Conference or Workshop Item:

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These are the author's presentation slides of a conference presentation made at the 20th Annual Green Chemistry & Engineering Conference.

UNIVERSITY OF LEEDS

Keracol
Functional, natural, sustainable

Industrial application of anthocyanins extracted from food waste

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[c]School of Chemistry, University of Leeds, UK

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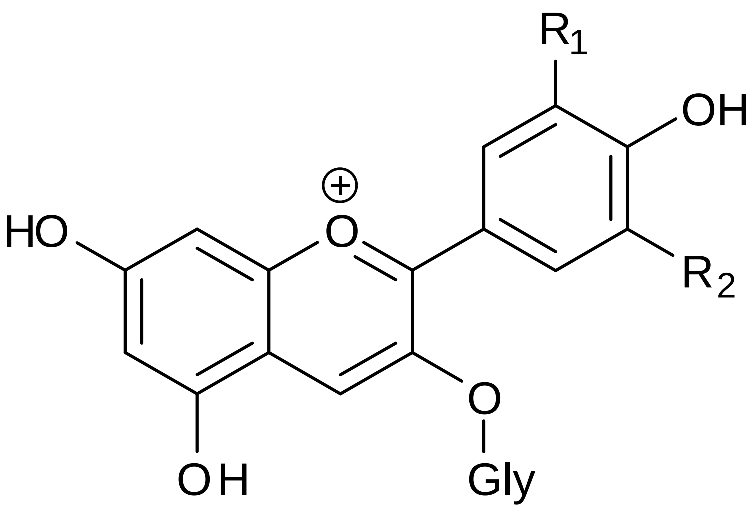
Anthocyanins

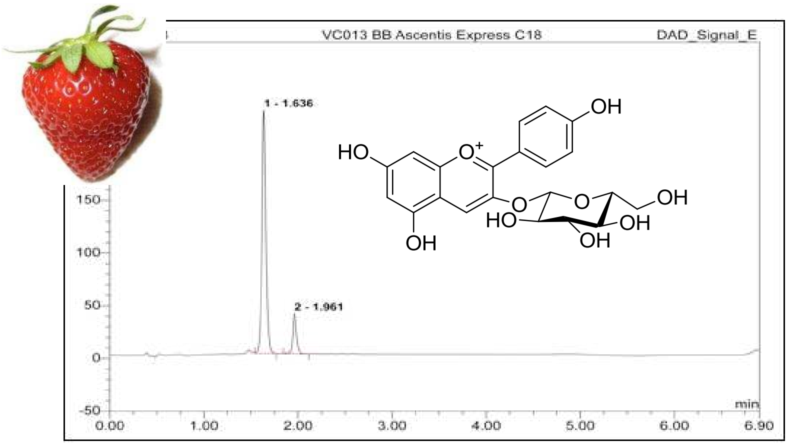
- Found in fruits, vegetables, flowers



Anthocyanin	R_1	R_2	λ_{max} @ pH3
pelargonidin	– H	– H	503
cyanidin	– OH	– H	517
peonidin	– OCH_3	– H	517
delphinidin	– OH	– OH	526
petunidin	– OCH_3	– OH	526
malvidin	– OCH_3	– OCH_3	529

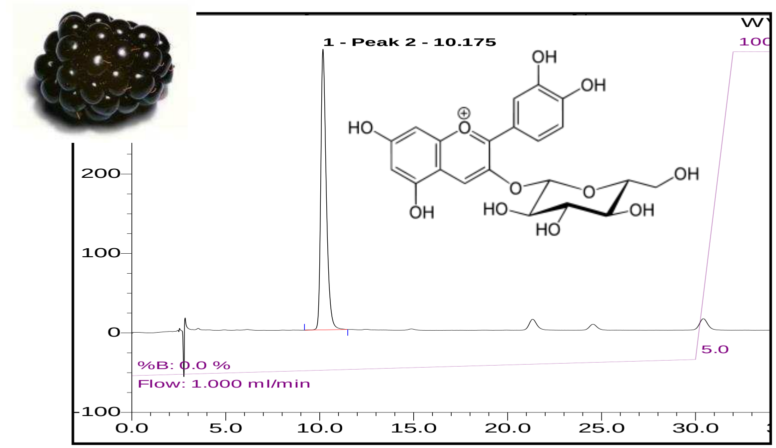
- Glycosylation typically at 3-O position
- In fruits, typically various mono- and disaccharides
- More complex glycosylation observed in other plants

STRAWBERRY (*Fragaria × ananassa*)



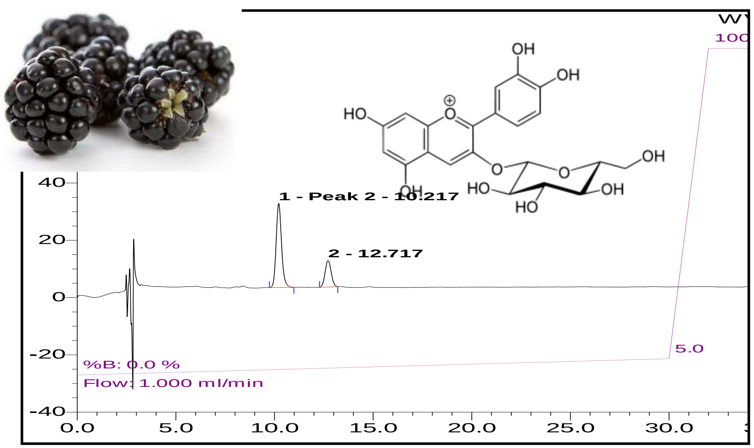
1. Pelargonidin 3-*O*-glucoside (86.35%)
2. Pelargonidin 3-*O*-rutinoside (13.65%)

BLACKBERRY (*Rubus fruticosus*)



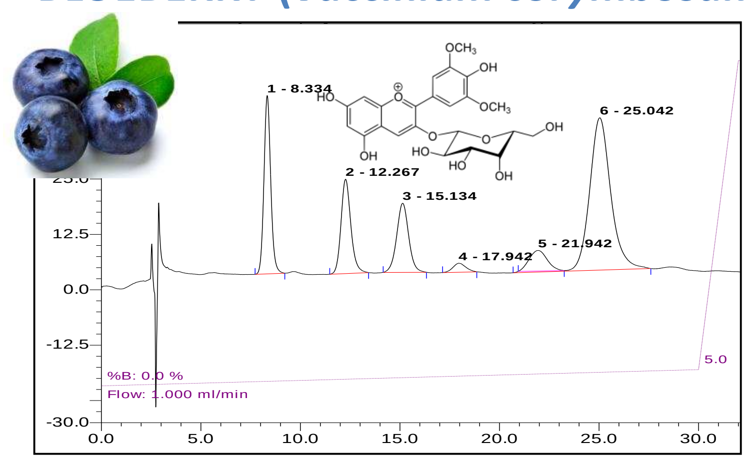
1. cyanidin-3-*O*-glucoside (>95%)

