project, contributing to a wider industry and academic based conversation on how to elevate sustainable craft based practices into circular (Ellen Macarthur Foundation, 2017) and viable production methods. The research considers how to transition from small-scale production to reliable large scale applications of natural dyes with industry impact. ao's extensive experience and research in this field allows distinct and innovative design outcomes. We now create fabrics using natural colour that are scalable, repeatable, compostable and of the quality demanded by industry. ao textiles show that naturally sourced materials and sustainable production techniques provide superb, high-quality results.

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Ormondroyd, G., 2018. Designing with Natural Materials. 1st ed. UK: CRC Press. Keywords: Circular, Colour, Textiles, Natural dyes, Fashion

Adsorption of red onion dye on cellulose substrates exploiting chitosan as a natural mordant

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Abstract

Biobased plant-derived colorants and biomordants have great potential for the development of more sustainable textile dyeing processes. Colorants isolated from biomass residues are renewable, biodegradable, and in general less harmful than synthetic colors. Interestingly, they may also bring additional functions to the materials. However, the extraction and purification of the biocolorants from biomass as well as their dyeing efficiency and color fastness properties require a more thorough examination. We extracted red onion (*Allium cepa*) skins to isolate polyphenolic flavonoids and anthocyanins and characterized the chemical composition of the mixture. To study the adsorption kinetics of the polyphenol extract *in situ* onto thin films of cellulose nanofibrils, CNF, we used a quartz crystal microbalance technique. The effect of different mordants on the adsorption behavior was investigated and compared with conventional dyeing experiments of textiles. These experiments enabled us to determine the interactions of the dyes with substrates and mordants. Chitosan showed high potential as a biobased mordant based both on its ability to facilitate fast adsorption of polyphenols to cellulose, and its ability to retain the purple color of the red onion dye (ROD) in comparison to the metal mordants. The ROD also showed excellent UV-shielding efficiency already at low concentrations (Grande, 2023).

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Keywords: red onion, flavonoids, chitosan mordanting, biopolymers

Consumers’ insight on the origin of colorants: “When it comes to natural colours, I think of something like red soil and indigo.”

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Abstract

Natural colourants of both organic and mineral origin provide a more sustainable alternative to oil-based synthetic ones. However, in the production of everyday consumer products, synthetic colourants are still widely used. For example, within contemporary textile industry, the use of natural colourants has been marginal, covering around one percent of colour production (Saxena & Raja, 2014). Similarly, the food industry continues to use synthetic colourants despite their negative effects on human health (Neves et al., 2021).

Consumers are concerned about the potential health risks related to colour-related chemicals in consumer products (e.g. Autio et al. 2023), particularly when they are in direct contact with the human body, as in the case of textile colours or food additives. They are also concerned about the potential harm the chemicals cause to the environment. According to Autio et al. (2023), consumers associate natural colorants with ecological sustainability, while synthetic colorants are linked to artificiality and environmental burden. However, the existing research is still limited in relation to consumers’ understanding of the origin of colourants and the meanings consumers attribute to them.

We study the meanings Finnish consumers (n=59) associate with the origins of colour in consumer products, approaching the topic from the perspective of qualitative interview data. According to our analysis, consumers possess a relatively broad insight of natural colorants and natural colour sources (such as beetroot and onion), whereas their knowledge of synthetic colorants is rather scarce. In relation to bio-based colours, consumers distinguish between plant-based and animal-based dyes, placing more value on colour sources of plant origin. Plant-based dyes are also associated with an ecological lifestyle, craftsmanship, and traditional ways of life. At the same time, synthetic colorants are linked to the chemical industry, laboratories, and toxicity.

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Keywords: Consumer, colour, synthetic, natural, sustainability

Re-envisioning decoloniality in the context of indian biocolor practices

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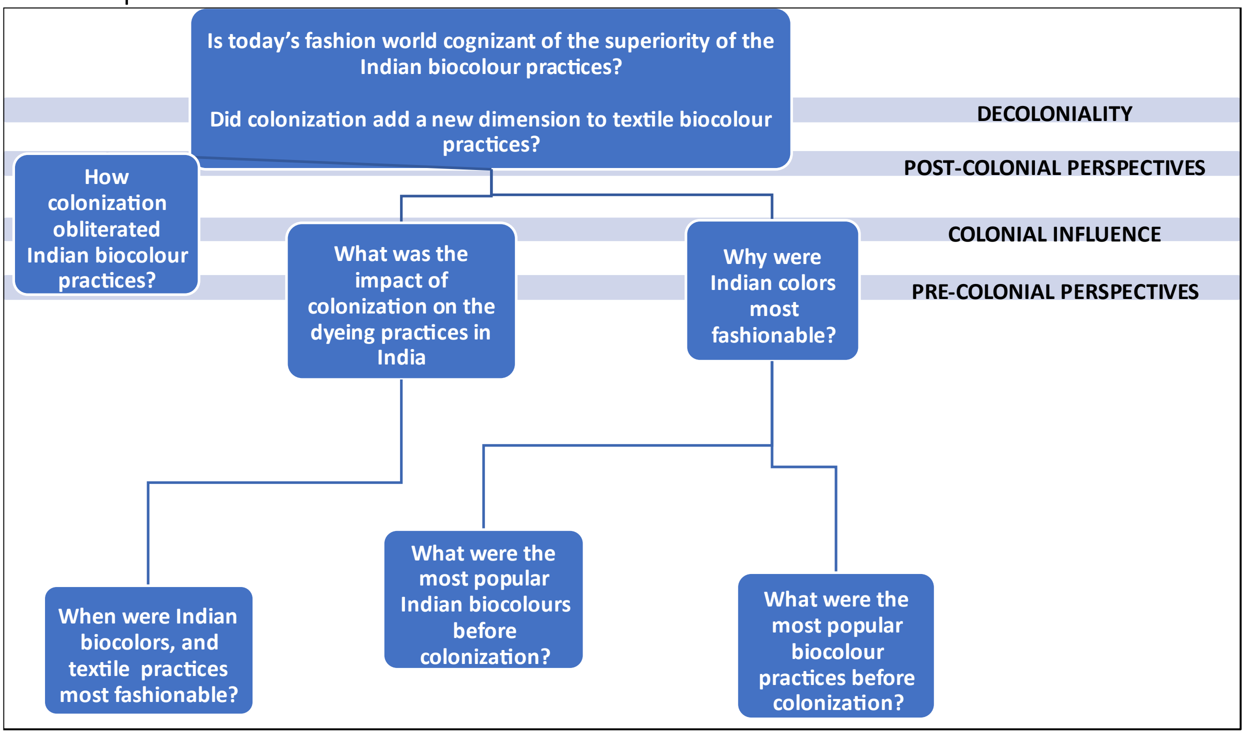
Abstract

Indian dyers' practices of natural bio-colours was key to their unrivaled mastery(Lemire & Riello, 2008), until Western chemical dyes were invented in the 19th century. The aesthetic significance of biocolours is written well, but aspects like socio-political exchanges, trade(Peck, 2013) and the influence of colonization on the Indian bio-colour practices are not documented. This research paper aims to understand the impact of colonization on indigenous Indian bio-colour practices and their choices as sustainable colourants. The major questions and arguments in the study are enclosed in the framework flowchart including Decoloniality, Post-colonial, Colonial influence and Pre-colonial perspectives. Some questions unfold “What was the impact of colonization on the dyeing practices in India Is today’s fashion world cognizant of the superiority of the Indian biocolour practices? Did colonization add a new dimension to textile biocolour practices? How colonization obliterated Indian biocolour practices?How colonization obliterated Indian biocolour practices?”



A detailed literature survey and textile museum archives reveal the first-hand accounts of several travelers, merchants, and scientific scholars, especially in the 18th and 19th centuries showing the innovation, resourcefulness, and ingenuity of the Indian communities(Global_Colors_dyes_and_the_dye_trade.Pdf, n.d.). This paper gives a detailed analysis on the Decoloniality perspectives revealing traditional Indian biocolours, mordants and practices derived from the used in India In today's textile dyeing industry, modern colour schemes portrayed by the brand's supply chain and customer choices are deeply rooted in colonialism . Indian bio colour practices and color palette choices need rethinking and envisioning and are extremely significant to portray a crucial component in the articulation of global textile practices. This research paper provides an important account of decoloniality comprehending colonial influence on Indian colour schemes, indigenous dyeing techniques, farming practices, dyeing communities, and their impact on the sustainable colour choices to meet future demands.

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Keywords: Socio-political exchanges, sustainable colour choices, Indian biocolour choices, decoloniality, indigenous dyeing techniques

Closing session

Ingrid Calvo Ivanovic, University of Chile

Sustainable colour design strategies: a framework for action

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Abstract

During recent years, it has become imperative to reflect on the social perspective, as the potential for fostering more sustainable user behaviour is a growing field of interest, together with a need for a careful evaluation of ethical issues. These concerns are also growing inside design practice and education, where colour is a fundamental resource. Design education should motivate a careful evaluation of a sustainable colour application, stimulating critical thinking and fostering concrete actions related to reuse, recycling, and recovering colours, among others. This paper proposes a reflection through eight strategies on colour sustainability to be addressed within design decisions. The strategies are (i) educating about colour management to optimize the technological processes of colour production and reproduction. Secondly, (ii) thinking critically about the impact of consumer culture and colour trends, which generate more waste and yearly exploitation of the planet's resources. Third, (iii) embracing colour relativity and lifecycles, as colours' fading, and evolution must be accepted and integrated as part of a product's lifecycle. Fourth, (iv) fostering identity through local colours can positively shape perceptions of territory and create reconnections with the land and landscapes. Fifth, (v) upcycling discarded colours, as industrial waste can contain valuable pigments which can be recovered, through experimentation that combines science and art. Then, (vi) exploring new ways of producing colour: colours and dyes that go beyond traditional pigments are emerging, such as bio colour and nanoscale colour, among others. The seventh strategy is (vii) embracing imperfection using visible recycled colour, as it can be a clever way to spark discussion and generate excitement about the prismatic potential of the things we throw away. Finally, at the core of the framework is (viii) promoting a sustainable colour mindset through the entire chain of decisions that are taken in a colour design process. This study aims to provide guidelines for design educators and practitioners to address these issues in the design process, and therefore, the strategies are presented through suggestions of concrete actions for their implementation.

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Keywords: colour sustainability, colour education, sustainable design, design education, sustainable decisions

Posters

Themes

- Sources and raw materials for sustainable colourants: Agro, biotechnology, aqua culture, food and forest
- Chemistry and technologies aiming for sustainable colours
- Safety and regulatory issues of bio-based colourants and products
- Bio-based colourants and materials
- Designing with biocolours
- Biocolours on markets and businesses
- Choices for sustainable colour - Societal and cultural perspectives
- Colour for the future

Sources and raw materials for sustainable colourants: agro, biotechnology, aqua culture, food and forest

Optimization of the heterologous production of anthraquinones in an industrial host

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Abstract

Anthraquinones constitute an important class of natural products that are known for their wide applicability in the field of textiles, food, cosmetics, paints, electronics, and pharmaceuticals, to list a few [1]. Emodin and endocrocin are common biosynthetic intermediates of anthraquinone compounds, found in many plants and fungal species [2] . Although, emodin and endocrocin are not of industrial interest, their structures could be further modified by a combination of O-methylation, oxidation, halogenation, dimerization, and glycosylation events to better adapt them for different applications.

We aimed to construct platform strains for emodin and endocrocin production by heterologous expression of the *Cortinarius- sp*. KIS3 polyketide synthase in different fungal hosts. To find the most suitable industrial host, we took advantage of the versatile synthetic expression system (SES)[3] , developed in-house at VTT, to construct and evaluate engineered strains of *Saccharomyces cerevisiae*, *Aspergillus oryzae*, *Yarrowia lipolytica* and *Trichoderma reesei*, for anthraquinone production. Furthermore, the Design of Experiment (DoE) strategy was used to optimize the cultivation conditions for the best production host. Our results highlight the important role of temperature, aeration, and media composition which in combination increased anthraquinone production by 12-fold. Generating efficient production strain for these anthraquinone precursors would open the way to modify these scaffolds *in vivo* or *in vitro* to tune their chemistry for diverse industrial applications.

