Naturally Red: Sustainable and achievable?

Future-proofing berryfruit
CLIMAFRUIT

The James Hutton Institute

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What are the rules of the game?

EFSA

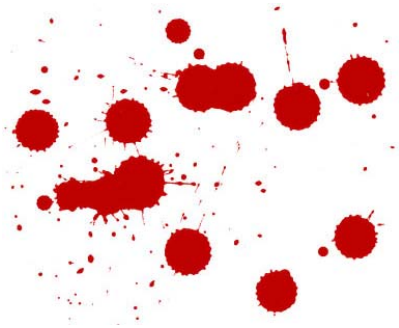
Under Commission Regulation 1331/2008 all food additives must undergo a safety evaluation by EFSA prior to their authorisation by EU risk managers. According to Commission Regulation 1333/2008, all food additives authorised for use in the (EU) before 20 January 2009 should be subject to a new risk assessment by EFSA.

- Food additives: New regulations on food additives, food enzymes and flavourings
- Regulation 257/2010 setting up a programme for the re-evaluation of approved food additives
- Food labelling - EU legislation

Delivered into at the EU level by several working groups within the Panel on Food Additives and Nutrient Sources added to Food

- Chemistry and Specifications - ANS Panel
- Exposure Assessment - ANS Panel
- WG 'A' Food additives and nutrient sources 2008-2011 - ANS Panel
- WG 'B' Food additives and nutrient sources 2008-2011 - ANS Panel
- Guidance on Food additives - ANS Panel
- WG on Toxicology - ANS Panel

Haemoglobin: Yes/No?



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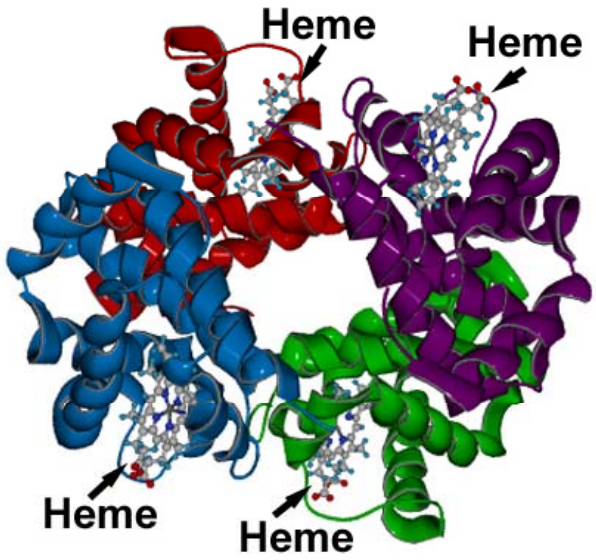
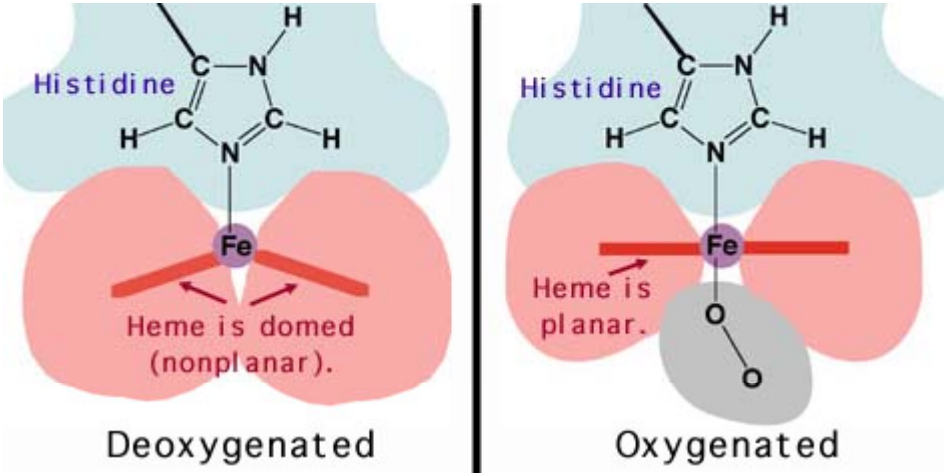
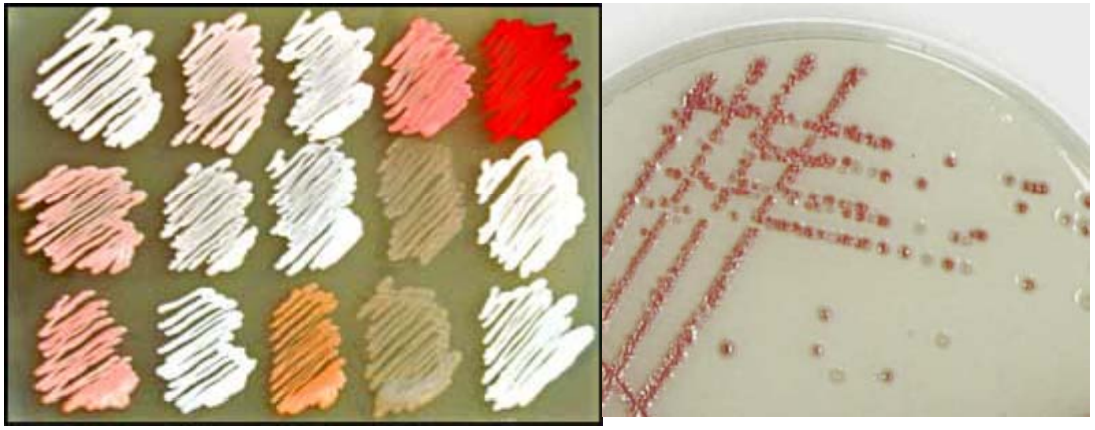


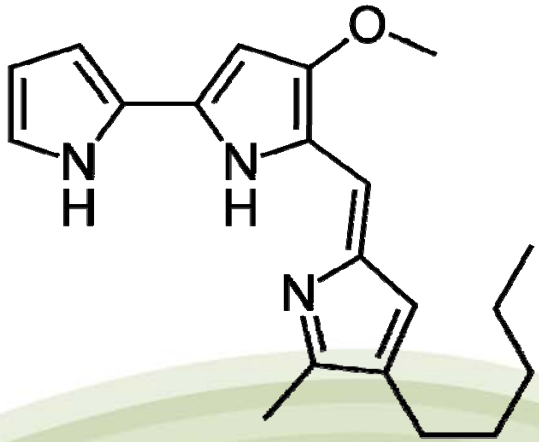


- Nice colour: vivid, deep etc. Red under high oxygen environments.
- Problems – Source, Degradation /Oxidation products reported to exhibit opioid, pro-bacterial and ACE inhibition properties.
- Ethical and societal issues.
- Too complex for purification. Allergenicity issues following degradation.

Bacterial routes to red



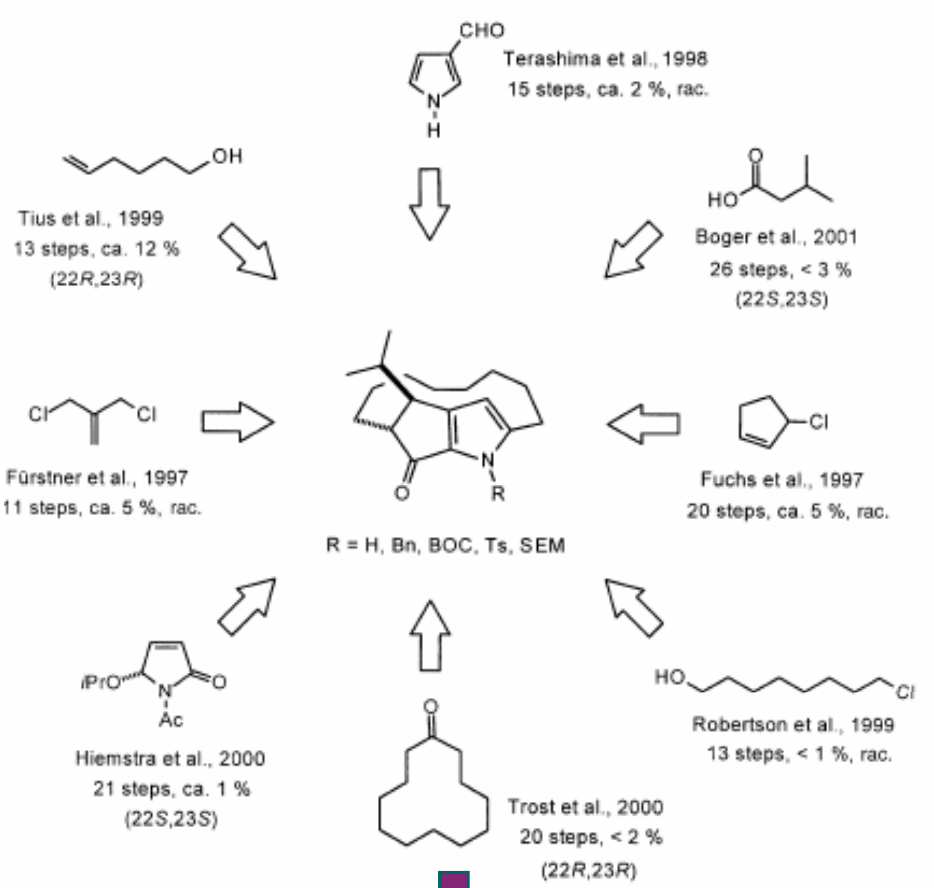
Biosynthetic and regulatory prodigiosin mutants of *Serratia*



Roseophilin and the Prodigiosin Alkaloids: the red pyrrole-containing pigment commonly found in Serratia, Streptomyces and Vibrio species has well-defined antibacterial properties.
Perez-Thomas et al (2003) Biochem Pharmacol 66, 1447–1452; Angew. Chem. Int. Ed. 2003, 42, 3582 – 3603

Raphael's “Mass of Bolsena” fresco in the Vatican.