BLACK MULBERRY (*Morus nigra*)



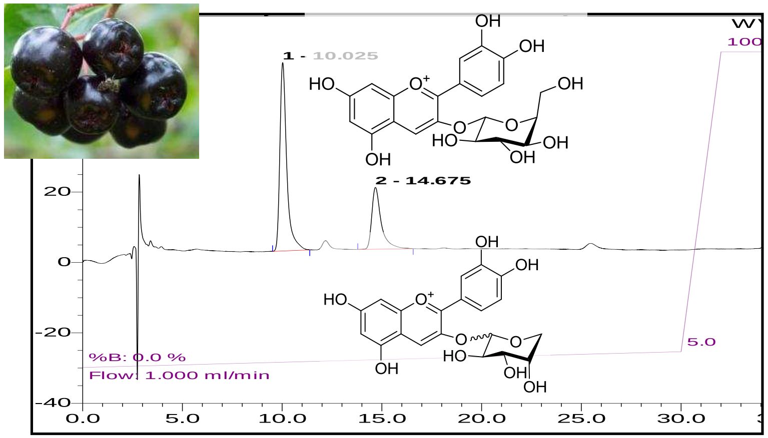
1. cyanidin-3-*O*-glucoside (73.83%)
2. cyanidin-3-*O*-rutinoside (26.17%)

BLUEBERRY (*Vaccinium corymbosum*)



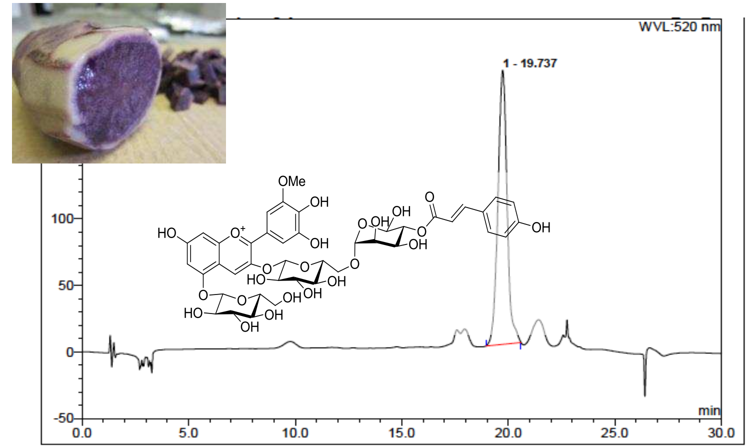
1. malvidin-3-*O*-galactoside (18.1%)
2. delphinidin-3-*O*-galactoside (13.86%)
3. delphinidin-3-*O*-arabinoside (12.38%)
4. petunidin-3-*O*-galactoside (1.74%)
5. petunidin-3-*O*-arabinoside (5.52%)
6. malvidin-3-*O*-arabinoside (48.38%)

ARONIA (*Aronia melanocarpa*)



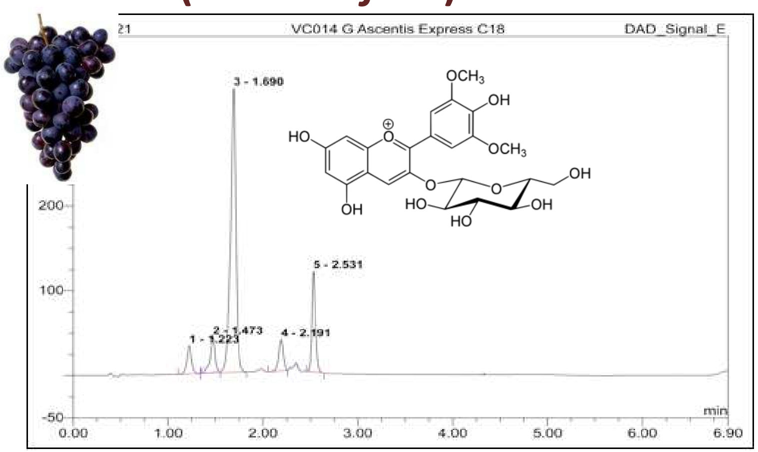
1. cyanidin-3-*O*-galactoside (68%)
2. cyanidin-3-*O*-arabinoside (30%)

PURPLE POTATO (*Solanum tuberosum*)



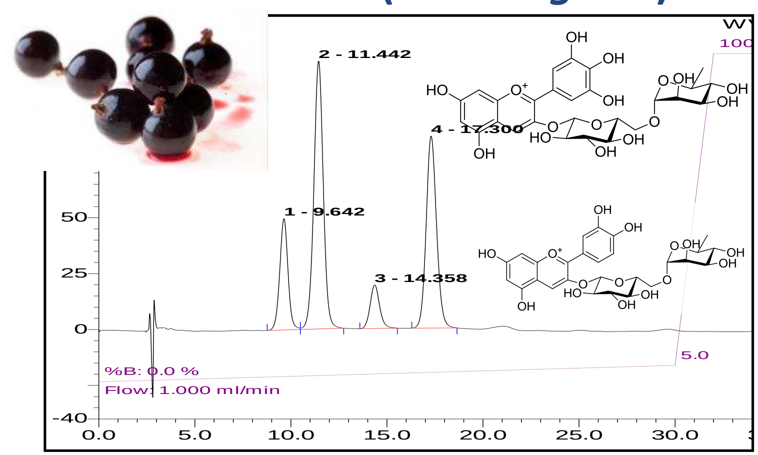
1. Petunidin-3-coumaroylrutinoside-5-glucoside

GRAPE (*Vitis vinifera*)



1. Cyanidin-3-*O*-glucoside (5.84%)
2. Delphinidin-3-*O*-glucoside (7.27%)
3. Malvidin-3-*O*-glucoside (65.30%)
4. Peonidin-3-*O*-glucoside (5.95%)
5. Petunidin-3-*O*-glucoside (15.64%)

BLACKCURRANT (*Ribes nigrum*)



1. delphinidin-3-*O*-glucoside (15.71%)
2. delphinidin-3-*O*-rutinoside (43.25%)
3. cyanidin-3-*O*-glucoside (7.03%)
4. cyanidin-3-*O*-rutinoside (34.00%)

Extraction & Purification

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Clean extraction

- Polar metabolites such as anthocyanins can be extracted using water, ethanol or blends of the two solvents

\+ Non-toxic solvents that allow efficient extractions in optimised conditions
Acceptable solvents for food or personal care and cosmetic applications
No-regulatory limitations

− Non-selective solvents
Free sugars, proteins and low-polarity metabolites are extracted too