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Keywords: Anthraquinones, Design of Experiment, Metabolic engineering, Synthetic biology

Fungal colorants as an alternative of synthetic resources: current developments, challenges, and opportunities

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Abstract

The global market for colorants is projected to reach €86.76 billion by 2030, with most of these colorants derived from non-renewable petroleum. The production process of synthetic colorants and its residuals can cause significant problems to environment and health. In light of these concerns, there is growing interest in natural colorants as a sustainable alternative. Fungal colorants are one of the promising candidates because of their high stability and lightfastness, their biocompatibility and relatively cost-effective production process. Till date, fungal colorants have been used for various purposes including food, cosmetics, pharmaceuticals, and textiles. However, the industrial productivity, scalability, and application possibilities of fungal colorants are not fully developed. The limitation of experiences and knowledge in these novel products creates a gap among research, commodity, and markets. To solve these problems, a multidisciplinary approach including fermentation yield improvements, extraction upscaling and application discovery is needed. In this comprehensive review we present the typical formation of fungal colorant value chains and discuss the technological bottlenecks together with recent breakthroughs. Especially, we describe the advancing precision fermentation applications in fungal colorants. Downstream of the value chain, we discuss the use of green chemistry in fungal colorant formulation and production. Across technological sections of the value chain, methods such as genome mining based target selection, fermentation control via Internet of Things (IoT) and super critical CO_2 based colorant extraction are especially described. Combined with the concept of circular economy, challenges of environmental impact and techno-economic studies on fungal colorants are also discussed. This review aims to help to estimate the current technological and economic potential of fungal colorants as a sustainable alternative to synthetic sources.

Keywords: fungal colorants, precision fermentation, green chemistry, biobased value chains, data driven natural product discovery

Bioactive compounds from plant -based unexploited resources as antimicrobial textile finishing agents

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Abstract

Various synthetic finishing agents are a substantial sustainability challenge in textile industry, thus there is a growing interest for bio-based solutions, while efficient utilization of bio-based side streams is a key focus in circular economy. In this research, the aim is to create concepts for the utilization of biomass waste from gardens, parks and greenhouses to produce active finishing agents for the textile industry[1]. In Finland alone, hundreds of thousands of tons of fallen leaves from parks and gardens are brought to waste management facilities every year. Rose production generates large amounts of green waste (on average 50 kg ha^{-1} d^{-1}) through crop maintenance, rejected produce and grading[2]. Current common practices of green waste management include incineration or landfilling, both posing negative ecological effects to the environment [3, 4].

We observed that extracts made of fallen autumn leaves and the rose cultivation waste contained various phenolic compounds and were possessing antimicrobial activity against e.g. human skin-related bacteria *Staphylococcus aureus*. These extracts are further assessed in textile applications. In addition, we investigate whether a commercial process can be implemented in a scale that fits into shipping containers, allowing the local utilization for different biomass streams.

Keywords: autumn leaves, rose cultivation, waste, antimicrobial agent

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UHPLC-MS analysis of microbially produced anthraquinone pigments

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Abstract

Due to the well-known environmental disadvantages of synthetic dyes, biocolourants have been investigated as alternatives [1]. Currently, the microbial production of anthraquinone pigments using synthetic biology approaches is investigated at VTT. Because of the structural diversity of the produced anthraquinone pigments, it was important to create a fast and reliable methods for their analysis.

New analytical methods were developed for the screening of new anthraquinone structures as well as for their quantitative analysis. The anthraquinone derivatives were analyzed by using a LC system coupled to a photodiode array detector and a high-resolution LC-MS system. Identifications anthraquinone species were based on their specific UV/Vis absorbances as well as on accurate masses and fragmentation patterns of the molecules which were compared to reference compounds (when available) and to literature data. Analyses were performed on an Acquity UHPLC and Synapt G2-S MS systems (Waters). Chromatographic separation of anthraquinones was obtained by reverse phase chromatography with a gradient elution. Mass spectrometry was carried out using electrospray ionization (ESI) in negative polarity.

Different types anthraquinones were identified in the microbial samples. The identified structures included oxidized, methylated and halogenated forms of the anthraquinone species. Also, anthraquinone glycosides and various types of dimers were analyzed by the method.

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Keywords: Anthraquinones, LC-MS analysis, Biocolours

Investigating natural dyeing and printing with mineral pigments and plant-based protein binder

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Abstract

This paper presents the initial phase of the ongoing research reassessing the societal approach to textiles, advocating for a change in the perception of the textile material and artifact, as well as the role of a textile designer. This change is seen as essential for sustainable and regenerative future development. The initial phase consists mainly of technological and material research within natural textile dyeing and printing. Choosing natural dyeing is driven by the motivation to engage in a process often challenged by its unpredictability and it is therefore a reaction to synthetic colorants designed to provide stable outcomes (Räisänen, 2023). The central focus of this research sits on the technological investigation of dyeing and printing textiles using mineral pigments sourced from diverse rocks and minerals in combination with a binder rich in plant protein. The selection of materials supports the unpredictability of the process and serves as the foundation to answer the question: *How textile designers could effectively reflect on research in new materials with the help of public debate in social, ecological, cultural and personal contexts?* The materials for the binder are mainly soybeans in a raw form or a form of soy milk. The waste generated during the production of soy milk has been also tested to create a printing paste. The pigments extracted from rocks and minerals are primarily sourced from rocks from the Danish island Læsø and the mineral schwertmannite found in the Slovak Ore Mountains region. Schwertmannite is possibly the main mineral found in waters affected by mining activities in the area (Kupka et al., 2012). Utilizing it as a colorant in textile printing not only presents prospective applications but also investigates the possibilities of the use of this mineral considered as waste filtered out from polluted water sources.

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Keywords: mineral pigment; protein binder; natural colorants; natural dyeing and printing

Structural elucidation and identification of biocolorants from natural sources

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Abstract

As a part of the BioColour project (biocolour.fi), our research group at the University of Helsinki characterizes various colorants from natural sources. Examples include anthraquinones from forest mushrooms, flavonoids from vegetables/food industry waste streams (onion skins and orange peel), and heterocycles from dye plants (woad, nettle). This poster compiles the structures of those natural colorants that are characterized in our lab to date. The drafted information brings a perspective to the attending researchers of the conference about our materials, targeted biocolorants, and the acquired methodologies. In this poster, we have included the molecular structure of each colorant (primarily contains anthraquinones, terphenyls, polyphenols, and nitrogen-containing heterocycles) along with their natural sources and mode of characterization so that it can be relatable to a broader audience or researchers working in similar fields. This poster has been put forward to bring together the scientific community working with natural product chemistry, color chemistry, dyeing, or similar allied fields to develop networking and discussion based on their interest.

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Keywords: Flavonoid, Anthraquinone, Polyphenols, Terphenyl, Indigo, Pigments.

Bikaverin production in *Fusarium oxysporum*

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Abstract

This study explores the cultivation of fungus *Fusarium oxysporum* to produce bikaverin, a red polyketide pigment with potential applications as a natural colorant. Aimed at finding sustainable alternatives for synthetic pigments, this research included identifying a suitable cultivation medium, scaling up the cultivation to a bioreactor level, extracting the pigment and evaluating the light fastness of the pigment.

15 different media were selected based on literature, and the best medium for maximizing bikaverin production was identified and utilized for bioreactor cultivations. The intracellular bikaverin pigment was extracted from the harvested biomass and assessed for its potential as a sustainable and environmentally friendly colorant. A major aspect of this assessment was the evaluation of the pigment's light-fastness, a key property for colorants applicability. The results of this research hold a promise for the use of bikaverin as a natural colorant in various applications. By demonstrating effective cultivation methods and the pigment's favorable media properties, this study contributes to the field of environmentally conscious alternatives to traditional synthetic colorants.

Keywords: Fusarium oxysporum, Bikaverin, Pigment

Preserving green brilliance: exposure of chloroplasts in freeze-dried fruits and vegetables and subsequent storage

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Abstract

Freeze drying is a recognized preservation method for green fruits and vegetables(Yan, Wu, Qiao, Cai, & Ma, 2019). Researchers are continuously striving to improve drying techniques in order to obtain long-lasting products that closely resemble fresh color in terms of sensory properties and nutritional value. Despite considerable progress in understanding the definition and principle of dehydration processes, with emphasis on chlorophyll loss as one of the appearance concerns, the chlorophyll-enriched organelles or so-called chloroplasts have often been overlooked in color degradation.

Here we show the varying degrees of deterioration in the stability of chlorophyll in fruits and vegetables after lyophilization. Neglecting to mitigate damage to the protective phospholipid membrane barrier and the subsequent diffusion of endo-enzymes may still impede quality preservation in dehydrated green foods. These findings highlight the critical importance of controlling damage to chloroplast structures when optimizing drying methods.

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Keywords: chlorophyll degradation; chlorophyll derivatives; illumination; LHC proteins; photodegradation

Antidepressant-like effect of saffron pigment in mice exposed to chronic, unpredictable mild stress

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Abstract

Saffron (*Crocus sativus L.*) has been used extensively as a spice and food coloring, and is believed to be efficient in alleviating and treating depression long time ago in Asian folk medicine and more is reported in recent researches. while few studies investigated the antidepressant effects of saffron pigment by mimicking various social stresses in human life depression model and also along with the pathways of NF-κB and CREB/BDNF/TrKB.

Saffron pigment (SP) was analyzed by UPLC-MS/MS and their stability under pH, food additives, thermal and light conditions was evaluated. CUMS was employed to investigated the anti-depression effect of SE in this study.

The results showed that crocins were the predominant pigment of saffron (~167µg/mg). Crocins exhibited high stability under food additives and pH conditions, with retention > 70% even under oxidizing (1‰ H_2O_2/$K_2Cr_2O_7$), acidic (pH=1), and alkaline (pH=11) conditions, and light exposure. In the CUMS model, SP supplementation ameliorated the depression-like behaviors in mice, including increased the sucrose consumption and total distance traveled in the open field test, and decreased the immobility time in both the forced swimming and tail suspension tests. These improvements may be associated with remission of neurological injury and neuroinflammation and recovery of neuroplasticity. Mouse brain histopathology showed more normal hippocampal and neuronal morphology in mice orally administered wiith SP. Reduced microglial activation, decreased levels of proinflammatory cytokines, and downregulated NF-κB pathway were observed, indicating that SE could relieve CUMS-induced neuroinflammation. Additionally, SE administration enhanced the expression and transcription of neurotrophic factors, which contributed to restoration of neuroplasticity impaired by the CUMS.

This study revealed that saffron pigment possesses high stability as a food colorant, in addition to providing food coloring, saffron pigments demonstrate potential antidepressant effects.

Keywords: Saffron; Crocins; Stability; Antidepressant; Neuroinflammation

High-pressure steam stabilization of black rice bran: a sustainable source of natural anthocyanins

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Abstract

Black rice bran (BRB) is rich in natural anthocyanins(Leonarski et al., 2023). However, due to the hydrolysis of free fatty acids, its edible quality is severely challenged, limiting the utilization of anthocyanins in BRB. In this study, we successfully inhibited the free fatty acids in BRB using high-pressure steam treatment, while maximizing the retention of anthocyanins. Using response surface methodology, we optimized the stabilization process of high-pressure steam treatment for rice bran. The results showed that when treated at 125℃ for 4 minutes, the content of free fatty acids significantly decreased, and the activity of lipase was reduced to 1/5 of its original level. At the same time, anthocyanins and other nutrients were well-retained under these conditions. After high-pressure steam treatment, the activity of anthocyanin lipase in BRB was reduced to less than 10%, and its shelf life was extended to 90 days, far exceeding the untreated 7 days. The treated BRB not only retained good nutritional value and anthocyanins, but also had a longer shelf life, making it an ideal natural source for edible pigments.

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Keywords: Black rice bran; Anthocyanins; High-pressure steam; Free fatty acids

Establishment of *Aspergillus niger* as a production system for azaphilone pigments from fungi

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Abstract

Fungi produce a wide range of colourful secondary metabolites (SMs), which have a high potential as natural colourants in several industries. However, just a handful is by now produced efficiently on an industrial level. This is due to several disadvantages that native producers of coloured SMs might bring along, including the parallel production of mycotoxins or several coloured SMs, a relatively slow growth rate during cultivation and the SM production just under specific, potentially unfavourable, conditions.

To overcome these disadvantages there are several strategies. One of them is the production in an industrially established heterologous host. Non-ribosomal generated SMs have the common feature that the required genes are often found in a continuous cluster in the organism's genome, which offers the opportunity to easily identify and transfer the whole biosynthetic gene cluster in a suitable host.