Synthesis Roseophilin and the Prodigiosin Alkaloids



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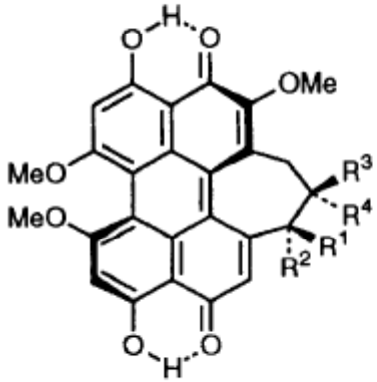
Too Expensive

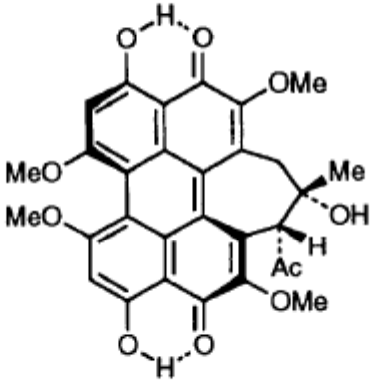
Fermentative Production

Definitely viable.
Antibacterial activity also.
Human bioactivity not fully tested.

Fungal Pigments



(74) R^1 = Ac, R^2 = H, R^3 = OH, R^4 = Me
(75) R^1 = H, R^2 = Ac, R^3 = Me, R^4 = OH



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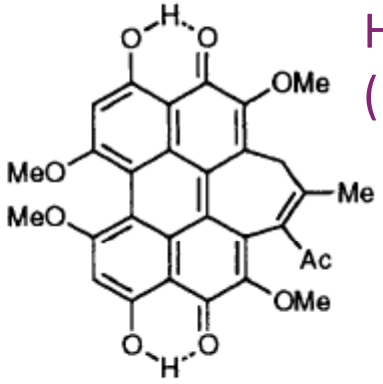
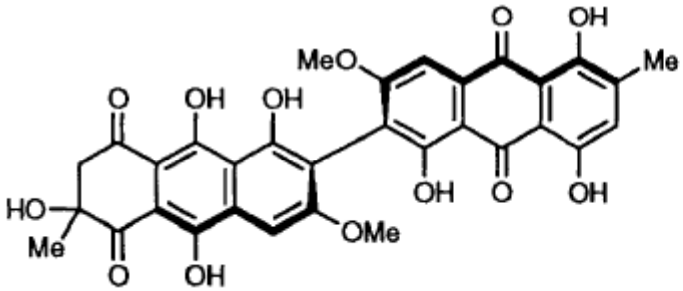


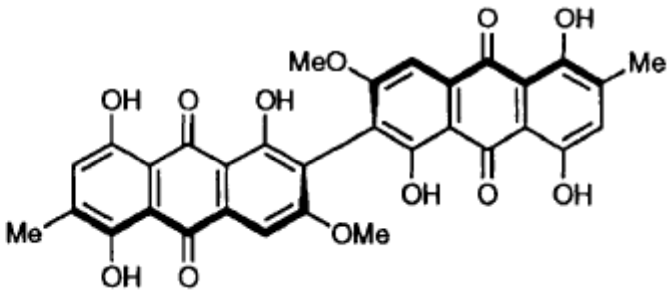
(77)

Hypocrella bambusae
(Bamboo fungus)

- 74 – Hypocrellin
- 75 – Shiraiachrome A
- 76 – Shiraiachrome B
- 77 – Shiraiachrome C

$HO^-_{(aq)}$





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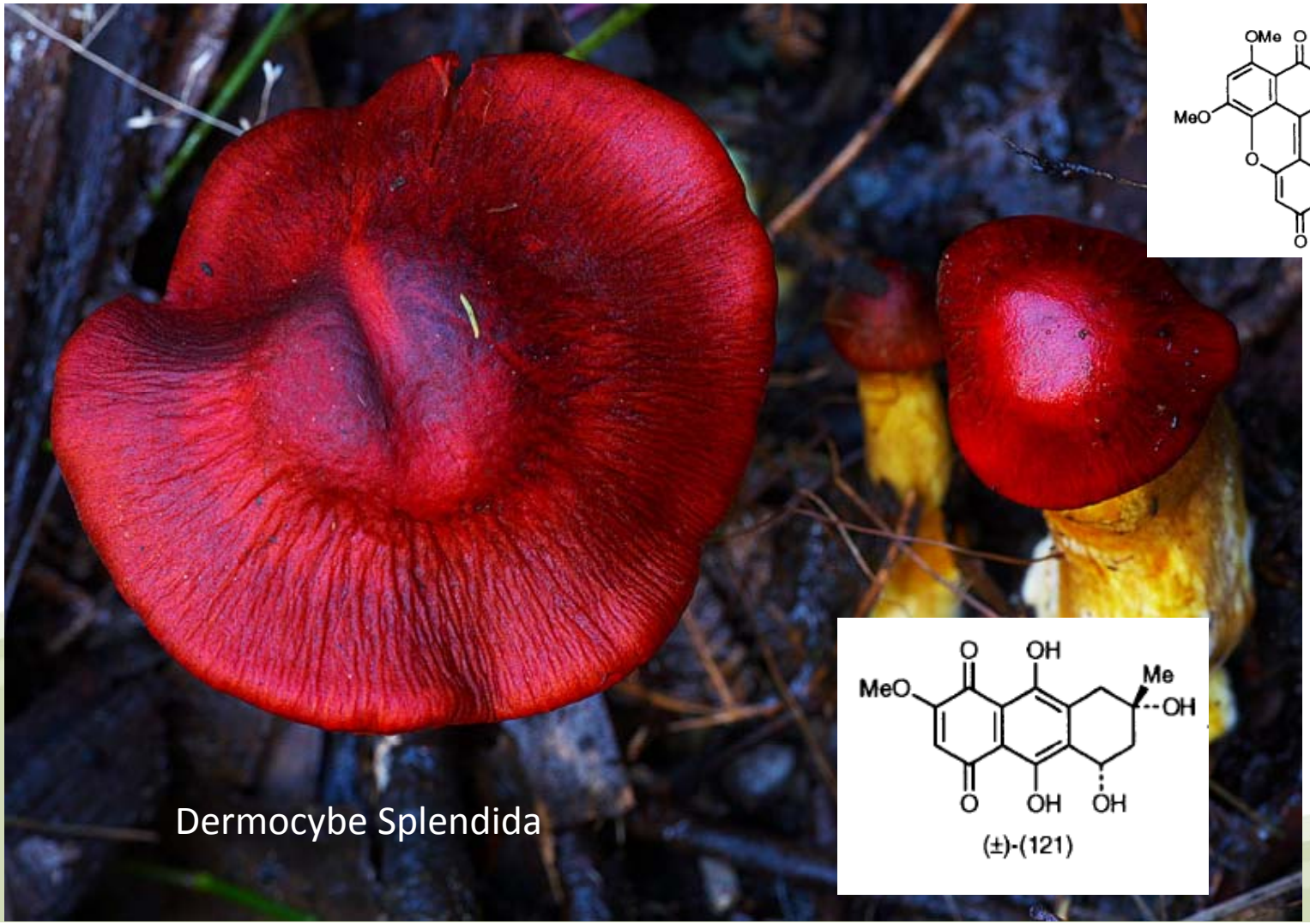
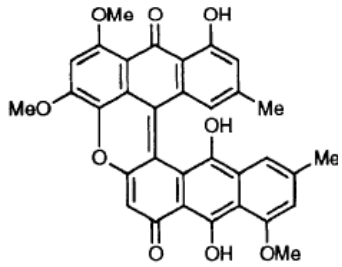
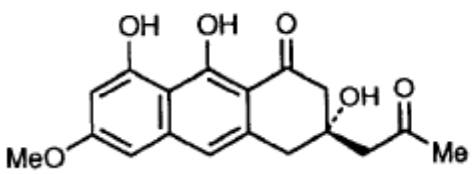
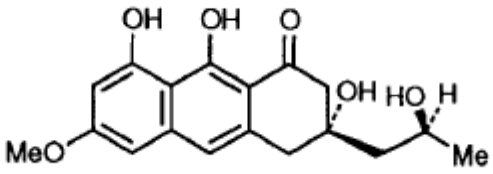


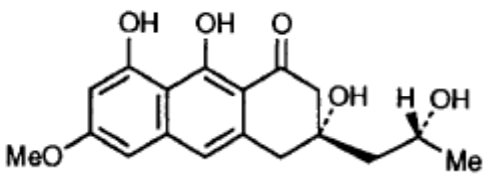


(±)-(121)

Dihydroanthocenones







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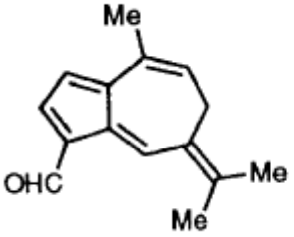

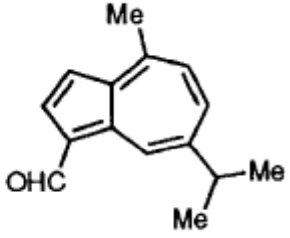

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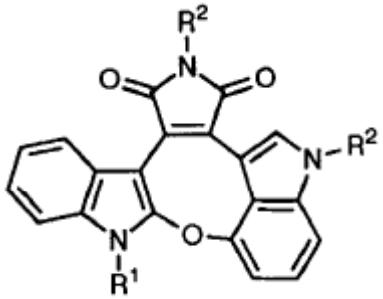
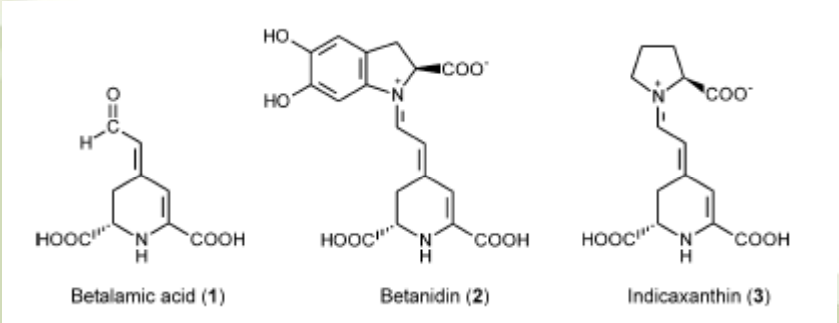


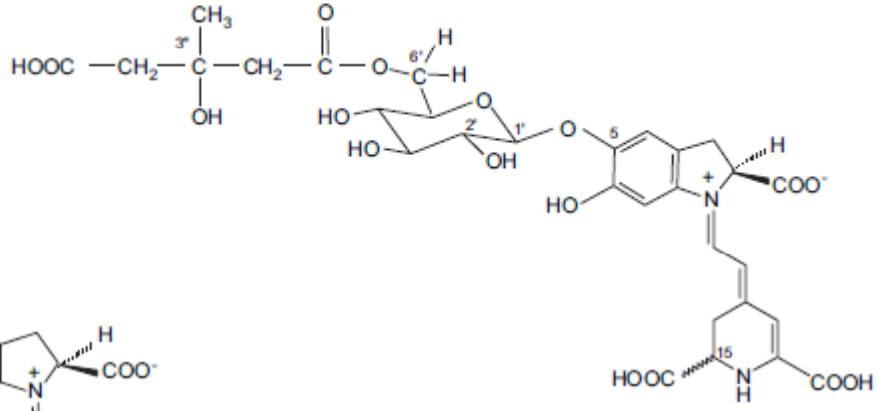
McGill, Nat. Prod Rep. 20(6):615-39.