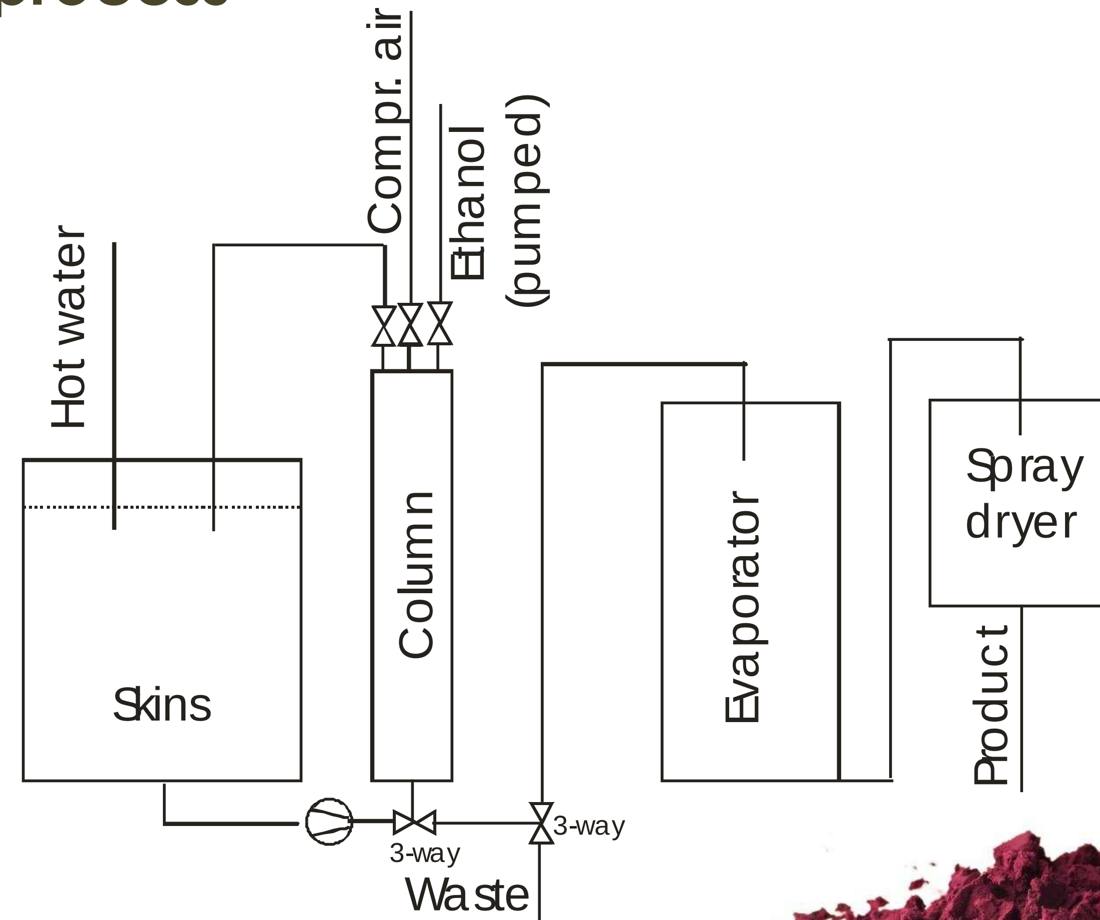
Solid-Phase Extraction (SPE): strategy for extract purification

- Anthocyanins interact with solid phase *via* H-bonding and hydrophobic interactions
- Resin allows for removal of interferents *via* preferential sorption of active
Free sugars removed with acidified water
Anthocyanins subsequently eluted with acidified ethanol

\+ Simple, safe and low cost
Allows high recovery of active
Reduces consumption of solvents

− Source needs to be loaded in water
Scale-up limitation?

Industrial-scale process



Case Study 1: Natural hair dyes

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Extract from blackcurrants (*Ribes nigrum*) grown in UK

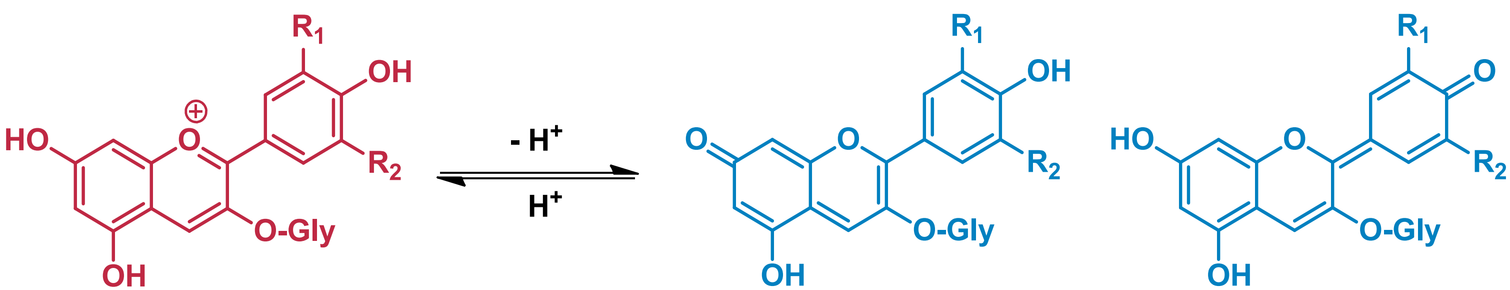
- sustainably sourced
- waste from blackcurrant juice process (Ribena)
- Extracted and purified using green technology
  - Aqueous process, clean, energy efficient
- High levels of both anthocyanins and flavonoids
- Patented[1] semi-permanent hair colorants and coloration process
  - Range of shades, fast to 12+ washes

1. Natural Hair Dyes, US8361167

Case Study 1: Natural hair dyes

Dyeing from acidic medium (pH 3-4)

- λ_{max} in aqueous solution at pH 3.0: cyanidin 517 nm; delphinidin 526 nm
  - purple/violet colour consistent with flavylium cation



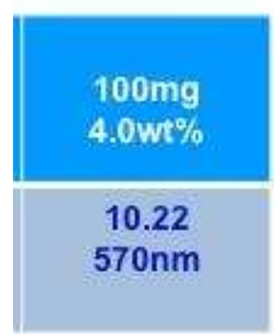
Flavylium Cation, pH = 1

Quinonoidal Base, pH = 7

- λ_{max} when adsorbed onto hair from aqueous medium:

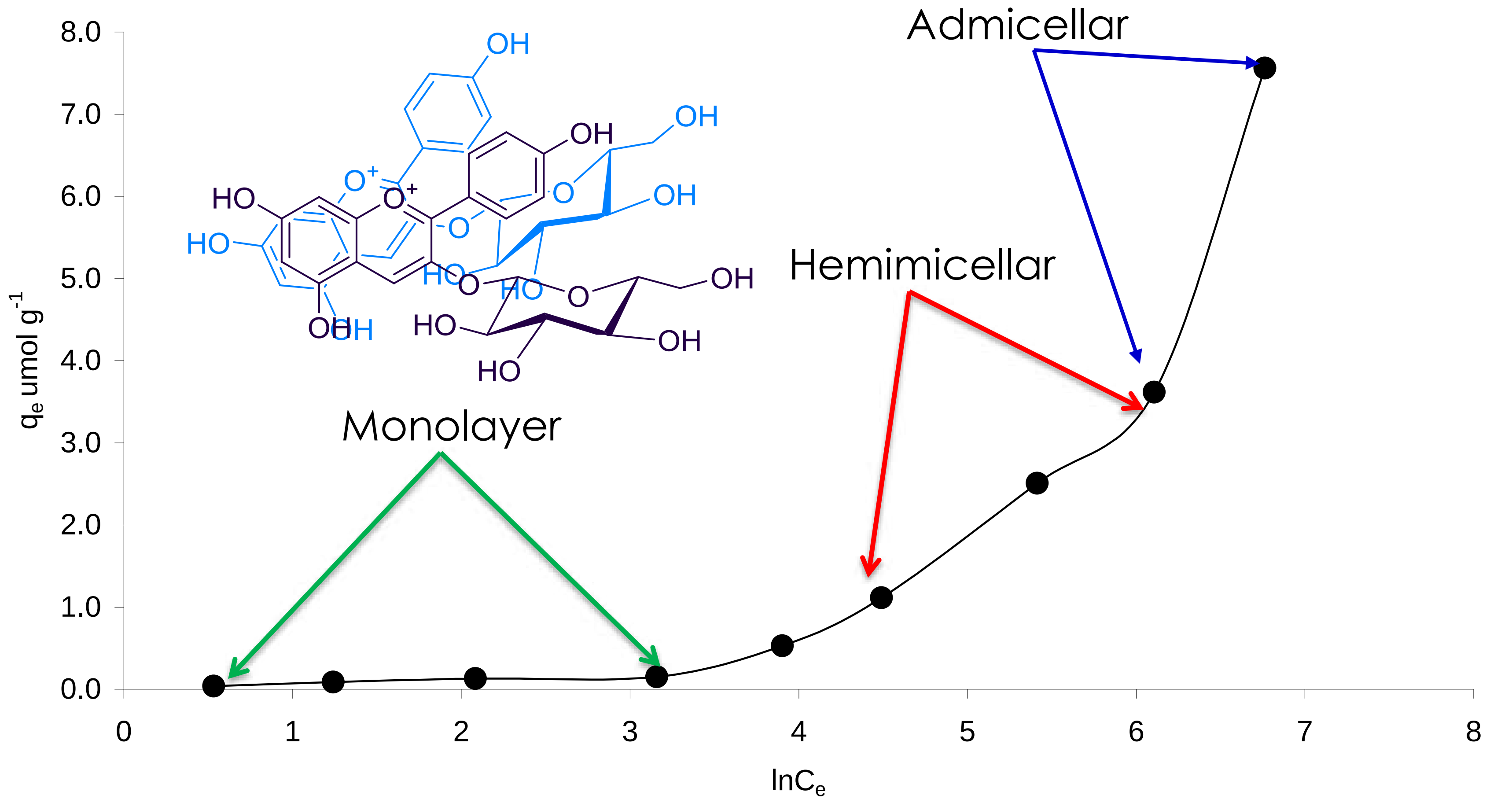
570-580 nm

  - Blue colour consistent with quinonoidal base
  - *in situ* neutralisation by basic sites on hair surface leading to formation of anhydrobase
  - Stable over 12+ washes, minimal colour loss, no colour change



Sorption studies

- sorption significantly in excess of theoretical monolayer capacity
- Formation of **hemimicellar** (side-by-side) and **admicellar** (stacking) aggregates



Blackcurrant anthocyanins

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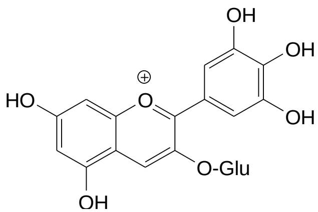
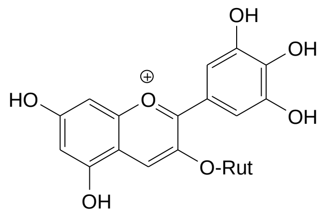
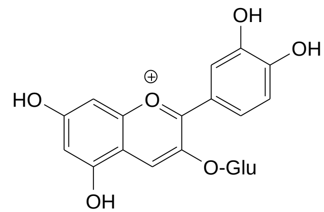