A well-researched and industrially established species is *Aspergillus niger*. With the production of citric acid alone, *A. niger* achieves a market volume of several million tons annually (Meyer et al., 2020). Furthermore, *A. niger* has proven to be an efficient heterologous producer for non-ribosomal peptide-based SMs with high titers (Richter et al., 2014). In addition, *A. niger* is known for its ability to produce several types of melanin pigments. The primary pigment, DHN-melanin, is synthesized via the 1,8-dihydroxynaphtalene pathway, which is a polyketide-based pigment (Jørgensen et al., 2011).

Given the capacity of *A. niger* to produce polyketide pigments in high quantities, we aim to establish *A. niger* as a heterologous producer of azaphilones, a promising class of natural dyes within the yellow to red colour spectrum, from the genus *Monascus*. Different strategies of gene expression will be investigated and the production of azaphilones will be benchmarked against optimal cultivation conditions for *Monascus* species.

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Keywords: heterologous production, azaphilones, polyketide pigments, Aspergillus niger, melanin

Correlation of cocoa powder colourability and its flavonoid profile

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Abstract

Dutching, or alkalization is an important processing step in the cocoa industry whereby alkali is added to improve cocoa flavour and to enable a wide range of dark colours to be produced. Because of this, alkalized cocoa powder is an important ingredient for many unique bakery, dairy, and confectionary applications. Due to the different growing conditions, cacao beans from different origins have different nutritional profiles. Consequently, this causes different bean origins to darken differently to the amount of added alkali, causing the need to classify beans into different darkening potential (colourability). Since the amount of alkali salts to be added will vary, in a factory scale, this can cause off-specification issues.

It still unclear what is the exact scientific mechanism behind the dutching process. It is likely that the dutching process is a complex reaction that involves many darkening pathways. Several research also showed that the dutching process causes a reduction in total polyphenol content, hinting in the involvement of polyphenols in the dark colour formation. Additionally, it is known that some flavonoids exhibit dark colours when complexed with different type of metal ions in high alkali conditions.

In this research, we attempted characterize the major phenolic cacao compounds from three different bean origins and establish a correlation with its darkening potential. Epicatechin, catechin, and 4-hydroxybenzoic acid concentration from the different bean origins were quantified through HPLC-FLD system. The cacao nibs were alkalized with potassium carbonate in a controlled condition. Its resulting colours were then measured (LCH colour space). Our results showed that beans with higher amount of measured polyphenols were able to be coloured darker and they have a stronger correlation with darker colours. This preliminary result shows the major flavonoid content in cacao bean may be able to be used to predict its colourability.

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Keywords: Cocoa, alkalization, colour, polyphenols, epicatechin

Towards a fungal-based colorful future: upscale of colorant production from several fungal species isolated from Dutch parks

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Abstract

Colorants and dyes have been part of human culture from its very beginning. From cave paintings to textile dyes, natural pigments have been ever present in society, either as a form of art and cultural expression or as a fashion statement. After the industrial revolution, many of these dyes and pigments started to be produced from petrochemicals, making them cheaper and more accessible than their natural counterparts. However, the production of synthetic, non-biodegradable dyes is a contaminating affair, negatively affecting both the environment and human health. In order to replace these toxic dyes, we need to find a sustainable and healthy alternative which has a competitive yield and is cost-efficient compared to petrochemical-based dyes. A possible solution for the high-volume, cheap production of biobased dyes is the cultivation of colorful fungi in large-scale bioreactors. These fungi can be easily isolated from nature, providing a wide array of color shades. Within this project, three different fungal strains (strain A, B and C) have been cultivated in 7 and 20L bioreactors for the production of biobased colorants. The colors obtained are shades of red, orange, blue and green, depending on the strain and cultivation conditions. To optimize the production of these colorants, both media composition and cultivation conditions have been optimized, partially with the help of Design of Experiments. Sequencing of the fungal genomes ensured that the fungal species do not produce toxins, thus proving that the colorants are not harmful when in contact with human skin. The extraction and application of these colorants as textile dyes will be explored with our consortium partners, analyzing colorfastness and durability. In all, this research represents a leap forward in the production, extraction and application of biobased fungal colorants for the textile industry, positively contributing to a biobased economy.

Keywords: biobased colorants, fungal cultivation, bioreactors, sequencing, textile dyes

Chemistry and technologies aiming for sustainable colours

Towards the identification of orchil- and crottle-lichen marker molecules on dyed wool samples

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Abstract

State of the art lichen dye analysis from textiles is a complex task as current analytical strategies are not fully compatible with the chemistry of the lichen chromophores. Lichens are composite organisms consisting of a fungus and a photosynthetic partner and unlike other colourants, the chromophores in lichen materials do not exist as such. These compounds need to be formed resulting from a chemical reaction of the lichen with other natural products [1]. The formation of the chromophores can occur in an alkaline solution for Orchil lichens (lichen purples) or in boiling water for Crottle lichens (lichen browns) and the compounds have shown to be unstable in acidic conditions. The sensitivity of lichen chromophores to acids forms a major drawback in the pretreatment of the samples prior liquid chromatography (LC) analysis, for which an acid hydrolysis step is usually applied to extract the lichen chromophores from the textile. The analysis itself, via reversed phase liquid chromatography (RPLC), comprises an acidified mobile phase. The acidity of the mobile phase can cause in situ degradation of the lichen chromophores, hence, it can lead to detection constrains. With our contribution we present the preliminary results obtained, using series of unaged Orchil lichen- and Crottle lichen dyes analysed via RPLC-DAD analyses. The research was aimed at enhancing the detection of lichen chromophores or marker molecules thereof by lowering the acidity in both the sample pretreatment step and in the reversed phase mobile phase. Artificial ageing and comparison to other analytical approaches is foreseen as our study is continuing, culminating in the Colour4Crafts project.

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Keywords: HPLC-DAD, Reversed phase, Lichen, Colourants, Dye analysis

Characterisation of a natural red pigment from a Basidiomycota strain

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Abstract

The demand for alternative food colourants, especially red dyes, is constantly growing, as synthetic dyes have a low consumer acceptance and natural dyes usually lack ideal properties in terms of stability or watersolubility. Screening of a variety of species from the phylum Basidiomycota revealed a promising candidate for the fermentative production of a water-soluble red dye. This fungus, PKH, produces a red pigment with an absorption maximum at 510 nm during cultivation in shaking flasks. The stability of the dye was investigated in the non-purified culture supernatant, revealing a pH-dependent colour change that ranged from purple in the neutral to bright red in the acidic range. Storage stability tests were conducted, demonstrating absorbance stability for over 70 days at 24 °C in the dark. Furthermore, the dye exhibited temperature stability up to a treatment of 100 °C for 30 seconds and oxidation resistance to the addition of hydrogen peroxide up to 30 mM. To assess the dye's suitability for colouring foodstuff, model food systems, such as fruit gummies, were coloured with PKH culture supernatant. Additionally, the red dye was isolated from the culture supernatant by extracting it with methanol after lyophilization. The methanol fraction was analysed by HPLC-DAD and revealed three major peaks, all with an absorption at around 500 nm. A dark red eluent was obtained after scaled-up purification via C18 hydra packed column chromatography with 50 mL bed volume. Upon concentration of the target fraction, a red to bronze-coloured precipitation occurred. This solid was filtered and appeared to be a pure isolate of the second major peak. Furthermore, experiments for structure elucidation were performed. In order to make the pigment actually usable, toxicological safety studies and upscaling of cultivation need to be carried out.

Figure 1: Submerged culture of PKH (A), methanol extract (B) and isolated pigment (C).

Keywords: red pigment, Basidiomycota, food colourant, characterisation, stability

Exploring Basidiomycota for sustainable food colour production

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Abstract

In recent years, there has been a growing demand for new natural dyes driven by an increased reluctance among consumers to buy foods coloured with synthetic dyes. Microbial production of these pigments as a more sustainable method is an alluring option. An interesting group of organisms for dye production are the fungi of the phylum Basidiomycota, to which most edible fungi belong. For instance in the review by Velíšek and Cejpek from 2011, 87 compounds and their derivatives were collected (Velíšek & Cejpek, 2011). However, for most examples, it is unknown, whether these pigments can be produced during submerged cultivation, as most of these dyes have only been described in the fruiting bodies of these species. From a biotechnical point of view, submerged cultivation is more efficient than the formation of fruiting bodies. Therefore, the fungal strain collection of the Institute of Food Chemistry and Food Biotechnology was screened for possible candidates for fermentative colourant production. From the ~500 fungi of the strain collection, 53 were chosen for screening on two different media in surface culture, yielding colours from green to red. Based on this, 22 candidates were selected for further screening in submerged culture. Deep brown, orange and red-coloured culture supernatants were observed for nine fungi. In order to optimise colour formation, various cultivation parameters were tested for these fungi, including cultivation temperature, light exposure, and the supplementation of amino acids. For some fungi, such as RDP and UMC, the supplementations have resulted in significant improvements in colour intensity. To fully utilize the potential of Basidiomycota for producing pigments, further studies on toxicology and process upscaling are necessary.

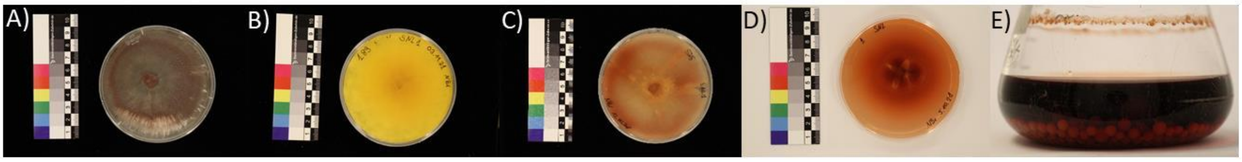

Figure 1: Example for the coloured surface cultures of TSP (A), NACP (B), PKH (C) and UMC (D) and submerged culture of UMC (E).

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Keywords: pigment production, Basidiomycota, screening, sustainable dyes, strain collection

Woad (*Isatis Tinctoria L.*) indigo blue extraction and dyeing with low inputs

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Abstract

Dyer's wood is a biennual herb, in the plant family Brassicaceae, successfully cultivated in the northern latitudes. Woad produces indigo-pigment precursors, the source of desired natural blue, in their leaves. Plant material, extraction efficiency and side reactions in the process affect the yield and purity of indigo.

In the basic process isatan-molecules are released from young, freshly harvested, washed leaves to hot water (>60°C). Then isatans are hydrolysed to indoxyls at high pH >10 at room temperature. Oxidation of the liquid leads to dimerization of two indoxyl molecules to blue indigo, which precipitates.

Dried indigo flakes can be stored and used for dyeing. Insoluble indigo must be converted to its water-soluble leuco state by addition of reductant, at high pH 11, in warm water (55°C), in a process called vat dyeing. Glucose can substitute toxic dithionite as a reducing agent (Vuorema, 2008). Water-immersed textile turns blue in the air as leuco-indigo becomes oxidized back to indigo. Some anaerobic, alkali-tolerant, fermentative and thermotolerant bacteria, e.g., *Clostridium isatidis*, can solubilise indigo, thus substitute toxic reductant, in traditional vat conditions (Milanovic, 2017). The bacteria may originate with leaf material from soil, and their suitability for dyeing processes is studied nowadays, especially in Japan.

Extraction and dyeing processes require a lot of water, energy, and chemicals for controlled cascade of temperature and pH changes. For these reasons plant-derived indigo is not necessarily a sustainable product.

As a part of BioColour project, alternative low-input methods for indigo extraction and dyeing have been studied in Natural Resources Institute Finland. Extraction without hot water was the key developed issue in the experiments. The results showed that the time needed for the extraction was usually longer and the dyeing gave lighter shades of blue compared to the basic processes. The resource efficiency of the low-input processes is discussed.

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Keywords: indigo, water extraction, bacterial reduction

Greening the blue: mitigating environmental impact of denim dyeing through indican

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Abstract

The denim industry, known for its iconic indigo-dyed fabrics, faces environmental challenges due to the traditional dyeing process involving indigo. Indigo, a vital component of denim dyeing, undergoes a chemical process where it is reduced to its soluble counterpart, leuco-indigo, requiring a potent reducing agent at high pH (Chavan, 2015) (Fig. 1A). This traditional dyeing method leaves a substantial environmental footprint, contributing to pollution of water and soil (Periyasamy & Militky, 2017). However, a promising and more sustainable solution has emerged: utilizing indican as a precursor for indigo dyeing (Hsu et al., 2018).