Betalains

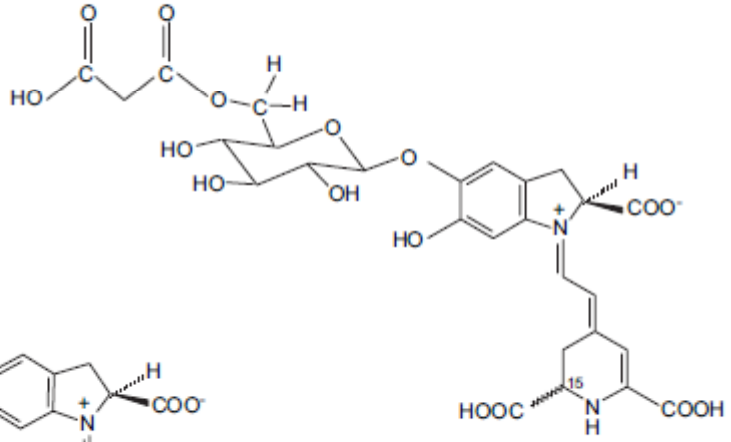
- Antiproliferative effects in human chronic myeloid leukaemia Cell line-K562. Phytomedicine 14, 39-746.
- Possible protection of LDL against ex vivo–induced oxidative modifications. Am J Clin Nutr 80, 941–5.
- *Recently implicated as contributing to a reduction in D-galactose-induced neurotoxicity in mice. Phytomedicine, 17, 527–532.
- Could be an ideal food colouring – stable(ish) and with no proven bioactivity. Trends in Food Science & Technology 15, 19–38.



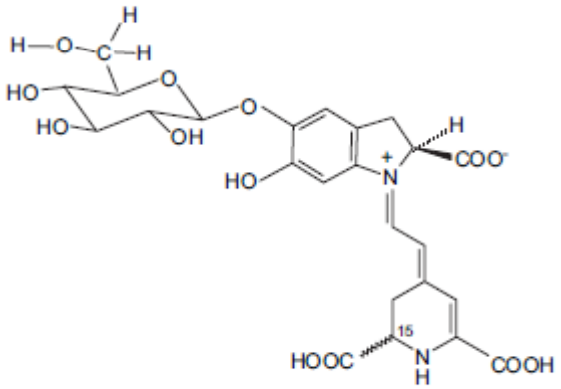
Betalains - Betacyanins



hylocerenin, isohylocerenin
[3-hydroxy-3-methyl-
glutaryl-(iso)-betanin]



phyllocactin, isophyllocactin
[malonyl-(iso)-betanin]



betanin, isobetanin

Betalains - Betacyanins

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- Microbially safe with minimal or zero coliform counts.
- Comparative colour addition = no taste impacts
- Degradation during storage greater in light.
- Glycosylation imparts increased stability in storage and solution.
- Acylation of the glycosyl portion imparts additional stability

- Aglycone < glycoside < acylated glycoside

- Stable over pH 3-7.
- Betacyanin losses in pitaya juice acidified to pH 4 is less than 10% during pasteurisation at 80 C for 5 min (Vaillant et al., 2005)
- Low and high pH are accompanied by different degradation pathways.

Azeredo . International Journal of Food Science and Technology 2009, 44, 2365–2376

Betalains – Betacyanins
Degradation pathways

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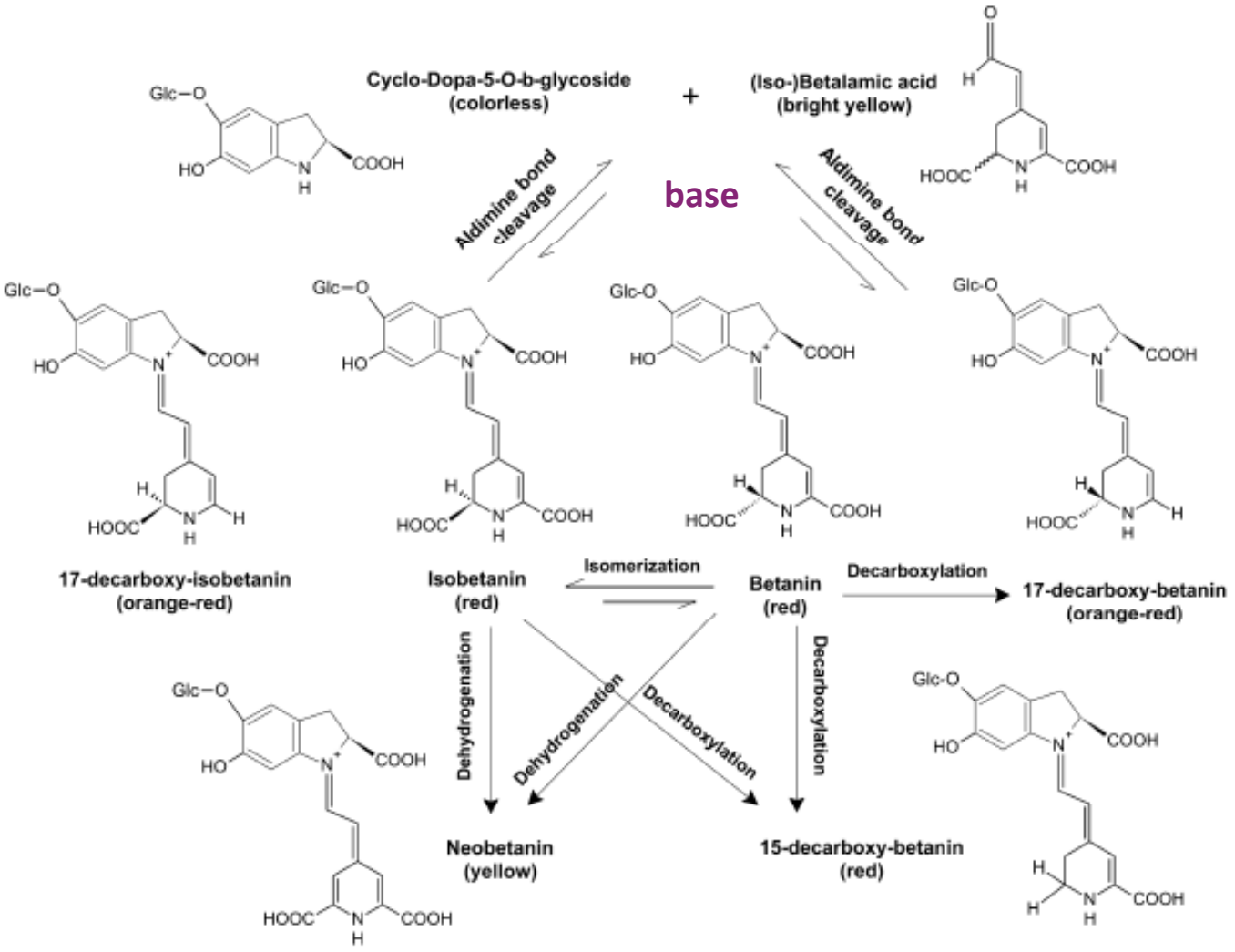

Betalains - Betacyanins

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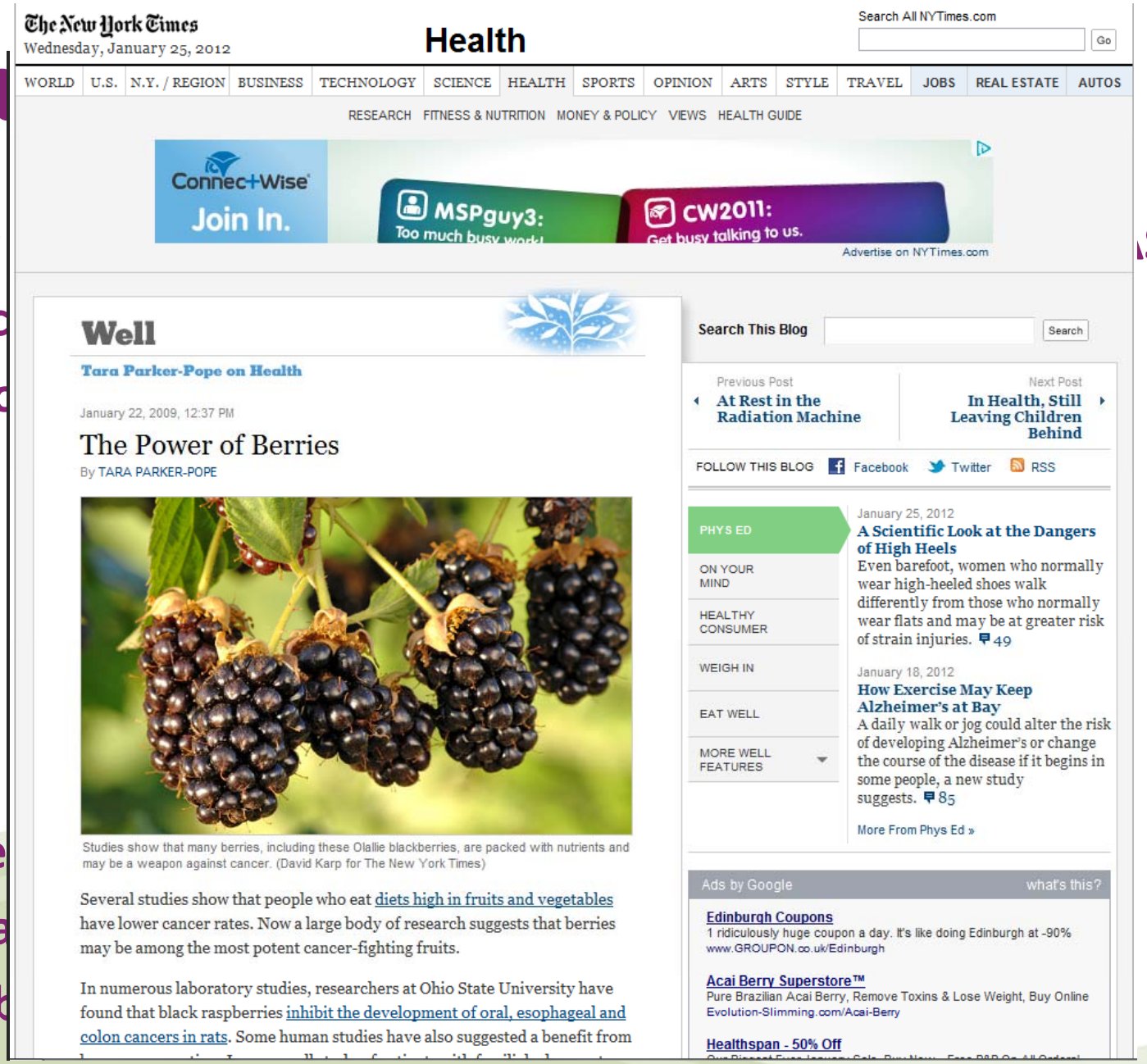
- Low and high pH are accompanied by different degradation pathways
- Light degradation absent in the anaerobic conditions.
- Heat may be problematic: isomerisation (colour shift to yellow), degradation.
- Amenable to cultured production: purity vs. amount.