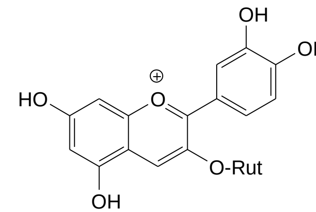
Delphinidin-3-Glucoside



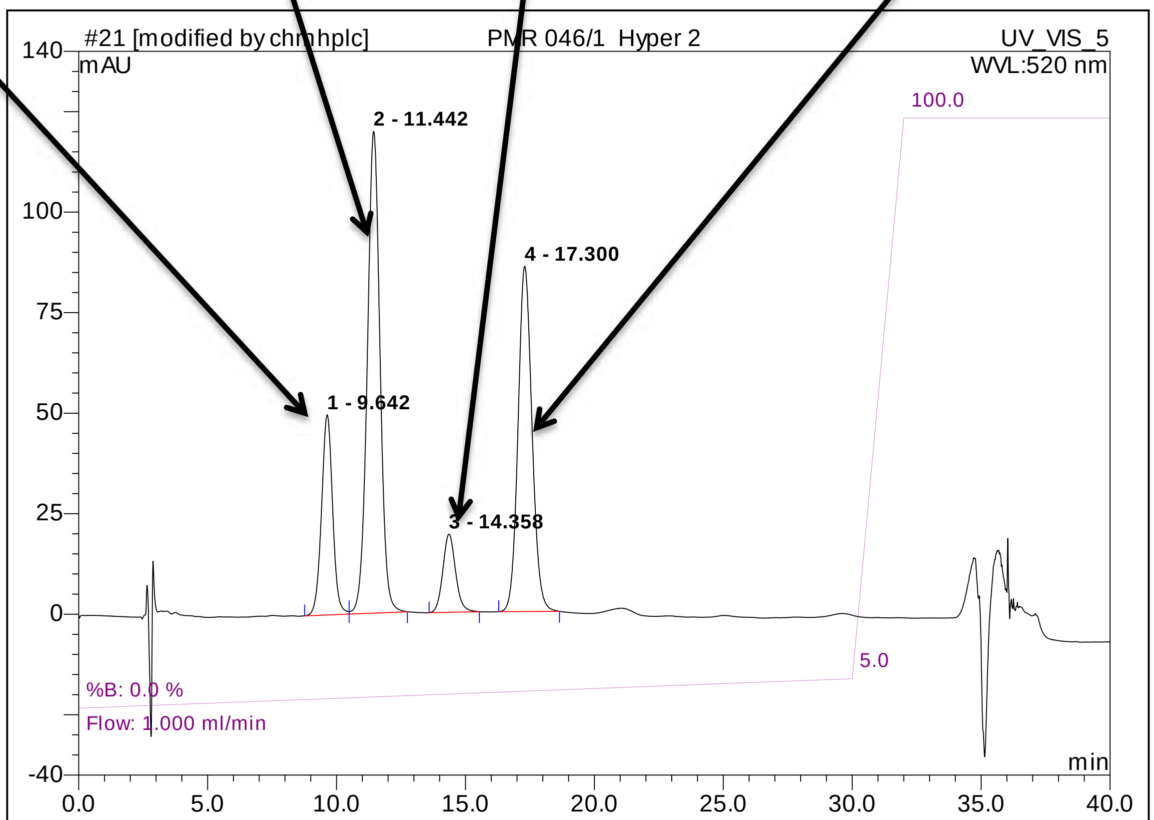
Delphinidin-3-Rutinoside

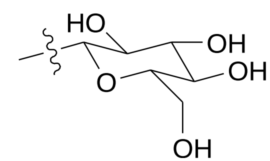


Cyanidin-3-Glucoside

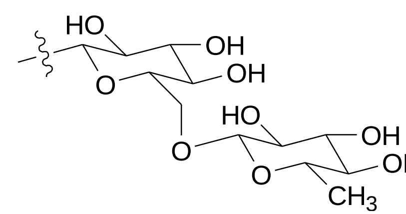


Cyanidin-3-Rutinoside





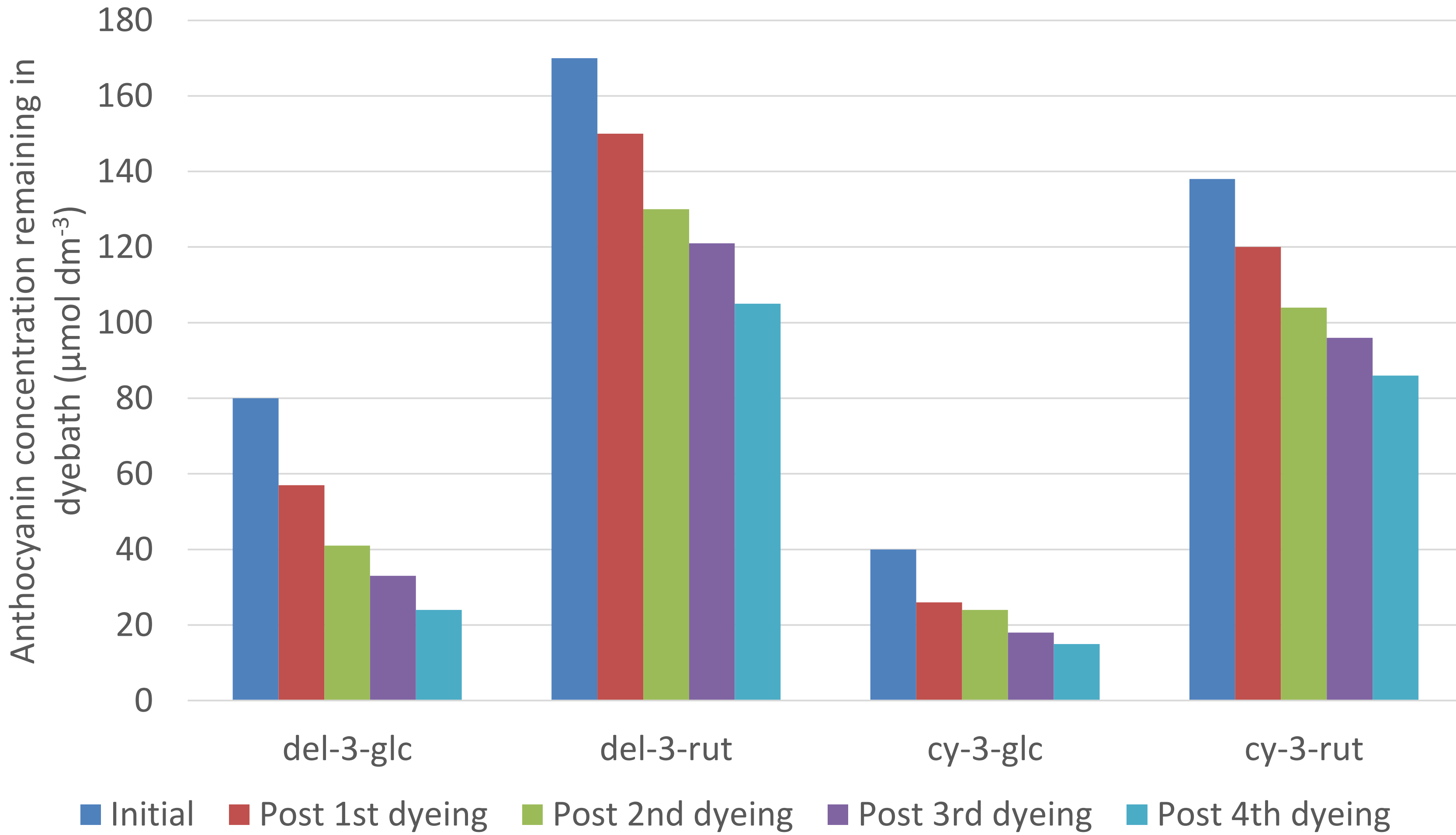
Glucoside Unit



Rutinoside Unit

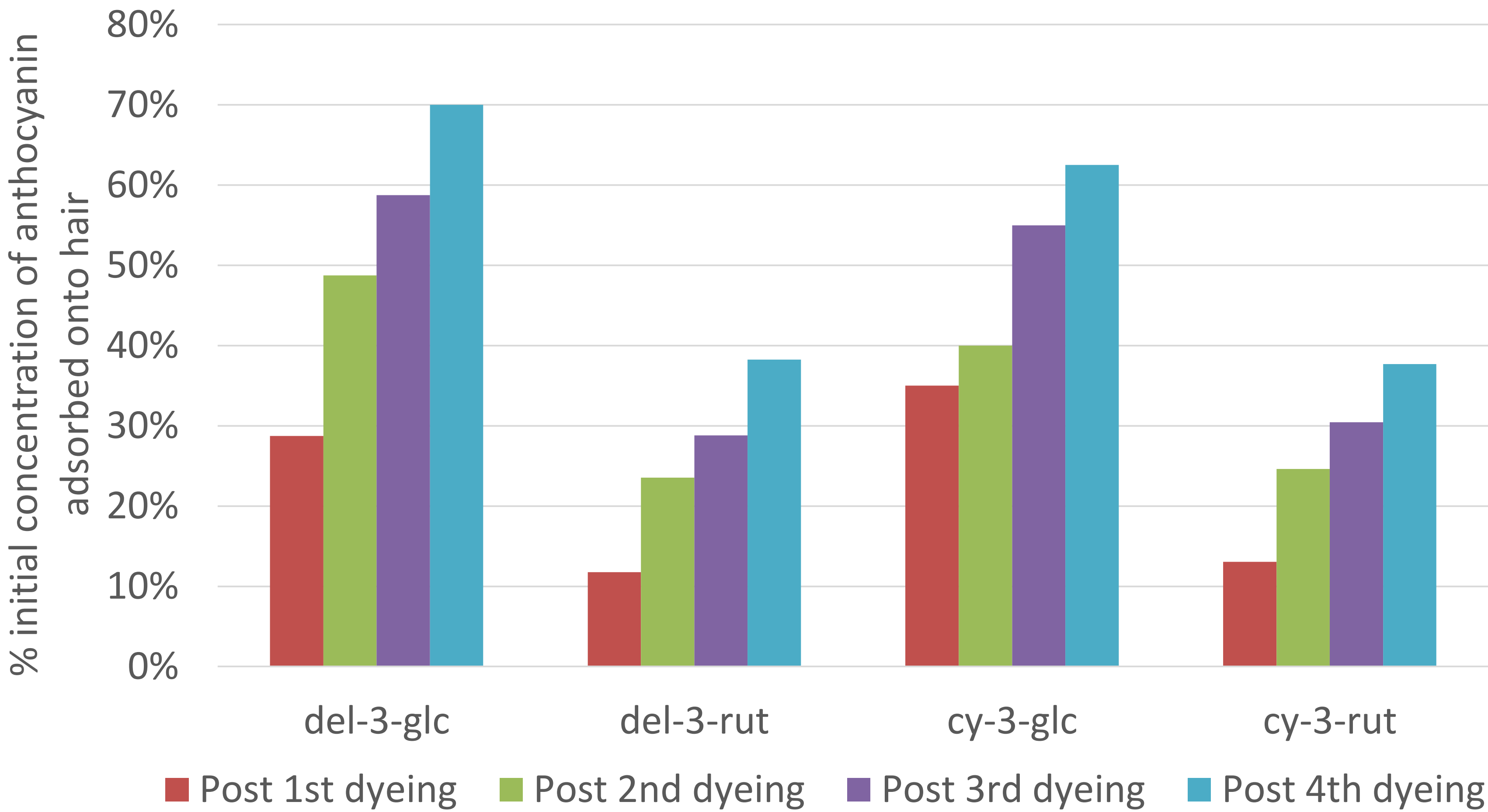
Successive dyeings from solution (amount remaining)

- All anthocyanins adsorb onto bleached hair