This research explores a dyeing alternative that utilizes water-soluble glycoside-protected indoxyl (Indican) as a precursor for indigo denim dyeing. Indican can be employed as a dyeing agent through enzymatic deprotection by glucosidase activity or photolytic cleavage (Fig. 1B), eliminating the need for environmentally harmful reducing agents. To assess the viability of this alternative, the research employs life cycle assessment and techno-economic analysis. These methodologies help identify and quantify the environmental and economic implications of the new approach compared to conventional dyeing methods.

By developing this low-impact alternative and evaluating its practicality, the study contributes valuable insights to the ongoing efforts within the denim industry to adopt more environmentally friendly practices and reduce its overall ecological impact.

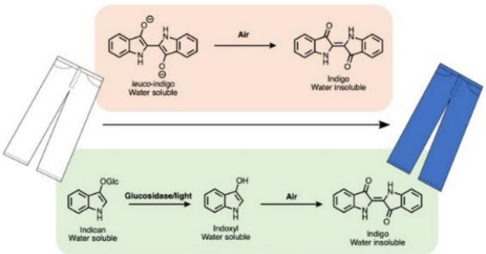

Figure 1, A) Indigo based dyeing. B) Indican-based dyeing

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Keywords: indigo, indican, biocatalysis, photocatalysis, sustainability

Development of a vibrant and stable anthocyanin-based blue colour in acidic pH

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As consumers become more conscious of healthy-eating and clean label foods, sales in natural food colors have also increased. However, it is challenging to find suitable natural blue colorants since blue edible materials are rare in nature. Anthocyanins are flavonoids abundant in plants and believed to exert health benefits including anti-cancer, anti-inflammation, and neuroprotective activities [1]. They are known to produce blue color in alkaline conditions, but their application as blue colorants is limited by their poor stability and limited food products in these pHs. Hydrangea flowers owe their color to the presence of delphinidin-3-glucosides (Dp3glu). These flowers can change color from red to blue by the complexation at pH 4.0 of three components: Dp3glu, caffeoylquinic acid, and Al3+ [2]. We successfully reproduced this blue complex with edible materials by combining Dp3rut from American eggplant anthocyanin, Al2(SO4)3 and Yerba Mate extract rich in caffeoylquinic acid.

The blue complex spectra had a lambda max 66 nm higher than the same anthocyanins at pH 4 and there was a positive cotton effect in circular dichroism. The accurate mass of this complex, determined by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry, was m/z 989.21229, with a 1.53 ppm mass error compared to the theoretical m/z [M+H]+ (C43H46AlO25+) (Fig 1.). The blue color was retained when the pH of the samples were adjusted to pH 3 to 5 showing stability for months at 4℃. The degradation kinetics fit first order kinetics (R2 of 0.91). Color stability has highest at pH 3.05, retaining over 60% color after 7 months at 4℃ while samples at pH above 5 lost over 50% of their color in 30 days. This project will help fill the gap of anthocyanin-based vibrant blue colorant for acidic food products.

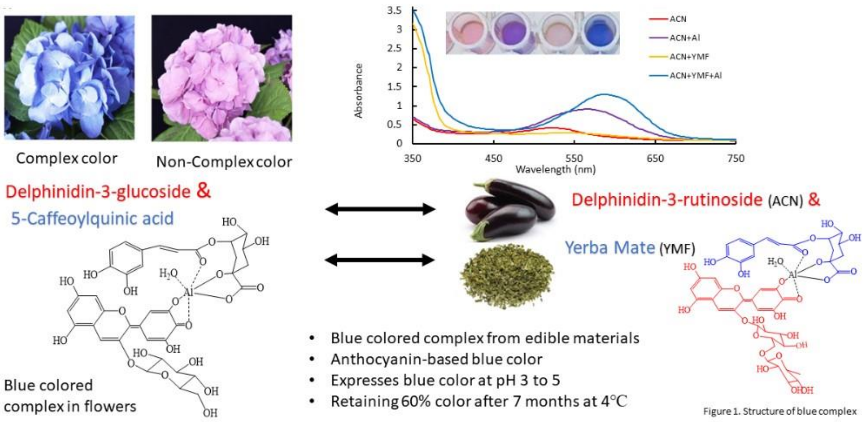

Figure 1. Structure of blue complex

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Keywords: Food Color, blue food, natural derived color

Digital printing of textiles using biobased dyes

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Abstract

Digital textile printing has experienced significant growth over the last 20 years. Indeed, digital textile printing now accounts for ~11% of global textile printing, with optimistic projections of a 10-14% compound annual growth rate from 2021 to 2030. This growth can be attributed to digital printing's competitive edge in reducing water and energy usage, as well as minimizing chemical waste and carbon footprint compared to traditional water intensive dyeing methods (Ugur Koseoglu, 2019). Typically, synthetic dyes are commonly used in digital printing due to their cost and favorable fastness properties. However, there is a rising trend in adopting bio-based dyes for textile coloration, especially in digital printing. Recent studies have explored the feasibility of using bio-based dyes in digital printing, focusing on formulations and application. While several bio-based dyes, such as annatto, gardenia yellow, indigo, curcumin, and madder, have shown promise for digital textile printing, challenges persist, including low fastness properties, limited shelf life, lower color yields, and print sharpness. The scarcity of research on developing ink for digital textile printing using bio-based dyes emphasizes the need for more exploration into potential colorants, their economic feasibility, and their impact on industrial competitiveness. Moreover, investigating alternative, eco-friendly options to organic solvents in digital textile printing and exploring surface modification techniques and pre-treatment methods for textiles can contribute to the sustainability and enhancement of color strength and fastness properties in the process. Considering these aspects, the promise of bio-based dyes in digital textile printing remains a realm of untapped potential. Therefore, a comprehensive review on this subject will be provided, offering insights into the dynamic intersection of technology, sustainability, and innovation within the digital textile printing industry.

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Keywords: Digital textile printing, biobased dyes, textile coloration, inks

Effect of glycosylation, acylation and pyranylation at cyanidin C-ring on its interaction with vitamin c in apple juice beverage matrix

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Abstract

Synchronous degradation between anthocyanin (ACN) and vitamin C (VC) was found in fruit and vegetable juice matrix. To investigate whether the C-ring 4th carbon of ACN is the key site of this interaction, cyanidin with four different C-ring 4th carbon modifications (3-glucosylation, 3,5-diglucosylation, 6’’-malonylation, pyranylation) were added to VC-containing apple juice, and the changes of ACN retention, VC retention, color, antioxidative property and differential metabolites were analyzed. The ACN retention was in the order of pyranylation > 6’’-malonylation > 3,5-diglucosylation > 3-glucosylation. The VC retention was in the order of 6’’-malonylation > pyranylation >3,5-diglucosylation > 3 glucosylation. The order of color stability was in agreement to that of ACN retention, and the order of antioxidative activity intensity was opposite to that of VC retention. The results showed that no matter what kind of modification, the addition of ACNs promoted the degradation of VC in apple juice matrix. Yet limiting the 4th carbon of ACNs could significantly improve the color and nutritional stability of ACN-added apple juice, indicating the 4th carbon of ACN was the critical site for the ACN and VC interaction, which indirectly supported the condensation theory. The shared differential metabolite of all apple juice matrices added with different ACNs was trans-hinokiresinol, which was likely generated from ACN skeleton reacted with certain compounds in apple juice. But due to the relatively large number of phenolic acids in apple juice matrix, neither 4th carbon condensed products nor other unique differential metabolites of ACN and VC were identified in metabonomic analysis. This study showed modification of the 4th carbon of ACN C-ring could affect the ACN and VC interaction to some extent, which provided valuable insights for the application of ACN C-ring modification in shelf-life quality control of typical fruit and vegetable beverages with the coexistence of ACN and VC.

Keywords: Anthocyanin; Vitamin C; Condensation; UPLC-ESI-Q TRAP-MS/MS; Color

Rapid phenotypic approach for screening major carotenoids in tomato fruits

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Abstract

Carotenoids are often associated with nutritional benefits and research studies have shown that high consumption of carotenoids may prevent certain types of cancer, cardiovascular diseases, and age-related macular degeneration. HPLC is commonly used for carotenoid analysis due to its accuracy and reliability, however it is time consuming and requires use and disposal of hazardous organic solvents. Raman spectroscopy could provide a rapid technique to profile carotenoids identifying different type of carotenoids during plant breeding. Our aim was to evaluate the feasibility of using portable Raman spectrometers to profile carotenoids in fresh tomatoes to support breeding programs. Fresh tomato (n=106) samples with different carotenoid profiles were harvested from Ohio State University Tomato Genetics and Breeding Program at Wooster, OH. Raman spectrum of tomato samples was collected using a handheld Raman spectrometer equipped with a 1064 nm laser. HPLC equipped with a photodiode array detector was used as reference method to profile carotenoids. Pattern recognition was used to generate classification algorithms for identification of different profiles of carotenoids in tomatoes. Supervised classification algorithms clustered tomatoes with different carotenoid profiles with high sensitivity and specificity. The main discrimination was associated with the band at 1510 cm-1 (C=C stretching), that shifted depending on the length of polyene chain and isomerization. Our research findings support the use of handheld Raman devices for rapid selection of unique genetic material based on different carotenoid profiles as a non-invasive method. Novel portable Raman systems may provide the tomato industry with a rapid method to evaluate carotenoid profile of tomatoes allowing for analytical flexibility since the units can be easily transferred for field applications.

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Keywords: Pattern recognition; handheld; Raman spectroscopy; tomato; carotenoids.

Stabilization of black rice anthocyanins by beta-lactoglobulin fibrils: morphology, interaction and thermal protection

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Abstract

The industrial exploitation of anthocyanins as natural colorants is limited by their low stability. β-Lactoglobulin (BLG) can self-assemble into fibrils, increasing its structural stability and exposing more functional groups, which may be beneficial to the thermal protection of anthocyanins.

In this study, 20mg/mL BLG was heated at pH2.0, 85°C for 16h to produce β-Lactoglobulin fibrils (BLGFs). It was analyzed for building blocks, driving forces, surface hydrophobicity and free sulfhydryl groups. The interaction of BLG or BLGF with cyanidin-3-O-glucoside (C3G, the major anthocyanin in black rice anthocyanin extract, BAE) was investigated through fluorescence quenching and CD spectrum. Thermal degradation kinetics was analyzed to evaluate the effect of BLGF on the thermal stability of BAE. The morphology of BLG, BLGF and their complexes with BAE was determined by TEM images.

The results indicated that BLGF was assembled by acid-hydrolyzed peptides mainly through hydrogen bonds, hydrophobic interactions and disulfide bonds. Fibrillation significantly improved the surface hydrophobicity of BLG and decreased the free sulfhydryl groups ($p<0.05$). The binding of C3G to BLG and BLGF followed a static model and was mainly driven by hydrophobic interactions. The half-life (T= 90 °C) of anthocyanins followed the order of BLGF-BAE (41.32 min) > BLG-BAE (24.30 min) > BAE (21.73 min). These results indicated that BLGF could effectively provide thermal protection to BAE due to the sufficient exposure of hydrophobic groups.

This study revealed the facilitation of fibrillation on the binding affinity between proteins and anthocyanins. BLGF was proved to be a potential nanocarrier of anthocyanins in the food industry.

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Keywords: β-lactoglobulin; Fibrillation; Fluorescence quenching; Hydrophobic interaction; Thermal stability

Chemical modification of natural dyes for enhanced interactions with textile fibres

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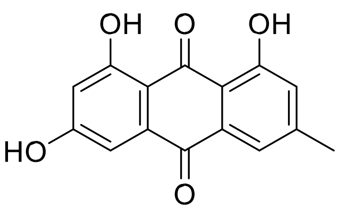
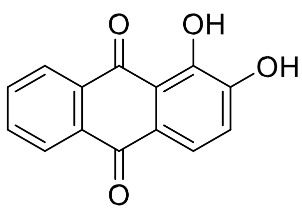
Abstract

Bio-based colourants are gaining more attention as green alternatives to synthetic dyes which have limitations such as environmental persistency and low biodegradability. However, natural dyes have poor brightness and fastness properties that they tend to fade easily. To overcome these, chemical modifications can be done to natural colour molecules which can bring about better interactions with textile fibres and it also helps to eliminate the use of metallated mordants in the dying process. This metal free dyeing process not only reduces the environmental impact but may also be more appealing for applications such as digital printing. Natural dyes such as quercetin, emodin and alizarin can be modified such that one or more of their hydroxyl groups are substituted with an organic moiety with various functionalities. The organic moiety can be modified to achieve various properties to accommodate the dying process and the nature of the textile. Thus, these modified bio-based colourants may offer a broader scope for potential dying applications.