Azeredo . International Journal of Food Science and Technology 2009, 44, 2365–2376

Ant

- Long
- Full c
- All go

- Rene
- Susta
- Can b



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Well

Tara Parker-Pope on Health

January 22, 2009, 12:37 PM

The Power of Berries

By TARA PARKER-POPE

Studies show that many berries, including these Olallie blackberries, are packed with nutrients and may be a weapon against cancer. (David Karp for The New York Times)

Several studies show that people who eat diets high in fruits and vegetables have lower cancer rates. Now a large body of research suggests that berries may be among the most potent cancer-fighting fruits.

In numerous laboratory studies, researchers at Ohio State University have found that black raspberries inhibit the development of oral, esophageal and colon cancers in rats. Some human studies have also suggested a benefit from

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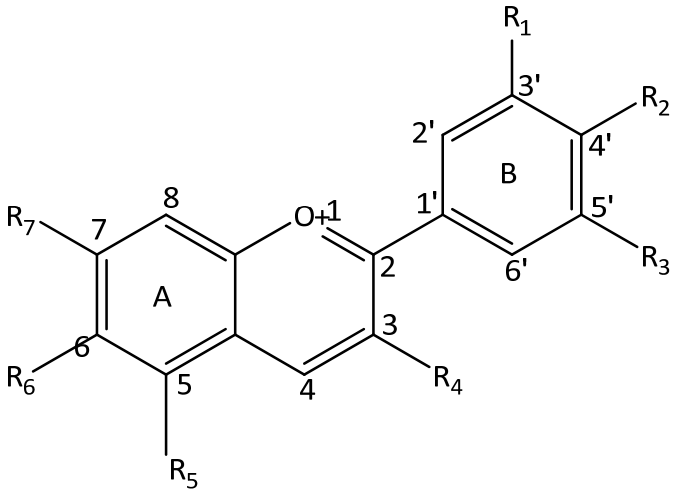

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Anthocyanidin	R1	R2	R3	R4	R5	R6	R7	main colour	E-number
Apigeninidin	-H	-OH	-H	-H	-OH	-H	-OH	orange	
Aurantinidin	-H	-OH	-H	-OH	-OH	-OH	-OH	orange	
Capensinidin	$-OCH_3$	-OH	$-OCH_3$	-OH	$-OCH_3$	-H	-OH	bluish-red	
Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH	magenta	E163a
Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH	purple, blue	E163b
Europinidin	$-OCH_3$	-OH	-OH	-OH	$-OCH_3$	-H	-OH	bluish red	
Hirsutidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	$-OCH_3$	bluish-red	
Luteolinidin	-OH	-OH	-H	-H	-OH	-H	-OH	orange	
Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH	orange, salmon	E163d
Malvidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	-OH	purple	E163c
Peonidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	-OH	magenta	E163e
Petunidin	-OH	-OH	$-OCH_3$	-OH	-OH	-H	-OH	purple	E163f
Pulchellidin	-OH	-OH	-OH	-OH	$-OCH_3$	-H	-OH	bluish-red	
Rosinidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	$-OCH_3$	red	
Triacetidin	-OH	-OH	-OH	-H	-OH	-H	-OH	red	

Sources of anthocyanins

Foodstuff	Anthocyanin in mg 100 g food
aubergine (egg plant)	750
black currant	130-400
blackberry	83-326
blueberry	25-497
cherry	350-400
chokeberry (aronia)	200-1000
cranberry	60-200
elderberry	450
orange	~200
radish	11-60
raspberry	10-60
red currant	80-420
red grape	30-750
red onions	7-21
red wine	24-35
strawberry	15-35

Antioxidant properties and anthocyanin contents of wild *Rubus* species

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Sample	*Colour*	*TEAC*[a]	*FRAP*[b]	*Ascorbic acid*[c]	A_{515}[d]	*Total phenols*[e]	*Anthocyanins*[f]	*A/P*[g]
R coreanus (A)	Orange	0.03±0.03	191±33	0.068±0.004	0.034	0.267±0.017	0.003±0.001	0.011
R coreanus (B)	Orange	2.24±0.08	3784±50	0.081±0.003	0.094	1.126±0.070	0.085±0.011	0.076
R coreanus (C)	Pale red	3.66±0.59	7765±96	0.081±0.005	0.106	1.259±0.047	0.086±0.006	0.068
R coreanus (D)	Red	7.98±1.61	5632±55	0.092±0.002	0.163	0.818±0.069	0.113±0.005	0.138
R coreanus (E)	Black	7.78±0.62	13078±241	0.102±0.003	0.197	1.217±0.031	0.343±0.012	0.282
R ursinus (A)	Black	11.71±0.75	55529±895	0.123±0.001	0.236	3.419±0.158	1.050±0.020	0.307
R ursinus (B)	Black	13.89±0.94	27333±494	0.164±0.005	0.272	2.021±0.130	0.619±0.044	0.306
R innominatus (A)	Light red	5.74±0.08	44260±552	0.079±0.007	0.105	3.264±0.071	0.792±0.011	0.243
R innominatus (B)	Dark red	11.53±0.54	33436±389	0.097±0.003	0.237	2.502±0.005	0.330±0.007	0.132
R ulmifolius	Black	15.47±1.34	34137±443	0.106±0.003	0.278	2.362±0.006	0.590±0.016	0.250
R parvifolius	Bright red	1.07±1.00	17436±205	0.069±0.008	0.064	1.717±0.041	0.116±0.012	0.068
R caucasicus	Dark red	25.32±1.28	65669±997	0.141±0.004	0.210	4.527±0.007	0.325±0.007	0.072
R niveus	Black	9.72±0.38	40799±780	0.099±0.003	0.275	2.991±0.028	1.186±0.017	0.397
R sumatranus	Yellow	2.52±1.07	13529±245	0.114±0.006	0.074	2.287±0.133	0.034±0.003	0.015
R tsangorum	Orange	3.47±0.50	2681±103	0.127±0.001	0.081	0.840±0.021	0.053±0.003	0.063
R hunanensis	Orange	10.06±1.16	20711±432	0.164±0.004	0.098	1.702±0.016	0.067±0.002	0.039
R lambertianus	Yellow	9.81±1.46	51289±635	0.079±0.003	0.029	3.173±0.003	0.001±0.001	0.001
R idaeus (Glen Lyon)	Red	17.25±1.03	25569±344	0.242±0.007	0.140	2.112±0.004	0.387±0.016	0.183

Cultivated v wild anthocyanin content can vary by 20-fold

J Sci Food Agric 80:1307±1313; J. Agric. Food Chem. 2010, 58, 7458–7464

Fruit extraction systems

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$H_2O/EtOH/H^+$

Dry and extract

Spray dry

Press and purify

Spray dry

High Power Ultrasound

- Dehydration & Extraction
- Greater specificity of extraction and yield

Fruit extraction systems

The Extract Company BerryPharma

The James Hutton Institute

Press

Clarification and/or ultra-filtration

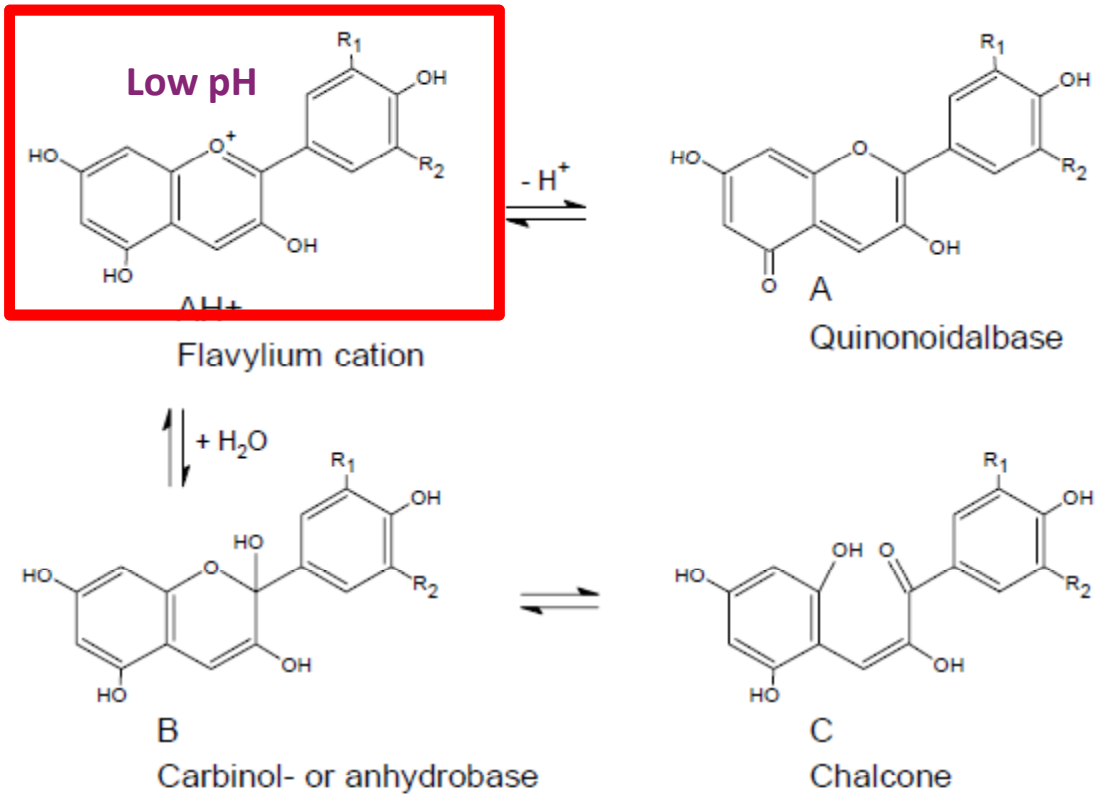
Presssing
Or Ethanol extraction

- Ethanol extraction
- Membrane purification (Size)
- Spray drying + maltodextrin