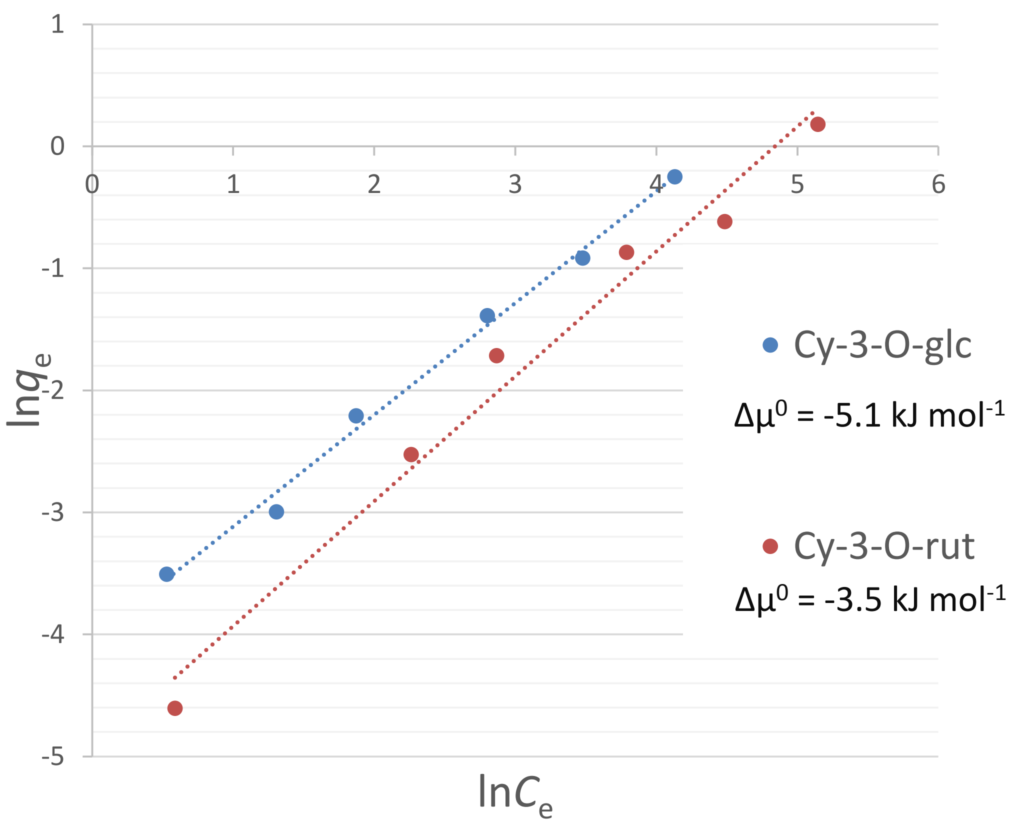
Successive dyeings from solution (amount adsorbed)

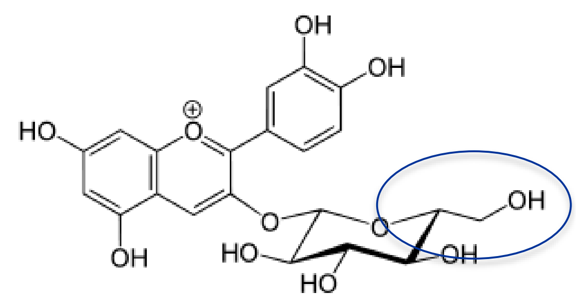
- Apparent preferential adsorption in favour of monosaccharides (glucosides) – *ca.* 2-fold over disaccharides (rutinosides)

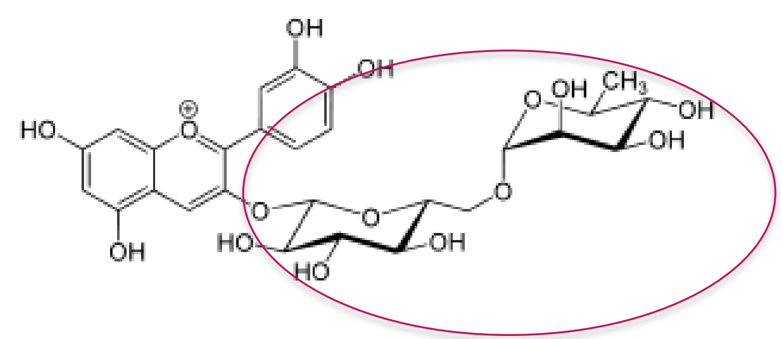


Blackcurrant glycoside sorption

- Isotherm study: cyanidin-3-*O*-glucoside higher adsorption energy in comparison with cyanidin-3-*O*-rutinoside
- Superior H-bonding through primary hydroxyl? Steric effects?