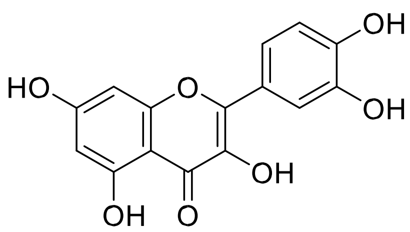
Keywords: Natural dye, Fabric, Bio-colour, Textile fibres, Chemical modifications



emodin



alizarin



quercetin

Safety and regulatory issues of bio-based colourants and products

Aquatic toxicity assessment of the natural dye alizarin

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Abstract

Alizarin is a natural anthraquinone extracted from *Rubia tinctorium* which has been used as a dye in several industrial processes including textiles (Santis & Moresi, 2007). As it can be present in the effluents, it is a potential aquatic contaminant. Alizarin is mutagenic in the *Salmonella*/microsome test with the addition of metabolic activation (S9) and it was toxic to the aquatic plant *Lemna gibba*, phytoplankton and fish (*Danio rerio*). In aquatic environment, alizarin can undergo photosensitization reactions causing photoinduced toxicity (Wang et al., 2009). This work aimed to complement aquatic toxicity data using algae, crustaceans and fish embryos. Light effect on the acute toxicity of *Daphnia similis* was also evaluated. Alizarin was dissolved in DMSO to its limit solubility (1.93 g/L) and tested at the concentration provided by adding 0.01% DMSO in the media as recommended by OECD (2011). A 72h exposure chronic toxicity test was performed with *Raphidocelis subcapitata*. Acute toxicity tests with *D. similis*, *Parhyle hawaiensis* and *Danio rerio* were performed for 48h, 96h and 168h, respectively. Data were analyzed applying generalized logistic models. Alizarin did not inhibit the growth of *R. subcapitata* or cause lethal effect in *P. hawaiensis* (IC50/LC50 > 193 µg/L) (Fig.1A). For *D. similis*, alizarin EC50 was 90.3 µg/L (Fig.1A). Constant light did not alter the toxicity (EC50 of 105 µg/L) but in the dark, there was a slight increase (EC50 of 68.6 µg/L). For *D. rerio* embryos LC50 was 39.6 µg/L (Fig.1A), therefore alizarin can be classified as category 1 for acute aquatic toxicity in the Global Harmonization System (GHS). D. rerio was the most sensitive organism for alizarin and light did not increase the toxicity for *D. similis* as expected. Comparing its toxicity to other similar natural anthraquinones for *D. rerio* emodin>alizarin>dermocybin and for *D. similis* alizarin>emodin>dermocybin (Fig.1B).

Acknowledgements: We thank Dr. Tova Williams from Wilson College of Textiles, NCSU, for supplying the alizarin and the São Paulo State Research Foundation (FAPESP) for the scholarship for A.R. (Process no. 2023/07768-3) and for G.A. (Process no. 2021/01204-5) and for M.B. (Process no. 2022/04482-9) and CAPES financial code 001 for N.F. Also, we thank the Ethics Committee on the Use of Animals (CEUA) (Process no. 5645-1/2020).

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Keywords: Anthraquinone, microcrustacean, amphipod, fish embryos, acute toxicity.

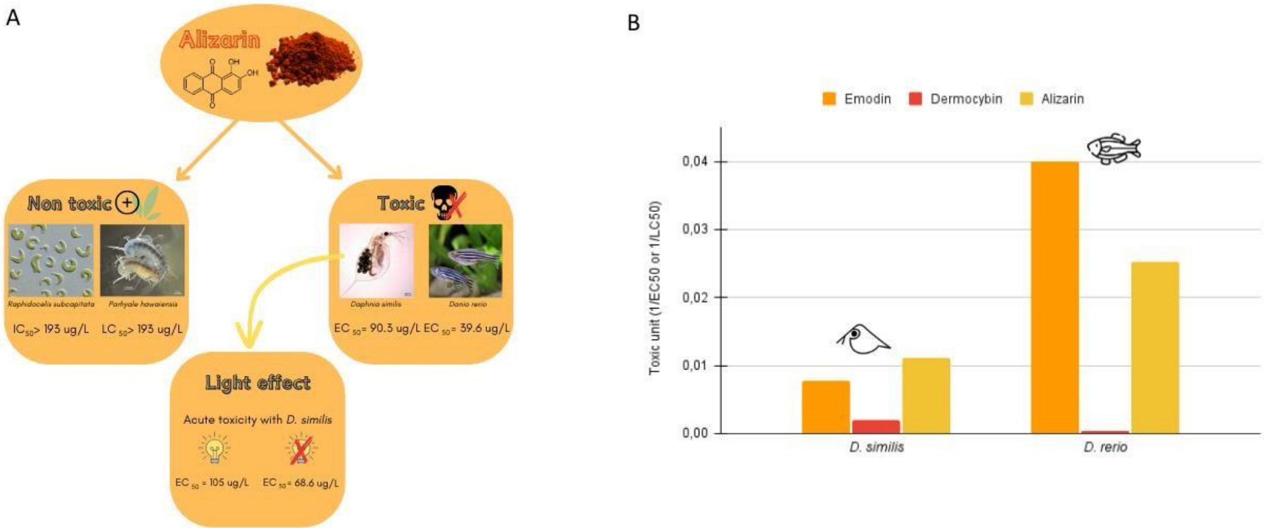

Figure 1. A: Rationale of alizarin toxicity testing; B: comparison of the toxicity of emodin, dermocybin and alizarin to Daphnia similis and Danio rerio embryos.

Soil toxicity of commercial natural indigo and aquatic toxicity and mutagenicity of its soluble fraction

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Abstract

Natural indigo is a very promising colorant and is considered a more sustainable alternative than synthetic indigo. Indigo can reach the environment through the discharge of liquid effluents generated during the dyeing process or the disposal as solid waste/dyed textiles in landfills potentially contaminating the aquatic and terrestrial compartments. This work aimed to characterize the toxicity and mutagenicity of a commercial sample of a natural indigo with terrestrial and aquatic organisms using different exposure scenarios. Indigo was tested mixed to natural soil and evaluated for chronic toxicity with the terrestrial organism *Enchytraeus crypticus*. It was also tested for acute toxicity mixed with reference sediment with an aquatic epibenthic organism, *Parhyale hawaiensis*. Because the dye is not soluble in water and partially soluble in DMSO we tested the soluble fraction of natural indigo in this solvent using *Daphnia similis* and also *P. hawaiensis* for acute toxicity and in the Ames test (Microplate agar). Its leuco form was obtained using a reducing agent in an alkaline medium. Indigo presented very low toxicity to *E. crypticus*, only at the highest tested concentrations (5.06 and 7.59 g kg -1 dry soil). And for P. hawaiensis a LC50 of 309 g kg-1 was calculated. The extract of indigo was not acutely toxic to both aquatic organisms and not mutagenic in the Ames test for *Salmonella* strains TA98 and TA100 with and without metabolic activation (S9). The toxicity of its leuco form was not caused by the dye itself, but by the increase of pH and electrical conductivity from sodium carbonate and sodium dithionite which were used to reduce the dye. The hazard data generated in this work can be used for future risk assessment of contaminated environments with this dye. The low toxicity and lack of mutagenicity observed for natural indigo suggest that the dye is relatively safe for the environment.

Keywords: Daphnia; Ames test; Parhyale; Enchytraeus

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Bio-based colourants and materials

Novel cyanine-type chromophores inspired to red hair pigments: smart sensors with acidichromic and hydrophobic-to-hydrophilic switching response

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Abstract

In recent years cyanine dyes, featuring peculiar chromophoric and fluorescence properties, outstanding biocompatibility and low toxicity to living systems, have largely been exploited as biological reporters and in other technological applications. This class of compounds typically features organic nitrogen centers, one of the imine and the other of the enamine type to form a push-pull system. In this frame, inspired by Δ2,2'-bibenzothiazine photochromic and acidichromic chromophore of red human hair pigments trichochromes, a new class of cyanine dyes termed trichocyanines was designed in which a highly tunable cyanine-type chromophore was implemented using the benzothiazine nitrogen as the acceptor moiety at high basicity allowing for a marked bathochromic shift even at slightly acidic pHs. Starting from this background, new red hair-inspired 1,4-benzothiazine-based scaffolds are disclosed herein, built upon a modular D-π-A architecture via condensation of the easily accessible 3-phenyl-2H-1,4-benzothiazine with indole-3-carboxaldehyde (Alfieri et al., 2020) or glyoxal (Alfieri et al., 2022). The cyanines thus obtained were characterized as 2Z-((1H-indol-3-yl)methylene)-3-phenyl-2H-1,4-benzothiazine) (1) and 2Z,2'Z-(1,2-ethanediylidene)bis(3-phenyl-2H-1,4-benzothiazine) (2) respectively, by complete spectral analysis. In both cases, a reversible acidichromic behaviour with a marked bathochromic shift upon acidification from yellow (444 nm at neutral pH) to violet (544 nm at pH < 3) or from red (480 nm at neutral pH) to blue (637 nm at pH < 4) (Figure 1) was observed on various fabrics, paper, polylactic acid films as well as on thin films of the cyanine exposed to acid/basic vapors and proved reversible over several cycles.

Figure 1: pH dependent chromophores of cyanine dyes **1** and **2**.

Noteworthy, the acidichromic behavior of 2 was associated to a remarkable hydrophobic-to-hydrophilic switch, that was probed in a filter permeability experiment.2 Other applications that are presented for 2 include the use as pH sensor paper for visual assessment of pHs below 0 and devices for monitoring food freshness, e.g detection of volatile amines generated by fish spoilage.

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Keywords: cyanine dyes; benzothiazine-based chromophores; acidichromism; pH sensors; hydrophobic/hydrophilic property

Development of cellulose nanocrystals-zinc oxide based bio-opacifiers; effect of particle size and drying conditions

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Abstract

Scattering enhancers or opacifiers are used to enhance the haze of different materials such as paints, paper, and cosmetics. Currently, the most used scattering enhancer (especially in paints and cosmetics) is Titanium dioxide (TiO_2) nanoparticles exhibiting a high refractive index of n= 2.5 (Caixeiro et al., 2017). However, in 2020, EU has classified TiO2 nanoparticles as a category-II carcinogen by inhalation, prompting a re-evaluation of their use in various applications (Yang et al., 2022). To cope with this situation a quest for a safe and sustainable alternative opacifier is underway using different approaches to create structural disordered anisotropy that is optimal for scattering maximum incident light. Cellulose came as a potential candidate for this purpose but its lower refractive index i.e., n=1.5 hinders it for such applications (Yu et al., 2023). This can be addressed by creating multiple scattering centers (pores and voids).

Similarly in current study Zinc oxide (ZnO) nano (100 nm) and microparticles (<5 µm) were used to create disordered anisotropy inside cellulose nanocrystals. ZnO nano and microparticles were used to see the effect of particle size on the overall light transmittance of cellulose nanocrystals (film). Briefly, cellulose nanocrystals in different concentrations (1.5 and 3 % w/v) were dispersed in water. ZnO nanoparticles and microparticles were supplemented to cellulose dispersions at different concentrations (1, 3, 5, and 10 w/w). Additionally to see the effect of drying conditions on light transmittance of the CNC films. Samples were dried in an incubator at 34±2 °C (CNC.inc) and freeze-drying conditions (CNC.frz). The obtained samples were analyzed for transmittance with UV/vis spectrophotometer and surface morphology with scanning electron microscopy.

As per results, the freeze-dried CNC films supplemented with microparticles revealed a significant improvement in opacity with increasing concentration of ZnO. For CNC.inc the opacity was recorded lower than that of CNC.frz. For CNC.inc samples compared to control, without any ZnO, the overall opacity increases with increasing concentrations of ZnO. The higher opacity of CNC.frz can be ascribed to the creation of aided porosity in freeze-drying conditions. However, for CNC.inc the self-assembly of nanocrystals led to a more transparent rather than opaque appearance.

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Keywords: Opacifier; cellulose nanocrystals; haze; Zinc oxide

Utilization of spray-dried wood bark extracts as dyes for fabric printing

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Abstract

Wood bark is the most common by-product of the sawmill industry, and currently mostly utilized by burning for energy without other purposes. In Europe only, the roundwood production in 2019 was 500 million m^3 of which the share of bark varied between 5 and 28 percent.[1] Because of its widespread availability at a low cost, bark has the potential to be employed in various applications, serving as a resource for energy production, but also the creation of several additional valuable products. The work contains a sustainable cascade use approach for large quantity biomasses that are currently underutilized but may offer a feasible substitute for example in the areas of textile and package printing.