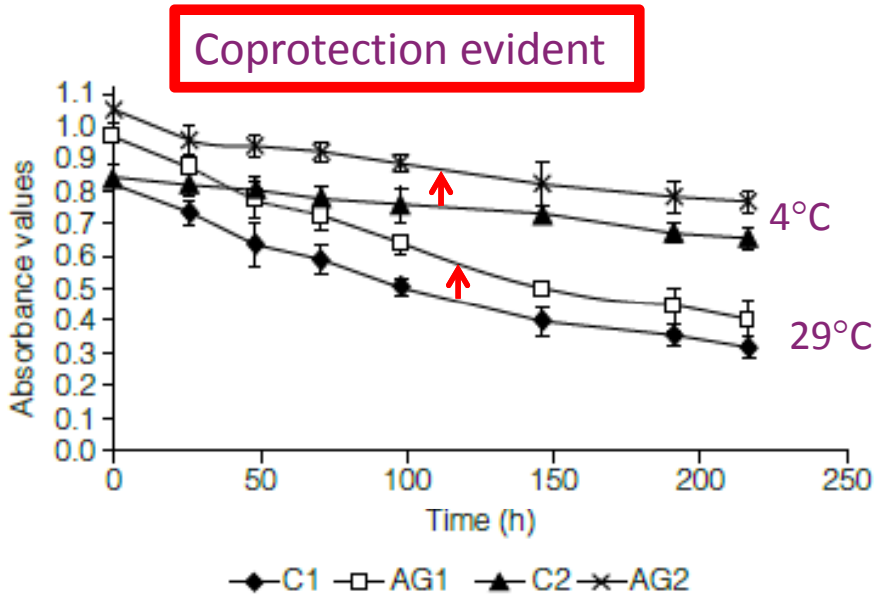
Different Purity Grades

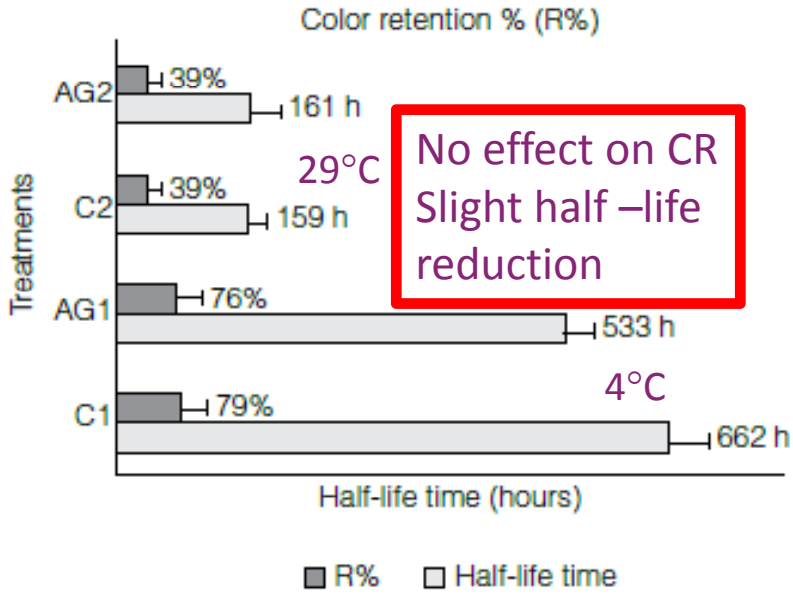
Anthocyanin stability

Increased anthocyanin concentrations also increases colour intensity by multifold. Change of cyanin concentrations from 10^{-4} to 10^{-2} produced a 300-fold increment of the colour intensity



Anthocyanin stability - Coprotection and temperature stability



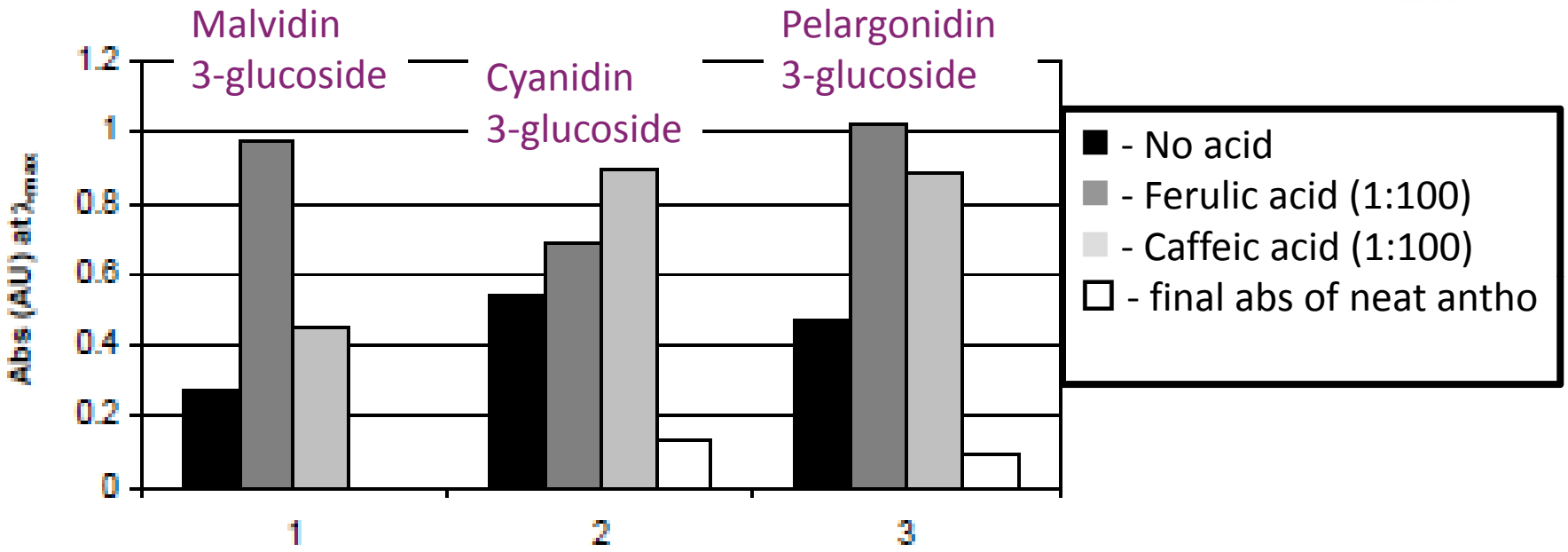


Anthocyanin extract of grape skin anthocyanins & gallic acid (model polyphenol) co-protection.

NB the majority of natural extract will be mixtures.

Braz. J. Food Technol., v. 11, n. 1, p. 63-69, jan./mar. 2008

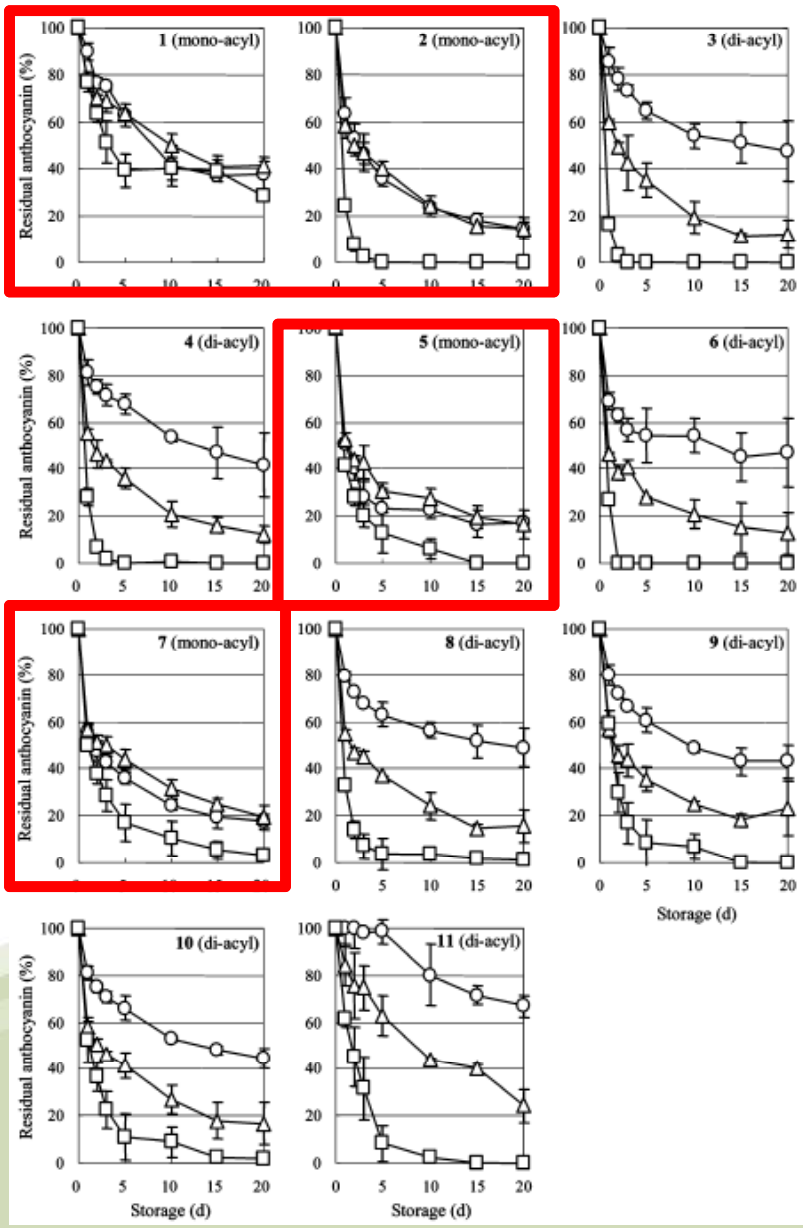
Anthocyanin colour- enhancement by polyphenols



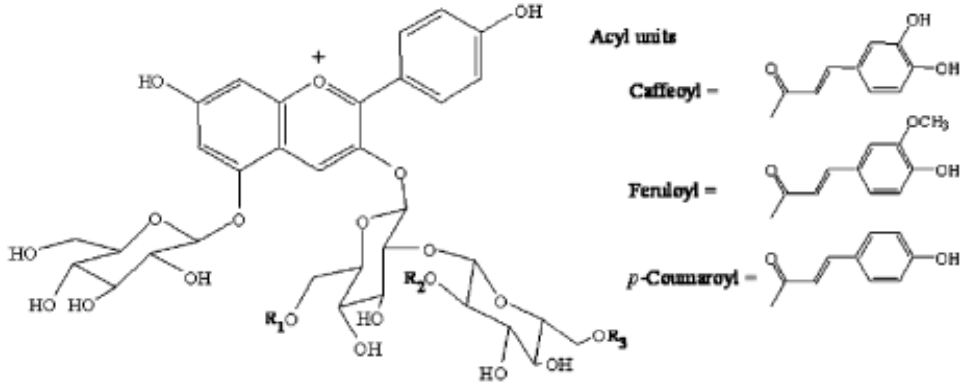
Anthocyanins + cinnamic acids for 6 months – Impact on Absorbance after 6 months

Maarit Rein 2005. Copigmentation reactions and colour stability of berry anthocyanins

Anthocyanin stability good – Modified anthocyanins better?