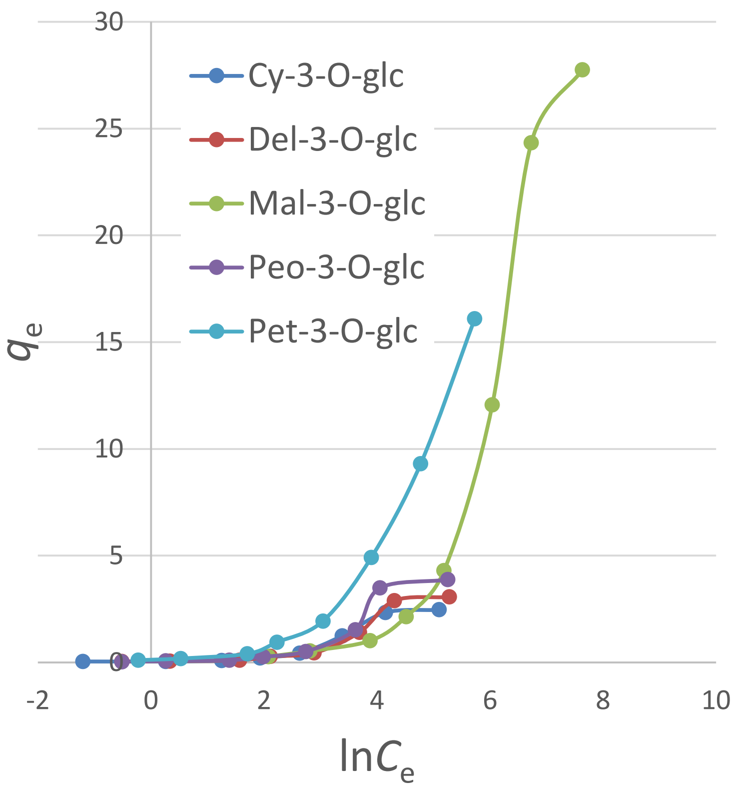


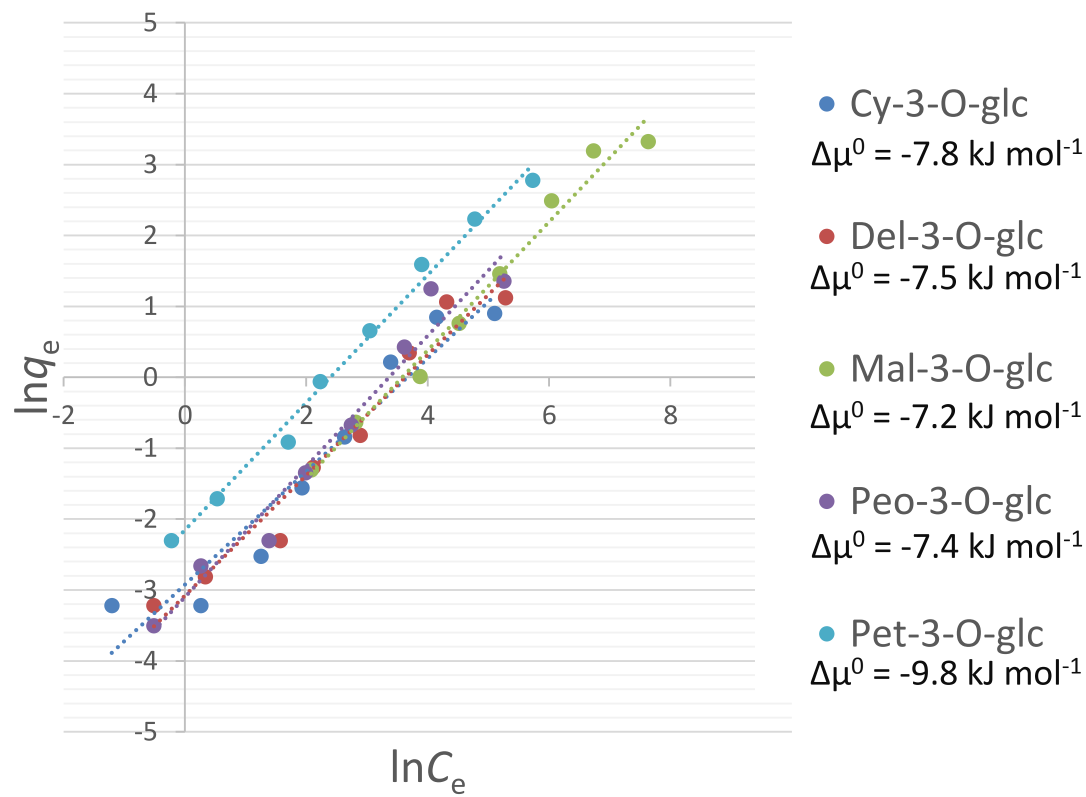




Grape glucoside sorption

- Isotherm study: Most glucosides show consistent sorption properties
- Unexplained sorption differences for petunidin-3-O-glucoside
- However, generally anthocyanin parent structure does not have significant effect on sorption – glycosylation more important





Case Study 2: Food colorants

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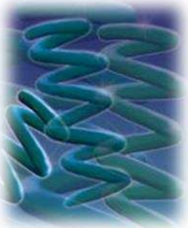
The Challenge

- Blue food colorants dominated by *Brilliant Blue FCF* (E133)
- Can induce allergic reactions
- Regulation a big issue
- Blue from nature most difficult to achieve

The Market Opportunity



- *B. Blue FCF ca*. 1,300 tpa
- Market value >$260m
- Industry → natural colorants
- Spirulina only current natural blue, but has application and stability problems
- Stable, natural blue highly desirable