The idea behind this research was to find a new value-enhancing application for humble wood barks by extracting valuable colors from Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) barks with simple and easily scalable hot water extraction. After extraction, wood bark extracts were spray-dried, and the suitability of the spray-dried extracts obtained for fabric screen-printing application was studied. Stability of prints pigmented with bark dyes were evaluated using a standardized light exposure test method (ISO 105-B02). Appearance and lightfastness of the fabric samples were assessed with both visual inspection and reflectance spectrophotometry before and after the light exposure test. Our results showed that the wood bark extracts are producing uniform, pleasant and earthy colors of which color stability during the lightfastness testing was promising.

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Keywords: Bark, lightfastness, screen-printing, stability

Microblu project: the search for blue biocolorants

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Abstract

Blue pigments are scarce in nature, so new sources of these colorants have attracted the interest from researchers and industries. In our laboratory, we have been investigating the production of blueish biocolorants from *Janthinobacterium sp., Antarcomyces pellizariae,* and *Fusarium oxysporum*, focusing on optimizing process conditions, downstream processes, and analytical tools for identifying the bluish compounds. *Janthinobacterium sp.* isolated from rice (1) produces a mixture of purple-blue pigments violacein (c.a. 92%) and deoxyviolacein (c.a. 8%), which have gained notoriety due to their biological activities reported so far, good tinting capacity and relatively good stability under some processing and storage conditions. The highest production was observed in culture medium composed by fructose and yeast extract, and the highest amount of pigments extraction was achieved using 70% ethanol. *Antarctomyces pellizariae* is an Antarctic fungus producing a blue biomass when grown at <15°C (2). In fact, tests in bioreactors showed no pigment production at >14°C. Transcriptomic approaches have been done to try to identify the genes and metabolic pathways involved in the production of this(these) blue compound(s), while chemometric approaches have been considered for identifying the compounds, but still with no conclusive results. *Fusarium oxysporum*'s red biomass, abundant in the red pigment bikaverin, distinctly shifts to blue upon heat treatment (3), which is also induced in the presence of specific amino acids and metal ions (1). We are investigating still key parameters (e.g. humidity, presence of O2, biomass age) influencing this color conversion (detailed in another abstract) and also a textile biodyeing procedure with this blue biomass. These microorganisms show promise as sources for the development of safe, stable and natural blue biopigments with applications in food, cosmetics, textiles, and others. Therefore, our research aims to provide practical solutions for industrial challenges, enhancing the availability of new biopigments.

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Keywords: Biopigments, blue colorants, microbial production, bioprocess, industrial applications.

Production of natural pigments by Streptomyces strains under submerged fermentation of potato chips industry waste and their application as biocolorants for different textile materials

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Abstract

Genus *Streptomyces* has been proven to produce different pigmented compounds, that are usually biologically active. These pigments are produced during the complex life cycle of this bacteria and are capable of coloring different materials, including various natural and synthetic textiles. If we consider the possibility of utilizing various agro or food waste as substrates for *Streptomyces* growth, bacterial pigments can serve as cheaper and biologically safer alternatives to chemical dyes.

The object of this study was the cultivation of pigment-producing *Streptomyces* strains: 15-10, BV365 and JS520, using growth media containing waste streams from a potato chip factory as a carbon source. Following the optimization of growth parameters for pigment production, the evaluation of dyeing properties of living bacterial cultures using different natural and synthetic textile materials was carried out. Finally, the evaluation of the antimicrobial and cytotoxic activity of extracted bacterial pigments provided valuable information on the functionality and eco-toxicity of the bioprocess.

Cultivation of the pigment-producing *Streptomyces* strains: 15-10, BV365 and JS520, was done following the standard microbiological protocol, using growth media enriched with waste from the potato chip factory. The testing of the dyeing properties was done by immersing Multifiber Adjacent Fabric TV Style 49 in a liquid medium, and then incubating with the pigmented liquid culture of bacterial strains. Bacterial culture extracts were evaluated for antimicrobial and cytotoxic effects using standard protocols. Results show that bacterial strain JS520 produced purple pigment in a growth medium comprised almost completely from waste from the potato chip factory, with addition of yeast extract in a final volume of 1.5%. The difference in cytotoxic activity of bacterial pigmented extracts was apparent, with low cytotoxic activity for the bacterial strain 15-10, moderately cytotoxic activity for the JS520, and high cytotoxic activity for BV365.

Keywords: Streptomyces, biopigments, textile-dyeing, bioconversion, bioactivity

Ecological treasures of Madagascar in traditional silk dyeing: *Pisolithus arhizus* ”taikininina” and *Aloe vaombe* ”vaombe”

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Abstract

Madagascar is called ” The Big Island”. It has a number of specificities, in particular, in the plant kingdom: 80% of all plant species are found only there, of estimated 12,000 species. Our previous results on five selected plant species from Madagascar Highlands showed a complete rainbow palette of natural dye. Various extraction processes involving aqueous decoction and ethanol UAE-extraction have been performed. Additional phytochemical studies: screening, polyphenol quantification, UV-Visible Spectroscopy characterization, and TLC analysis of extracts have also been undertaken. The above selected plants were also examined for their antioxydant, anti-inflammatory, and antimicrobial activities. Accordingly to the traditional malagasy dyeings, two species have been retained as valuables dyeing resources: *Pisolithus arhizus* ”taikininina”, and *Aloe vaombe* ”vaombe”. Three common fibers are used for dyeing experiments: two kinds of silk: *Boroceras cajani* ”lamba landibe” (*), domestically silk *Bombyx mori* ”landikely”, and cotton. For *Pisolithus arhizus*, dyeing trials displayed vibrant colors ranging from golden yellow to dark brown. The colors range from rose, rosewood and purple-red were obtained from ”vaombe”. These colors depended not only on colourant concentration, but also on the mordants used. ”Vaombe” a medicinal plant native to the southern part of the country, has also been cultivated in the Highlands by craftsmen. The valorisation of its waste, by-product of gel extraction used in medicinal product, offers a dual ecological and economic advantage.

(*) As regard the Malagasy ritual, a dead body is wrapped with what is traditionally called ” lamba landibe”, it’s a grave cloth or shroud. Nevertheless, ”lamba landibe” has its own natural colour because of its original fiber from cocoons. It is really a luxury fiber over the big Island (2).

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Keywords: Phytochemistry, pharmacological property, Malagasy crafts, natural dye, mordants

Enzymatic synthesis of indigo

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Abstract

Indigo is the well-known dye that gives rise to the iconic blue denim look. It has been used since ancient times when it was extracted from plants. When the demand exploded with the Industrial Revolution, the industry turned to the more convenient chemical synthesis of the dye pioneered by Adolf von Baeyer (Baeyer & Drewson 1882). The synthesis is based on aniline, a petroleum-derived chemical that is suspected carcinogenic and has high acute toxicity (Mocquard *et al*. 2022). The synthesis also includes several other problematic chemicals, such as formaldehyde, hydrogen cyanide, and sodamide. The markets for denim, and thus indigo, are expected to grow, and so is the associated pollution, posing a threat to the health of people and the planet. We search for alternative routes and processes to overcome this challenge. There are numerous examples of *in vivo* approaches to indigo biosynthesis, but the biocatalytic alternative is less explored (Han *et al*. 2011).

The enzymatic cascades explored are based on basic renewable building blocks anthranilic acid, tryptophan, and indole (Fig 1A). Even though longer enzymatic cascades are more challenging in terms of process optimization, we consider them valuable as they might result in faster processes and use less impactful starting materials. To examine this, we proceed with a life cycle assessment and techno-economic analysis to identify the environmental and economic drawbacks of this approach by comparing it to the conventional synthesis methods (Fig 1B). We work continuously to improve our processes and will present the projects' current stage, including technical maturity, economic, and environmental performance.

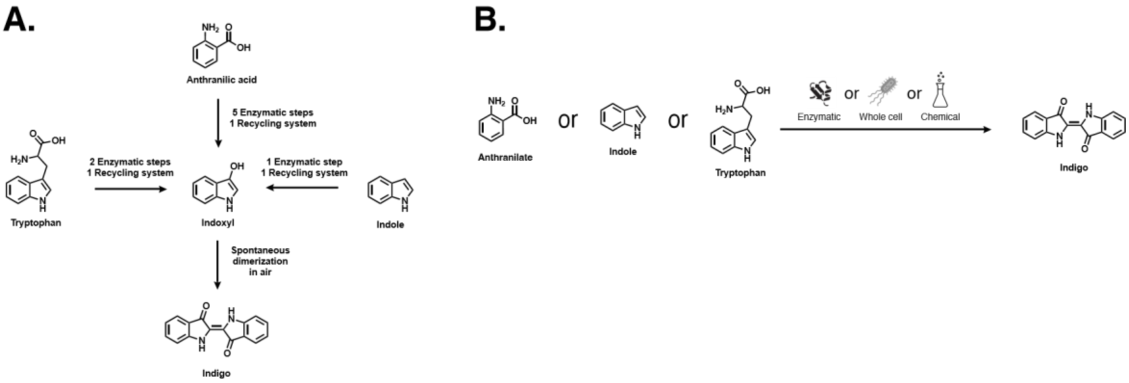

Figure 1 A) Three routes for enzymatic indigo synthesis B) Scenarios considered for indigo synthesis that will be assessed for environmental impact.

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Keywords: Biocatalysis – Cascade – Indigo – Life-cycle assessment

Investigating the red-to-blue colour change in the biomass of *Fusarium oxysporum* CCT7620

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Abstract

The interest in microbial pigments have increased due to their biodegradability, natural status, eco-friendliness, and the possibility of yielding rare colors. With its diverse colour range, species from the genus *Fusarium* spp., especially the polyketide bikaverin-producing strains, emerges as a promising new source of biocolorants. Bikaverin's color varies with pH, transitioning from red in acidic solutions to blue-violet in alkaline solutions. The studies on this pigment are still scarce, but this molecule has already been used in textiles and also as antimicrobial compound. Moreover, our team discovered that the red biomass from *F. oxysporum* turns blue at some conditions, such as heat treatment. A hypothesis tested by our research group suggests an interaction with cell wall proteins, supported by amino acids like arginine and histidine inducing a blue colour after heat treatment. In this sense, some m/z values such as 487.14, 517.15, 529.15, and 545.15 were found in violet extracts, which MS/MS spectra presented fragments of arginine. The present study also explored the influence of metal ions on bikaverin-rich *F. oxysporum* biomass revealed a color change even at acidic pH. Metal ions generated pigments ranging from black (Fe^{2+}, Co^{2+}) and blue (Zn^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}) to purple tones (Cu^{2+}), although the possible interaction mechanism was not yet investigated. Biomass color change appears to be linked to oxygen, moisture, and fermentation age. While our initial hypothesis attributed the heat-induced blueing to oxygen presence, nitrogen gas injection experiments yielded unexpected color conversion, requiring further investigation. Additionally, we propose a color shift via adducts or complexes involving bikaverin, amino acids, and metallic ions. These hypotheses are being investigated for confirmation. Ongoing chemical studies aim to reveal more, progressing towards commercializing this innovation. These findings, together with toxicological studies, might enable the adoption of this blue pigment complex in food, cosmetic and textile applications.

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Keywords: Fungal pigments, bikaverin, colour change, metal ions, industrial applications

Lactic acid bacteria assisted production of anthocyanin derived food colorants

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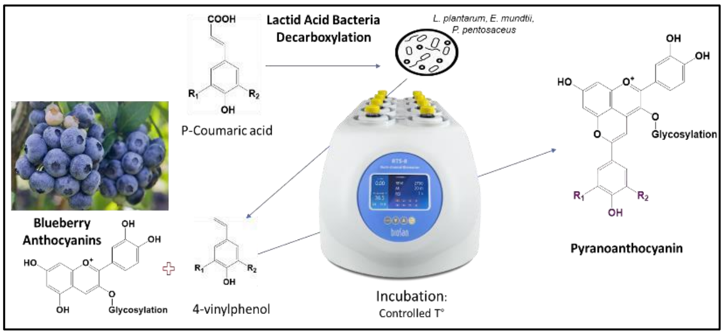
Abstract

Color plays a critical role in food selection, with consumers demanding more natural ingredients, plant pigments offer a feasible alternative to synthetic colorants. Anthocyanins (ACNs) are water-soluble pigments responsible for the red-to-blue coloration in some plant materials; however, their application in food is limited by their lower stability. A promising alternative is found in the color stability of red wines [1], ACN-derived pigments called pyranoanthocyanins (PACNs). Their formation occurs slowly during fermentation by the interaction of hydroxycinnamic acids (HCAs) or 4-vinylphenols (4VP) with grape ACNs. This study aimed to efficiently produce PACNs through simultaneous bacterial production of 4VPs and reaction with blueberry ACNs.