○ - pH 3; △ - pH 5; □ - pH 7



1	R_1=H	R_2=H	R_3=Feruloyl
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7	R_1=Feruloyl	R_2=H	R_3=H
8	R_1=Feruloyl	R_2=H	R_3=Caffeoyl
9	R_1=*p*-Coumaroyl	R_2=H	R_3=Feruloyl
10	R_1=Feruloyl	R_2=H	R_3=Feruloyl
11	R_1=Feruloyl	R_2=Feruloyl	R_3=H

Monoacylation – Feruloylation at R_3 best

J. Agric. Food Chem. **2007, 55, 3692-3701**

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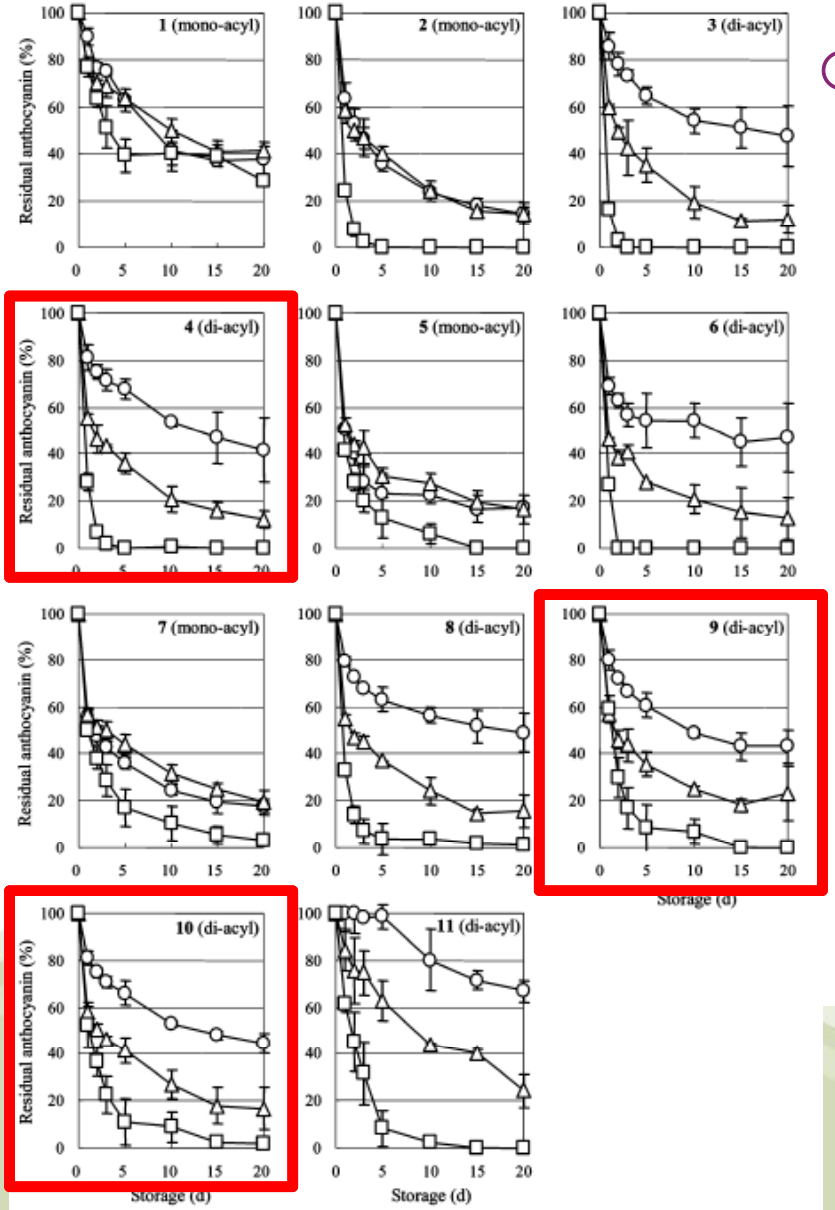

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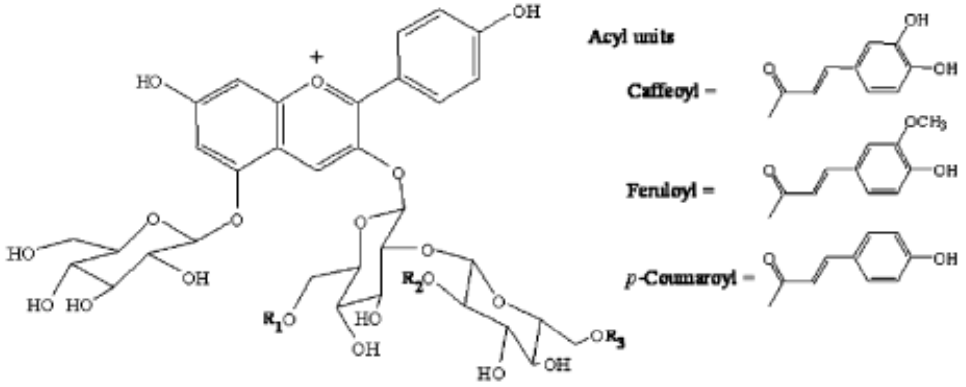

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Feruloyl at R_3 + acylation at R_1: Little benefit

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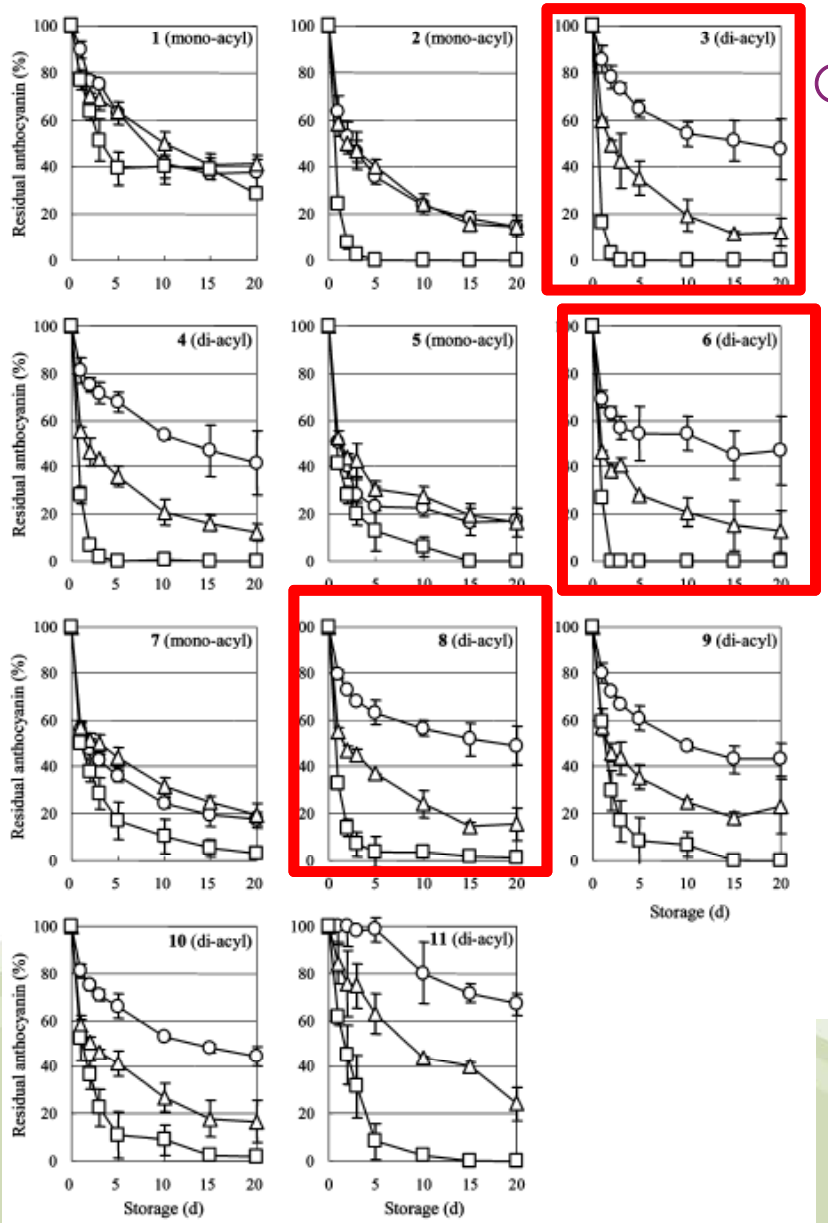

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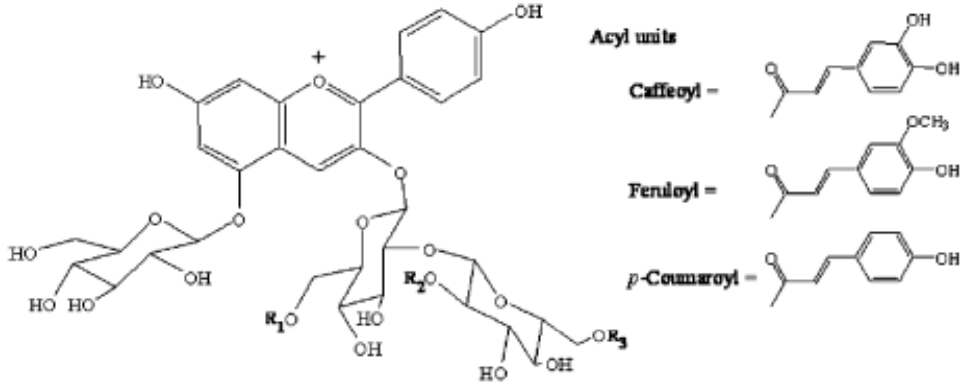