The technology

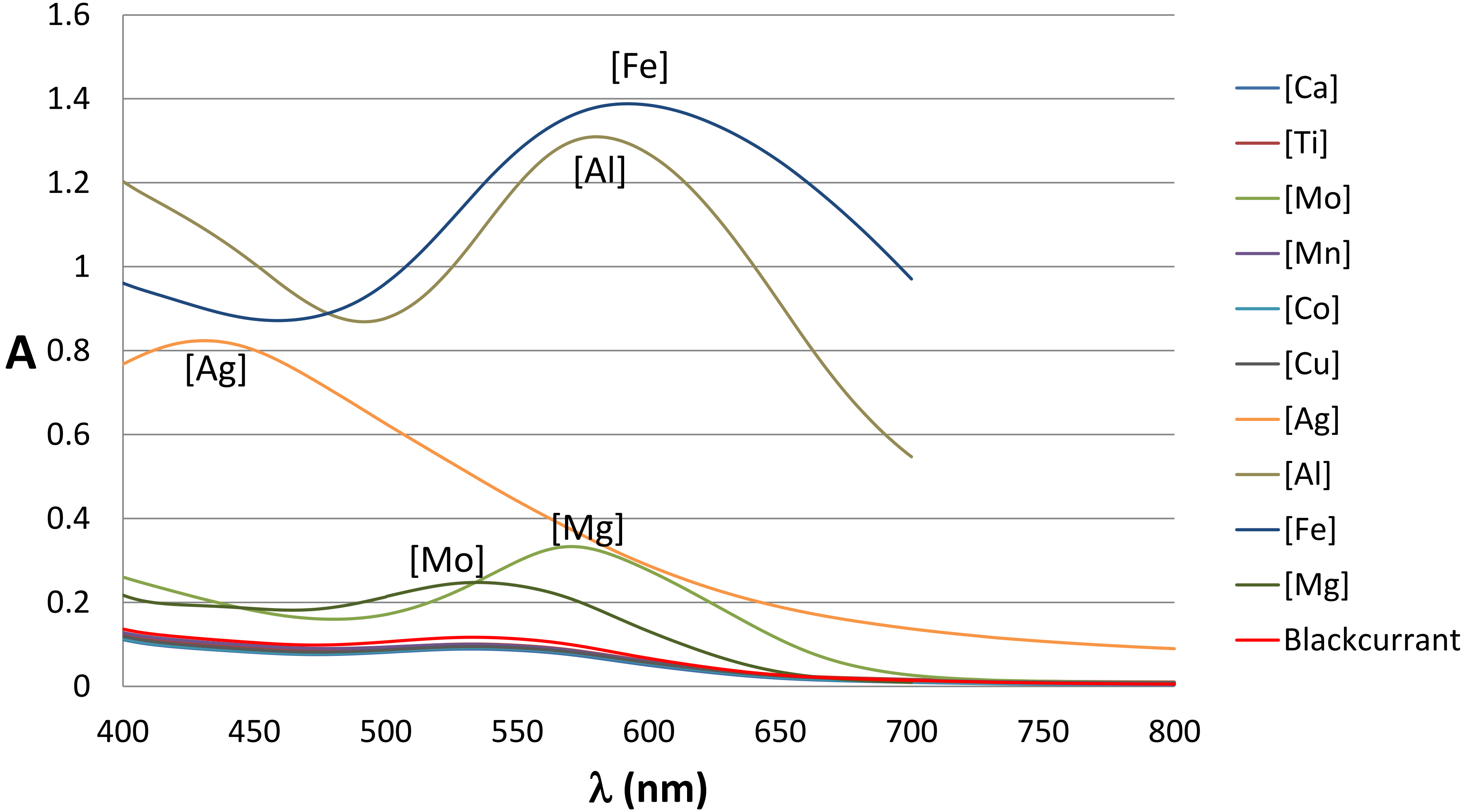
- Anthocyanins extracted from sustainable source plant materials
- Lake pigment formed using novel "biomimicry" process
  - inspired by plant pigment formation in flowers
- Pigments in a range of colours suitable food application
- Both water soluble and water insoluble pigments are possible

Plant sources identified

$\xrightarrow[Mg^{2+},\ Ca^{2+}]{Fe^{3+},\ Al^{3+}}$

Pigments formed (water-soluble and water-insoluble)

Formation of metal complexes with blackcurrant anthocyanins



Stability of complex to pH changes

- Stability of Al-complex in aqueous solution with changing pH
- Blue colour is stable down to pH 2.8; only below pH 2.8 does complex break down and revert to the red form (non-complexed)

<--1 M HCl					Original	1M NaOH-->						
2.0	2.1	2.3	2.5	2.8	3.8	4.1	5.7	7.1	8.4	9.5	10.5	12.2
Red	Red	Red/ Purple	Blue/ Purple	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue	Blue

Stability of complex to citric acid

- Very low concentrations of citric acid cause a colour change to red, significantly higher than pH 2.8
- Effect with malic and lactic acid was not as extreme, but still a colour change was observed at relatively low concentrations
- Acetic acid could be used in much higher concentrations before any colour change was observed
- Effect not directly related to pH - citric acid has a propensity to complex Al^{3+}, abstract the metal from the colorant complex, and cause the complex to break down

Acid	Ratio (mmol acid per g colorant)				
Citric	0.00	0.11	0.53	1.60	3.71
	Blue	Blue/ Purple	Red/ Purple	Red	Red
Malic	0.00	0.27	0.78	1.34	7.39
	Blue	Blue/ Purple	Red/ Purple	Red	Red
Lactic	0.00	0.70	1.67	2.78	10.89
	Blue	Blue/ Purple	Red/ Purple	Red/ Purple	Red
Acetic	0.00	3.67	8.50	11.17	13.33
	Blue	Blue	Blue/ Purple	Blue/ Purple	Blue/ Purple

Example formulations in confectionary products

No acid

Citric acid

Acetic acid

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Formulation of inks for egg coding applications using dyes extracted from waste food products

- Inks developed using colorants extracted from natural waste materials[2]
- Increase in the information placed on an egg
- Reduced environmental and toxicological impact
  - Some current concerns over erythrosine
- **Enhance security, safety, and traceability**

2. Ink Composition, WO2015128646

Case Study 3: Marking eggshell

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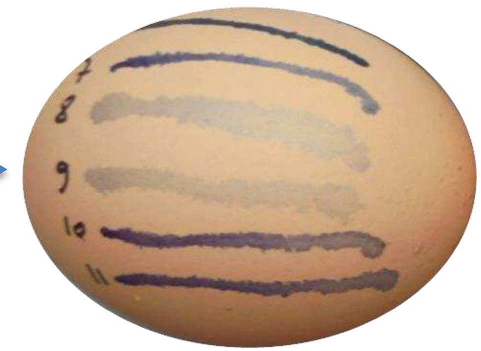
Cy-3-O-glc
Cy-3-O-rut
Del-3-O-glc
Del-3-O-rut

Fe^{3+}

black

Al^{3+}



blue

Cy-3-O-gal
Cy-3-O-arab

red

Case Study 3: Marking eggshell

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New inks technically superior to erythrosine

- Good adhesion, excellent water fastness, no penetration of colorant into egg interior
  - Binding with Ca^{2+} in $CaCO_3$ eggshell forms stable complex (known that Ca^{2+} involved in anthocyanin complex formation in blue flower petals[3])
  - Also protein in eggshell matrix may contribute to interactions with anthocyanins
- Provide high print definition to achieve text and barcoding
- Increase in the information placed on an egg
- Technology being trialled by egg producer in UK

3. Shiono *et al.*, *Nature*. **2005**, *436*, 791

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