Lactic Acid Bacteria (LAB) strains (*L. plantarum*, *E. mundtii*, and *P. pentosaceus*) were grown separately in a commercial MRS medium at 32˚C. Cells were collected by centrifugation after reaching stationary phase and seeded at an optical density of 0.2 (OD_{600nm}) into a chemically defined medium (pH 4) containing highbush blueberry anthocyanins and p-Coumaric acid (pCA; 1:2 molar ratio). The mixture was incubated for 72 hours total, 24 hours at 32˚C, and 48 hours more at 45˚C. pCA bioconversion to 4VP and PACN formation were monitored by HPLC-PDA-MS/MS analysis. Color was calculated using ColorBySpectra based on spectral changes recorded in a plate reader.

Select LAB strains decarboxylated pCA completely into 4VP within the first 24 hours of incubation, as a detoxification mechanism against HCA's antimicrobial activity [2]. The bioconversion and the increase in temperature facilitated an efficient PACN formation since 4VP is a more reactive cofactor than pCA and 45˚C favored interaction between reactants. After 72 hours of incubation, the final pigment profile contained up to 50% PACNs, revealing a hypochromic shift in their λmax of ~506nm compared to ACNs (525nm).



PACN formation assisted by LAB could enable efficient colorant production from diverse plant materials containing the essential reactants.

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Keywords: Pyranoanthocyanins, p-Coumaric acid, 4-vinylphenol, Blueberry.

Curcumin based yellow, red and blue colourants

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Abstract

Europe absorbed approximately 87 million tons of packaging waste per year (2020). Industrial composting is often used for biobased packaging materials, such as PLA. Non-biodegradable synthetic colourants are released during composting, as well as after littering of packaging material. Many contain toxic components that can accumulate in the environment. When recycling paper, these colourants also end up in wastewater. Partners from Ireland, Germany, Belgium and the Netherlands started a project in 2020 to address this issue.

During this project we have developed yellow, red and blue colorants based on the yellow food colorant curcumin (E100). Curcumin is the yellow ingredient of the spice turmeric, and the compound can easily be extracted from the Curcuma longa plant. By means of straightforward chemical modifications of curcumin we have been able to produce red and blue colorants as well as yellow colorants with enhanced UV-stability. The newly prepared curcumin based compounds have been applied as colourants in color master batches using PLA and have tested upon its lightfastness using a QUV setup. From these results the best colourants have been selected for the production of packaging material prototypes. Yellow colourants are suitable for indoor packaging materials only as they are more prone to fading compared to the red colourants. The lightfastness results from some of the red colourants in which low ΔE values were obtained after prolonged QUV testing prompted us to start the follow project starting this year in which we will develop a curcumin based colourant route to market. The presentation will show the latest results from both projects, which will include the application of yellow, red and blue colourants in plastic materials.

References: https://vb.nweurope.eu/projects/project-search/curcol-curcumin-based-sustainable-colours/

Keywords: natural colourants, curcumin, colour masterbatch, packaging

Yellow onion (*Allium cepa*) as a UV protective colorant

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Abstract

Natural plant dyeing is considered an eco-friendly dyeing technique, as it utilizes dyes and colorants obtained from various parts of plants such as stems, roots, leaves, and peels for dyeing textile materials (Che & Yang, 2022). With the growing concerns regarding the health and environmental impact of synthetic dyes, researchers are turning to nature for sustainable alternatives. Flavonoids, which are known for their antioxidant properties are widely used as natural colorants in the textile industry. Exploiting the substantial flavonoid-rich waste produced by the food processing industry, this study investigates how yellow onion dye (YOD) obtained from yellow onion skins can be used to dye cellulose nanofiber (CNF)-based films, intended for use as a packaging material. The study centers around achieving optimal color intensity and UV shielding properties utilizing various mordanting agents.

Dyeing experiments conducted following the protocol outlined in (Grande et al., 2023) on red onion dye (ROD), reveal the important role of YOD concentrations in achieving desired shade and UV shielding properties in CNF films. To study the pretreatment condition, the skins were extracted in hot water, and both dried and freshly extracted batches were used in the dyeing process. The efficiency of biobased mordants, such as polyelectrolytes and organic acids was compared to metal mordanting in terms of the substrate color tone and intensity. This study also briefly explores cotton fabric dyeing using YOD, suggesting that the desired color for cotton fabric may only be achievable with the use of various mordanting agents. The study emphasizes the need for further investigations, particularly regarding the lightfastness and wash fastness properties and their potential enhancements by utilizing acid-mordanting procedures. However, promising dyeing results for CNF films were achieved even without mordanting agents when using a fresh dyeing solution.

Keywords: natural dyeing, flavonoids, biobased mordants, added value, UV shielding,

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Anthocyanin-embedded silk fibroin nanofibrils with controlled scale serve as novel stabilizers for pigments

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Abstract

The outstanding functionalities of protein nanofibrils benefit from their hierarchical structures. Silk nanofibrils (SNFs), derived from edible silk fibroin, are considered effective matrices for the stabilization of bioactive agents. Anthocyanins (ACN), as natural pigments with health benefits, exhibit relative instability. The hierarchy of SNFs manifests diverse secondary structures and β-sheet crystals, which may improve the stability of anthocyanins.

In this study, polymorphic SNFs with various secondary structures and crystallinity were prepared by electrospinning-acid hydrolysis (SNFs-I), salt-acid hydrolysis (SNFs-II), or an acidic-ultrasonication combined method (SNFs-III). The interaction between these SNFs and ACN was characterized using multiple microscopy and spectroscopic techniques, along with molecular docking, to assess their structural, thermal stability, and digestibility properties.

Results showed that SNFs-I exibited a nanocrystal morphology featuring a rod-like structure, with diameters of 10-20 nm and lengths of ~1μm. The SNFs-II exhibited a size range of 2-4 nm, with contour lengths extending to several microns. SNFs-III, obtained based on the H-firoin peptide, had a diameter of 2-3 nm and a length of about 500 nm. Binding with ACN altered the structure of all three SNFs with nanofibril aggregation, β-sheet disruption, and decrease in crystallinity, where SNF-III-ACN complex exhibited a disciplined entangled fibrous network with the highest content of β-sheet and crystallinity. SNFs effectively improved the chemical stability and bioaccessibility of ACN. SNF-III showed the best protection, with a 4.04-fold increase in thermal protection compared to ACN alone. The peptide sequence of SNF-III, characterized by (GA) and (GAGAGS) compositions, has a molecular weight of ~10 kDa. Molecular docking indicated that ACN was embedded in the hydrophobic cavity of a β-sheet structure by hydrophobic forces, thereby enhancing the stability of ACN.

These results elucidate the characteristic peptide structure of SNFs that stabilizes ACN and lay the groundwork for further research on peptide-based stabilization of natural pigments.

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Keywords: Anthocyanins; Silk nanofibrils; Stabilization; Topology protection; Peptide sequences

Bio-based colourants from buckthorn and chokeberry

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Abstract

Plant-derived colorants are in their nature biodegradable and compatible with the environment, as well as being less allergenic and less harmful than their synthetic counterparts. Apart from their inherent dying properties, plant-based colorants exhibit additional characteristics, including antioxidant and antimicrobial activities that can be utilized in cosmetic, textile, and food industries. Owing to their chemical composition, these substances may become less stable when exposed to elevated temperatures, light, and varying pH levels. This prompts research and innovation efforts directed toward improving the stability of colorants derived from plants.

In this study, we used two berry species, buckthorn (Hippophaë rhamnoides L.) and chokeberry (Aronia mitschurinii) to study their chemical composition and characteristics. These berries were specifically selected for their cultivation in Finland, and because their dyeing properties have not been extensively explored. After pigment extraction, we employed High-Performance Liquid Chromatography (HPLC) and Quadrupole Time of Flight Mass Spectrometry (QTOF-MS), FTIR, and NMR to analyse the chemical composition of the extracts. We unveiled a diverse array of chemical compounds, prominently found within buckthorn berries classified under the carotenoid group and flavonoids, responsible for imparting vibrant pigmentation to the berries. Chokeberry on the other hand was rich in anthocyanins giving rise to intense colours and variations in their colour spectrum. The effect of pH on the variations in the colour hue and intensities was measured with UV-VIS spectroscopy.

The absorption characteristics of bio-based pigments on natural fibers derived from cellulosic and protein-based substrates were elaborated and optimised, particularly to enhance our comprehension of how these pigments express their dyeing characteristics across diverse material substrates and how we can improve their colour fastness.

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Keywords: colorant, plant-derived, buckthorn, chokeberry, textile

Sustainable production of betalains in engineered *Yarrowia lipolytica*

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Abstract

The oleaginous yeast *Yarrowia lipolytica* has emerged as a leading cell factory for synthesizing various valuable compounds including lipids, fatty alcohols, lipases, and carotenoids. With several strains attaining Generally Recognized as Safe (GRAS) status from the FDA, its utility extends to diverse applications such as food supplements and sweetener production.

Betalains, encompassing betacyanins and betaxanthins, serve as hydrophilic vacuolar pigments responsible for the vibrant coloration in roots, fruits, and flowers of plants within the Caryophyllales order. Betanin, derived from red beets, holds approval as a natural red food colorant (E162) and is recognized for its antioxidant properties, potentially offering health benefits.

In light of growing consumer concerns regarding the safety and sustainability of synthetic food colorants, there's an increasing demand for natural alternatives. However, the low betanin content in beetroot poses significant challenges in terms of extraction efficiency and resource utilization. To address these issues, a sustainability-driven biotechnological approach was developed, leveraging *Y. lipolytica* to produce betalains, including betanin and its isomer isobetanin. Through meticulous metabolic engineering and fermentation optimization, significant yields of betanin and isobetanin were achieved, showcasing the feasibility of this approach.

Moreover, life cycle assessment revealed that the fermentation process significantly reduces land, energy, and resource requirements compared to traditional betanin extraction methods. Finally, techno-economic assessment underscores the potential economic viability of betanin production via fermentation in existing market conditions. This highlights the pivotal role of *Y. lipolytica* as a sustainable platform for the production of natural food colorants, addressing both consumer preferences and industrial sustainability goals.

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Keywords: Betalains, Betacyanins, Betaxanthins, Fermentation, *Yarrowia lipolytica*.

Designing with biocolours

Designer's dream - alder buckthorn (*Rhamnus frangula*)

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Abstract

For a designer working with natural dyes, alder buckthorn (*Rhamnus frangula*) is a rich source of colours. Contemporary dyeing manuals tell that with the boiling method and alum mordant the leaves of alder buckthorn give yellow and golden yellow on wool. The early 20th century dye books advise to treat the skeins with cold, alkaline wood ash lye to turn yellow hue reddish.

During the Middle Ages immature berries of *Rhamnus* species were used to dye intensive yellow. It is less known that the ripe berries of *Rhamnus frangula* can also give bright green and turquoise colours, as shown in our dyeing experiments.

Finnish and Estonian folklore mention alder buckthorn as a source of red. Experimental research has revealed that this colour cannot be obtained with the boiling method alone. Our intensive experimentation has proven that by fermenting bark at room temperature for weeks produces first brownish, then dark red, dark maroon, salmon pink and pink colours on wool. Alternatively, the bark can be pre-treated by burying the bark for weeks or months in soil whereafter it can be used in dyeing with the boiling method. This procedure yields bright reds, oranges and purple on wool with and without alum mordant.

The potential of *Rhamnus frangula* as dye is not yet fully explored. To gain more knowledge of alder buckthorn's character the dyed wool samples will be analysed by HPLC to detect main colouring compounds. Also, the obtained colour will be determined by CIEL*a*b* values, and colour fastness tested according to the ISO standards. Currently it is known that vivid hues can be achieved on protein fibres wool and silk, but on cellulose fibres the hues are paler. When dyeing both white and grey wool, the number of different colours can be multiplied.