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Caffeoyl at R_3 + acylation at R_1: Little benefit

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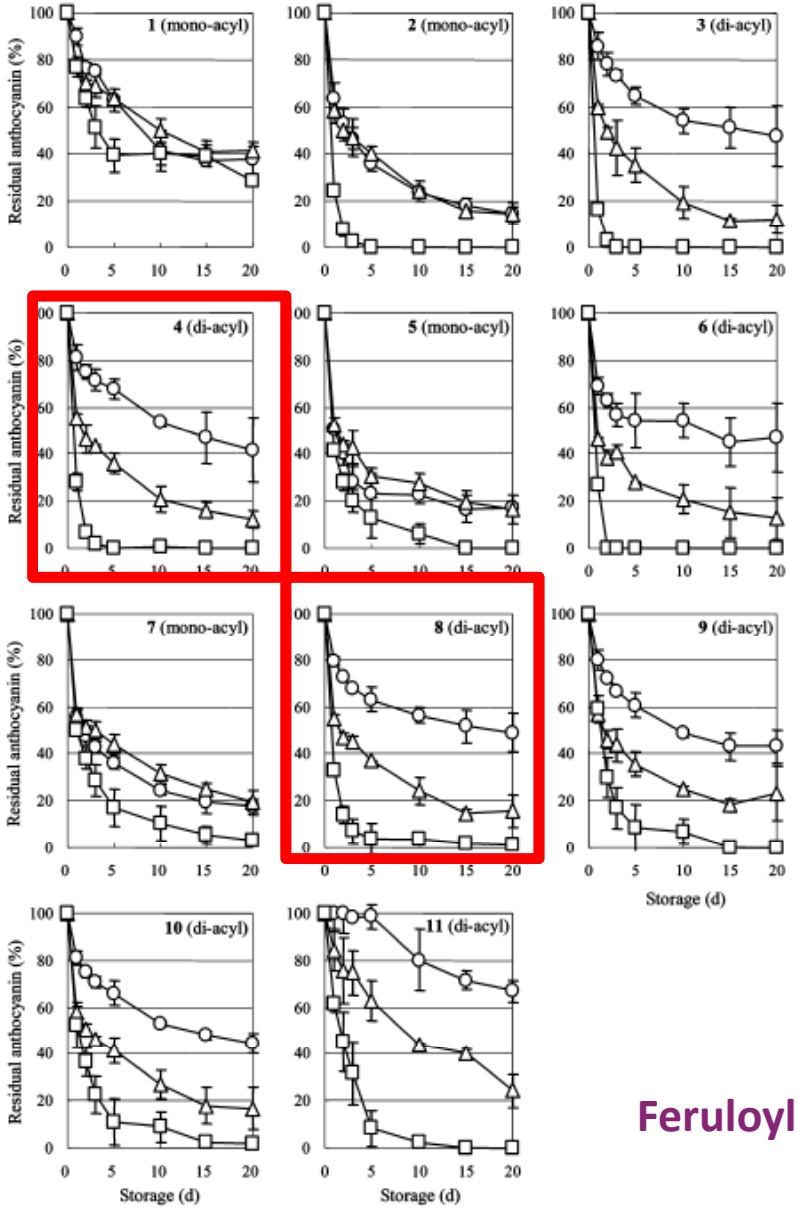

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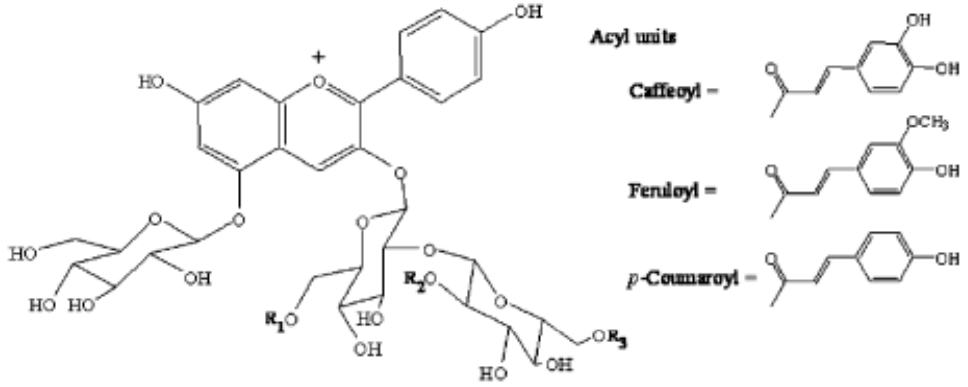

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Feruloyl – Caffeoyl positional shifts: R_1 & R_3 interchange

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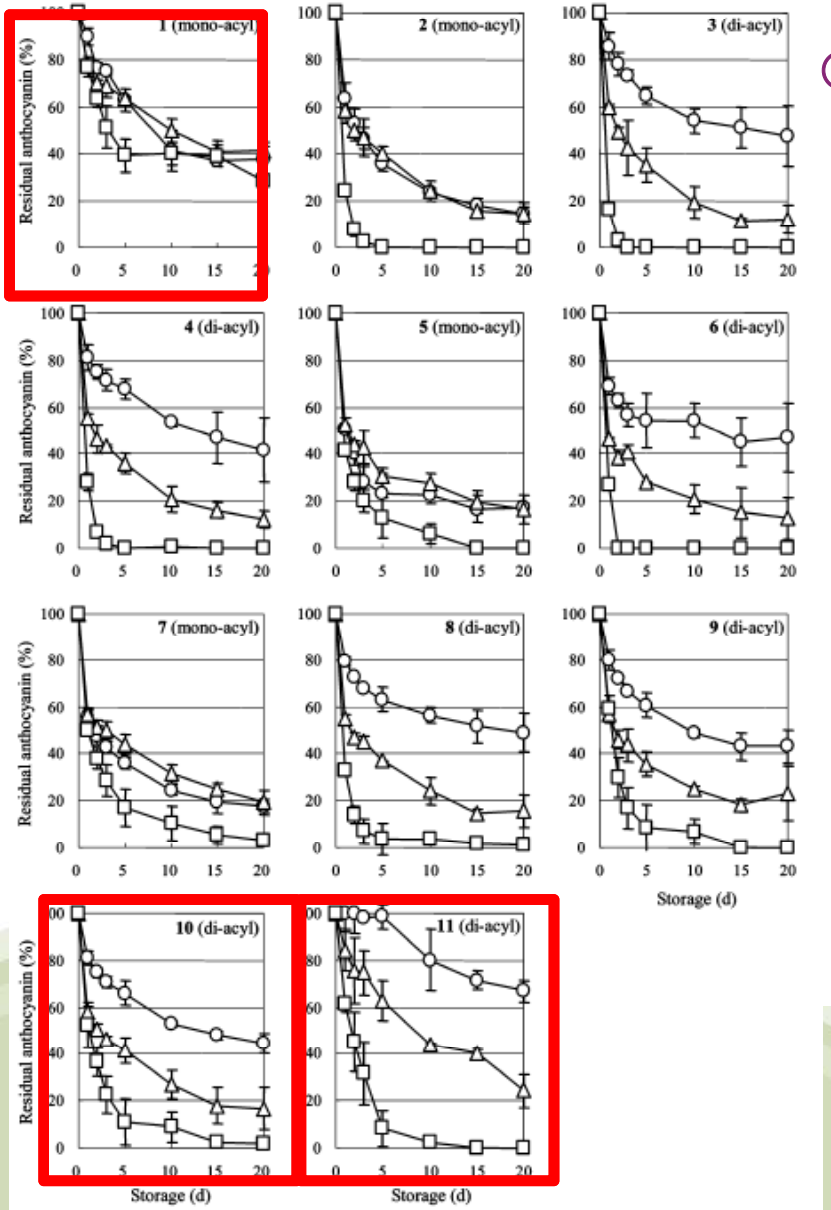

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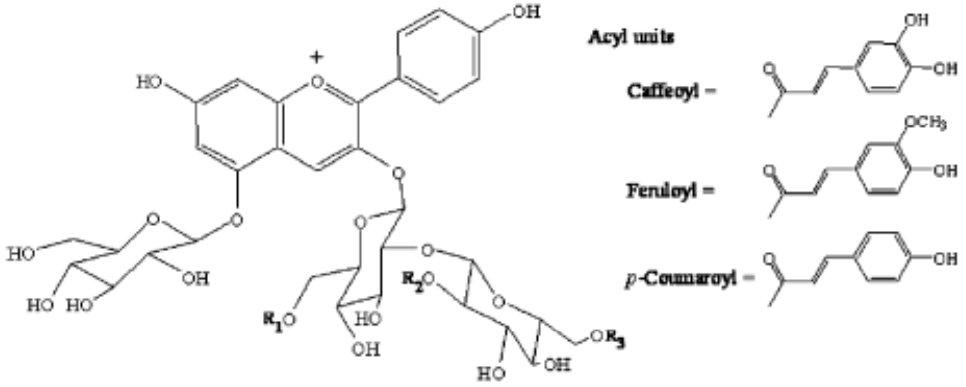

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10	R_1=Feruloyl	R_2=H	R_3=Feruloyl
11	R_1=Feruloyl	R_2=Feruloyl	R_3=H