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Keywords: Plant dye, traditional recipe, low-energy dyeing, experimental research, colour quality

Designing with natural dyes – design students' perspectives

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Abstract

This article explores the use of natural dyes in textile and clothing design by design students. Until the mid-19th century, natural dyes were a common dyeing method in textiles, but synthetic dyes began to replace them through industrialization, and their reign has continued until these days, especially in the mass production in the textile and clothing industry. The industrial production and use of synthetic dyes have caused environmental problems that have raised the attention of industry, research and education towards natural dyes as a possible environmentally and human-friendly option. Striving for sustainable and responsible manufacturing requires changes at various points in the textile and clothing industry's value chain, beginning from the design stage. This article focuses on the designer's role and decision-making in initiating change from the perspective of design students. Designers influence the way of thinking and attitudes about natural dyes, their use, meanings and future. This article presents a study conducted for design students (N = 11) of the University of Lapland, which aimed to identify specific perspectives related to design with natural dyes. The data were collected through a survey in 2023 from the students of the Experimental Textile Expression course. The main question we look at is: "What issues should designers take into account when they use natural dyes?" A content analysis suggests integrating natural colours into a design requires a new way of thinking and a change of mindset from trend colours to colours that are available from nature. For example, traditional trend forecasting does not follow the colour cycle or the availability of natural dyes. Understanding the unique properties of natural dyes, including hue, saturation and colour fastness, emerged as an important aspect of the successful realization of the products.

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Keywords: natural dyes, craft, textile and clothing design, designer, sustainability

Presenting production development projects with naturally dyed local wool

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Abstract

This conference paper presents insights and outcomes from a decade-long exploration of production development projects built around producing naturally dyed local wool products. Beginning with the Aurinkokehrä collection in 2014, these projects evolved through collaborations with dye extract producer Green'ing owner Patrick Brenac. Notable initiatives include creating a 1000-color tone recipe chart for business clients, piloting natural dye recipes on industrial cone dyeing machines, and founding a specialized dye-house to enhance the availability of Finnish wool for crafters as well as manufacture scale production.

The paper delves into the challenges and successes encountered during these ventures, such as adapting industrial processes to natural dye extracts, identifying weak points in the production chain, and addressing the demand for slow, small-scale production. Key considerations include the rhythm of spinning production when natural wool color bases are applied, and the challenge of recipe repeatability as well as effective dye process development.

The results highlight the significance of sustainability values, cultural impact, and social wellbeing in crafting a narrative around naturally dyed wool. The paper concludes by emphasizing the importance of a flexible production process and acknowledges the role of science in answering questions about reputation, fastness, and the necessity of recipe repeatability.

In conclusion, this comprehensive exploration offers valuable insights for those engaged in natural dyeing and small-scale industrial textile production, providing a roadmap for navigating challenges and promoting the significance of sustainable practices in the textile industry.

References: [URN: fi:amk-2014120518622](https://urn.fi/URN:NBN:fi:amk-2014120518622)\]

Keywords: textile design, collection, production design, industrial dyeing process, dye extracts

Living colour systems – a literature review and analysis of microbial colourant research for textile dyeing and printing

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Abstract

The global reliance on petroleum-based synthetic dyes in the textile industry is a major contributor to global pollution and the current state of the climate emergency. However, there is a solution found in the fermentation of microbial colourants derived from bacteria, fungi, and algae. These colourants can be produced in large volumes in short timespans, reducing water and energy usage and minimising waste output(Colorifix, 2022). Their production is not affected by seasonal changes, does not require large land areas for crop cultivation and has potential to use surplus from other industries as feedstock.

Research on microbial colorants for textile applications was first published in the 1990's(Herbertz et al., 1995) and has since grown significantly. This review will encompass past and ongoing research in this field, considering studies from both science-led research and developments from design-focused practitioners. It is important to highlight the divide between design-led and science-led research approaches, with science-led start-ups focusing on scalable industrial processes and design-led research often emphasising collaborative working relationships with living organisms to develop creative processes, design tools, and equipment(Collet, 2023).

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Keywords: microbial colourants, textile industry, dyeing, printing, sustainable coloration

Glycosylation impact on application potential and color of 10-guaiacyl-pyranocyanidins

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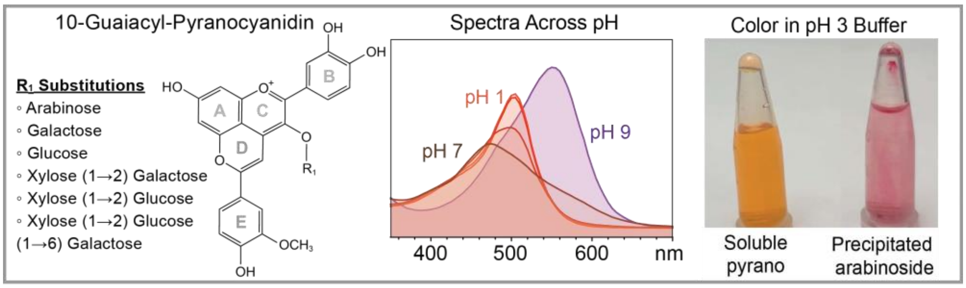
Abstract

Pyranoanthocyanins are a diverse class of nature derived colorants with potential for use in foods and cosmetics. They have excellent stability to pH and bleaching (Oliveira et al., 2006), and they could be produced from a variety of anthocyanin sources including agro-industrial byproducts as an environmentally sustainable colorant . The sub-class of 10-guaiacyl-pyranoanthocyanins may form efficiently (Miyagusuku-Cruzado et al., 2023), but they often precipitate in water. Our goal was to evaluate how glycosylation patterns, which affect polarity, impacted 10-guaiacyl-pyranocyanidin color expression and stability in isolated systems and applied in food emulsions.

Six unique 10-guaiacyl-pyranocyanidins were formed from cyanidin anthocyanins with different C3 glycosylations and 4-vinylguaiacol (Figure) (Miyaguskuku-Cruzado et al., 2023). Each pyranoanthocyanin was isolated, dried, and identities checked with HPLC-PDA-MS/MS. Color expression and stability were spectrophotometrically measured across pH 1 to 9 and in different buffers. For application, 10-guaiacyl-pyranocyanidin-rich extracts differeing in glycosylation profiles, selectivly made from *Aronia melanocarpa*, *Sambucus nigra*, and *Daucus carota*, were mixed into oil in water emulsions (20 or 60% oil) stabilized with lecithin and xanthan gum. The opaque color was measured with a spectrocolorimeter.

Glycosylation patterns had minimal effect on color with all pyranoanthocyanins producing similar orange hues (λmax = 507 – 510 nm). This orange color was stable up to pH 7 but bathochromically shifted above this pH (Figure). Increasing glycosylations improved solubility, with the 3-arabinoside, a pentose, readily forming pink colored precipitate (Figure). In emulsions, 10-guaiacyl-pyranocyanidins from all three anthocyanin sources produced uniform color, and adding the color to the oil (with lecithin) or aqueous (with xanthan gum) phase did not impact the final color. By adjusting glycosylations, 10-guaiacyl-pyranocyanidins can be designed to work in a range of products differing in polarities with little impact on their color performance. This opens new opportunities to use pyranoanthocyanins as colorants.



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Biocolouring with *Beta vulgaris*: exploring colour impermanence as a design premise through contemporary costume design

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Abstract

Colour is a fundamental quality of costume, however, the synthetic colourants favoured, largely due to their predictable and stable colour results, in contemporary costume practices are often developed from petrochemicals through processes that cause damage to the natural environment and its inhabitants. What happens if the unstable and shifting qualities of biobased colourants are allowed to orient an explorative costume design process? This paper presents some of my explorations with *Beta vulgaris,* the beetroot vegetable, as a case study for costume design with impermanent biocolourants, where material circularity, aesthetic unpredictability, and messiness became central premises for the material approach. It invites critical reflection on how impermanent biobased colour can be understood and appreciated as a performative agent while being sensitive to the urgent need for more environmental sustainable design practices.

These explorations are situated within my doctoral research project 'BioCostume: Experimental Costume Design with Biobased Co-Actants' (2020-) which is located at the intersection of contemporary costume design, systems thinking and biobased material development; of aesthetics and ethics. It explores how ecologically informed material practice in costume design can contribute towards new perceptions and understandings of material agency, where ecologically responsible and accountable praxis is considered a value with implications beyond the performance itself.

Biocolours on markets and businesses

Towards a circular bioeconomy: closing open loops in the transition from synthetic to bio-based colourants in the textile and packaging industries

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Abstract

The circular bioeconomy seeks to utilize renewable biological resources, as well as their waste streams for the production of value-added goods (Carus & Dammer 2018). However, as Salvador et al. (2021) have pointed out, bioeconomies are large and diverse, and mainly dependent on local characteristics such as the availability of specific resources and logistical factors. Currently, the production of colourants is based on non-renewable materials (e.g. Pereira & Alves 2011), which creates an opportunity to alter colourant production towards one more fitting of the circular bioeconomy. In our study, we elucidate the obstacles and prospects for a circular bioeconomy of colours, which could be achieved by a replacement of mainstream synthetic colourants with bio-based alternatives in the textile and packaging industries to some extent. Our study employs a qualitative approach with data collected from 13 semi-structured interviews with business experts. Our interviews discussed themes ranging from value chain collaboration to how production sustainability could be enhanced by biocolourant use. The preliminary results suggest that actors involved in the making of biobased colourants lack established ways of cooperation. The lack of networks between actors, along with issues related to technology gaps and consumer knowledge, hinder the adoption of biobased colourants. However, there are key opportunities for forerunners to seize waste streams for the creation of value, as actors who diverted their waste streams towards colourant production considered it both a financial and marketing opportunity. Our study therefore outlies ways in which actors involved with biocolourant production could drive the transition to a more circular colour system forward.

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Keywords: sustainability, circular economy, closed-loop systems, biocolourant

Choices for sustainable colour – Societal and cultural perspectives

Biocolours and textilecraft around the lake Tuusula

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Abstract

Nature provides inspiration for artist. For craftsmen it also provides material resources like dyes. Natural dyeing is universal and globally known, but at the same time traditions are very local. Local plants and dyeing recipes as well as cultural customs are intertwined into global craft heritage (Primetta et al. 2019). In this study we want to highlight the natural dyeing traditions with local dye plants from the perspective of one dyer and artists who lived at the Lake Tuusula surroundings, in Finland, in the beginning of the 20th century.

In the fall 2015 we received a large collection of yarns dyed with the plants of the Tuusula Rage. The collection was made by a local crafter and dyer Laila Siitonen during the years 1960-1970 (Vyyryläinen, 2018). We investigated her dye sources, dye recipes and obtained colours as well as the history of the dyer herself. Furthermore, we combine this knowledge with the artists who lived at the Lake Tuusula surroundings in the beginning of the 20th century. Lake Tuusula is famous for its art community which included several painters, writers, poets, and composers. We are especially interested in the works and family life descriptions of female artists, such as Jenny Soldant-Brofelt, who is also known to have dyed with natural dyes, and whether these yearns were used for handicrafts.

We will shed light on the artists life at Tuusula Lake from a new perspective: how artists of the early 1900s and their families (wives) used plant dyes in their artistic works and home crafts. The research utilizes the art and artefact collections in Tuusula and Järvenpää, e.g. The collections of the Paloheimo Heritage Foundation and the museums.

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Keywords: Biocolour, textilecraft, natural dyeing, local plants, Lake Tuusula

Colour for the future

From traditions to innovative design in an holistic approach. Japan as a study case

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Abstract

Based on the observation of traditional dyers and priestesses in Japan, mainly in the Ryukyus Islands, focusing on traditional feminine practices involving local flora, and including the observation of techniques, customs and certain types of ritual, the study will concentrate on colouring and care practices using plants. Using a holistic approach, the article will look at material and colour design as an opportunity to propose new approaches to the textile industry, including considerations for the well-being of people and the environment. Considering the concept of Cradle to cradle® as a point of view to define the manufacturing process of favourable colours, the conference will question the circularity of the life of materials, including the manufacturing process. The ways in which plants are considered, manipulated, named and integrated into a creative process, whether material (mainly dyed textiles) or immaterial (animistic care practices involving the so-called 'invisible' world), will be observed and noted. By capturing the chromatic realities of the Japanese islands, I will be able to propose maps and visuals that combine cultural data with potential for the future. A diagram summarising the relationships observed between the different dimensions making up dyeing and care practices will be proposed in order to assess the potential human-environment-plant interactions activated by colour. In conclusion, the author proposes a holistic approach to colour and material design, based on the observation of dyeing and healing traditions, as part of fieldwork practices in Japan, with a view to developing innovative design projects. Design ideas will be put forward, including different types of medium relating to socio-economic sectors such as textiles, paper, make-up and cosmetics.

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Keywords: holistic approach, care design, color territories, natural dyes, color ranges


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