Only Feruloyl – Big impact of R2 acylation

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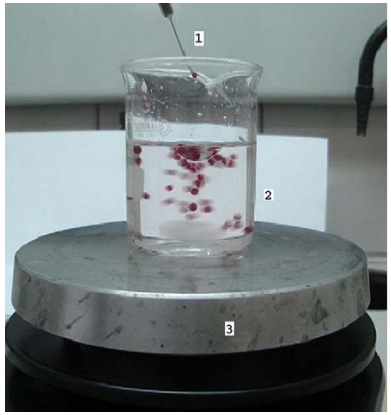
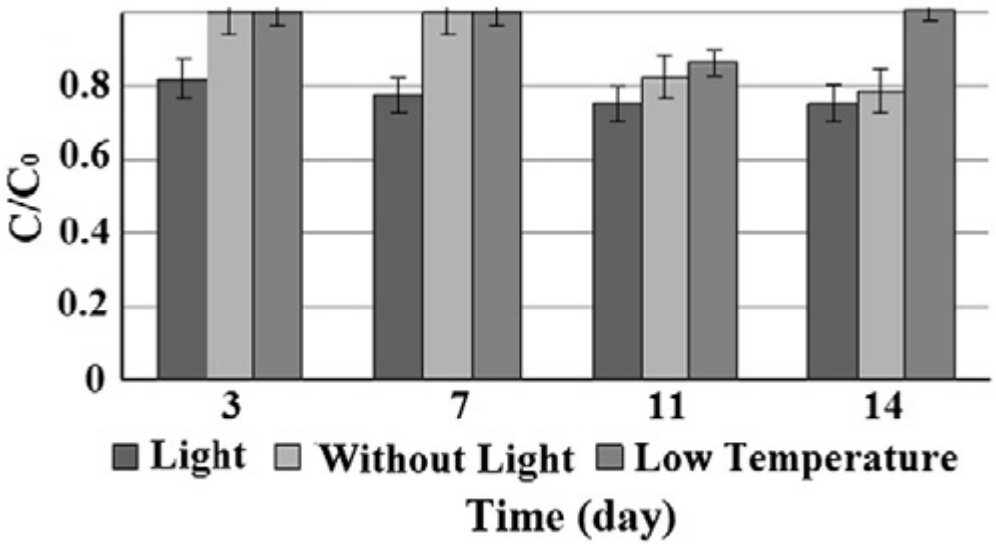
Anthocyanin Encapsulation

The James Hutton Institute

Aliginate encapsulation
Alginate is an inert food safe polysaccharide



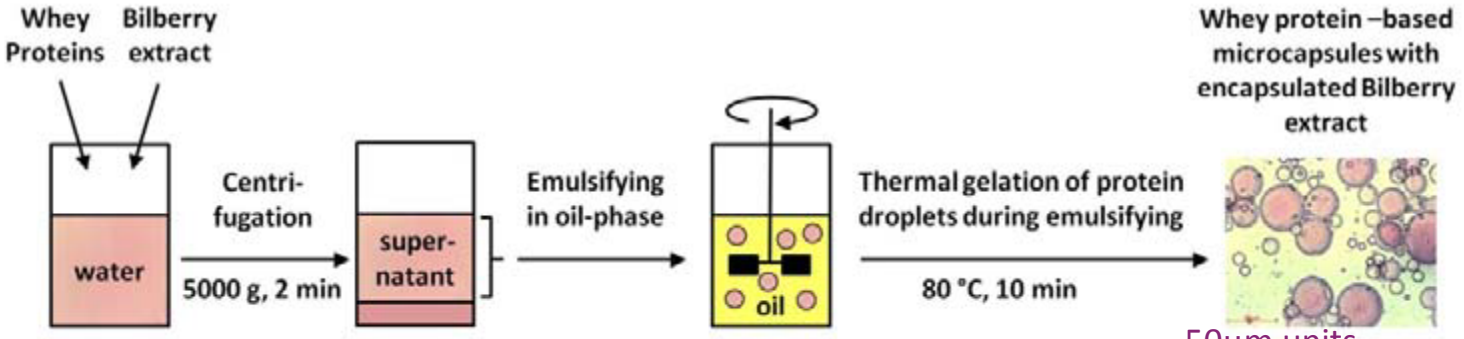
Stability to light and temp



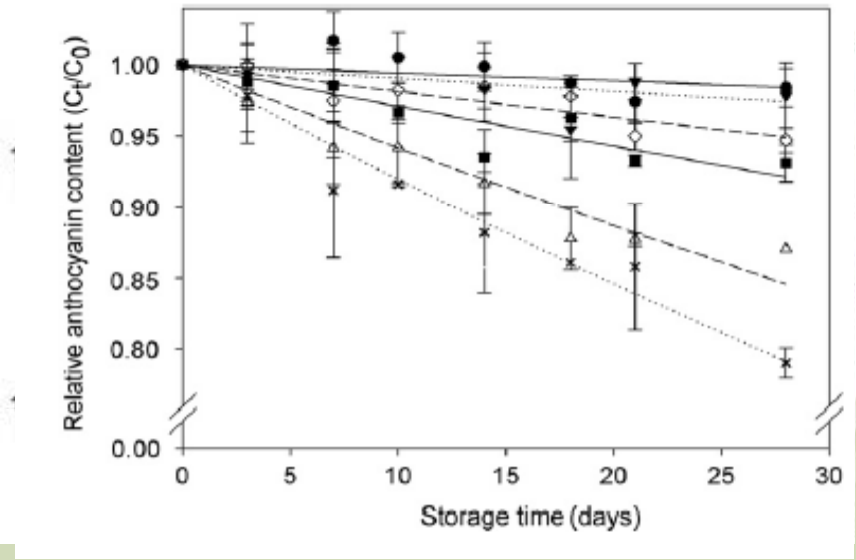
Anthocyanin Encapsulation

The James Hutton Institute

Whey proteins - Generally food safe, amenable for inclusion in baked goods, pastries etc



50µm units
Much smaller soon
Nanoencapsulation?



- Gel - pH 1.5, 4C
- Gel - pH 1.5, 20C
- Solution - pH 1.5, 4C
- Solution - pH 1.5, 20C
- Gel - pH 3, 4C
- X Gel - pH 3, 20C

Betz and Kulozika. Procedia Food Science 1 (2011) 2047 – 2056

Anthocyanin bioproduction

Can we get pure compounds, tailor mixes and differential acylation?

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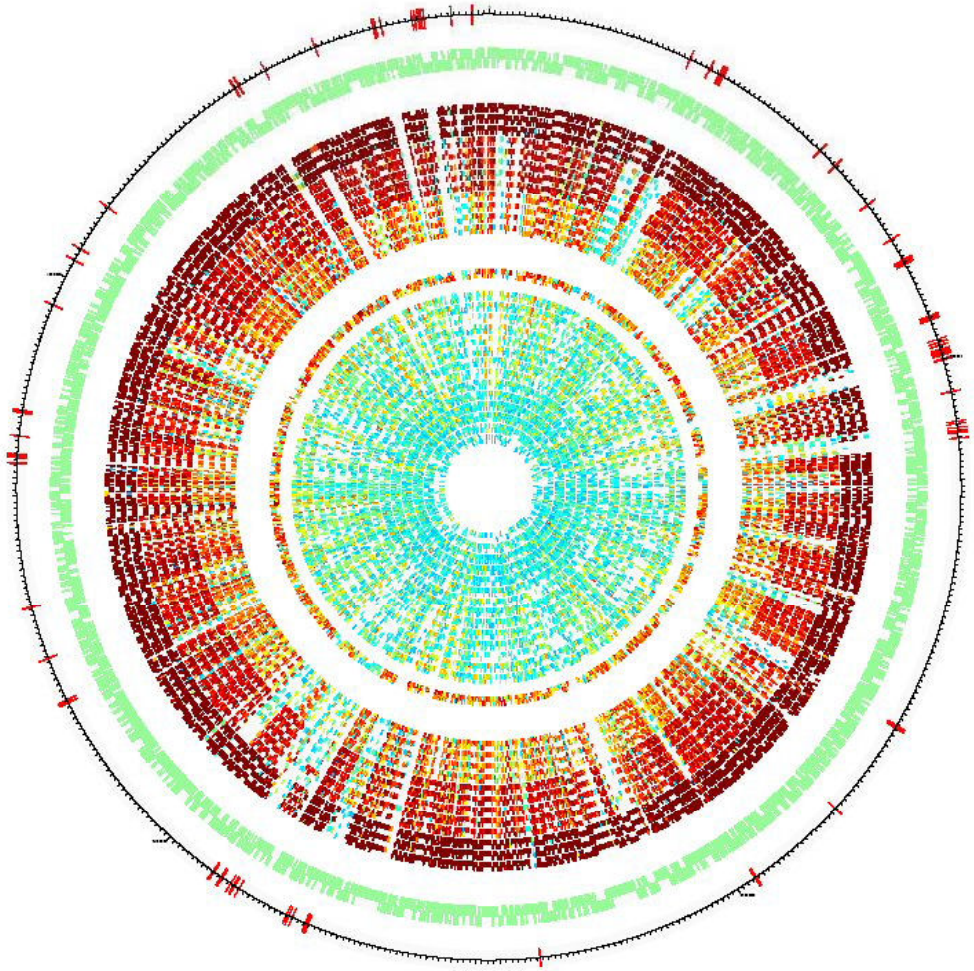
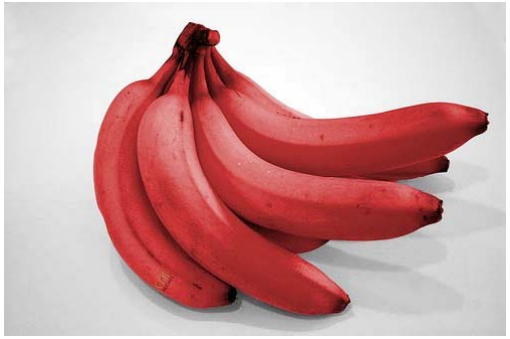


- Linkage maps being completed
- Extension of mapping population for confirmation of QTLs
- Mapping of key genes the general and specific anthocyanin pathways.
- This can be exploited as fruit or used in bacterial and fungal (& plant?) fermentation systems
- Ultimately we can design the colourants we want and define the quantity.

Genetic (AFLP, SSR, Next gen sequencing) ↔ Metabolomics (high throughput multiple metabolite analysis

In Conclusion



The James Hutton Institute

- The biological sources of red are still expanding and there to be exploited (marine sources still to be addressed).
- The chemistries involved are diverse and will determine the end use (Hydro/lipophilicity $\rightarrow$ aqueous or oil foods).
- Stabilities good but can be optimised.
- The ability to use crops is attractive as the waste is an attractive feedstock.
- The newer extraction procedures (ultrasonic etc) will maximise recovery and minimise degradation.
- Colour compounds can be the target trait in crops e.g. Chokeberry.
- The advances in genetic breeding ($\pm$GM) can realise the industrial requirements.
- More specific targets (glycosylation, acylation etc) can be satisfied via fermentative approaches: plants, fungi and bacteria.
- Renewable, sustainable and ***SAFE*** red can be achieved and is within our grasp.