


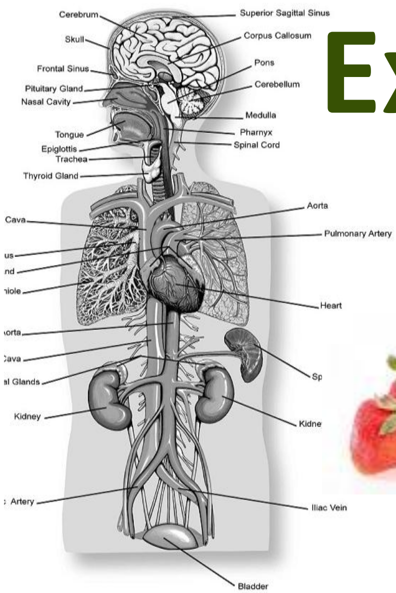

Examining human health effects of berries



Gordon J. McDougall

Crop Productivity and Utilization

The James Hutton Institute

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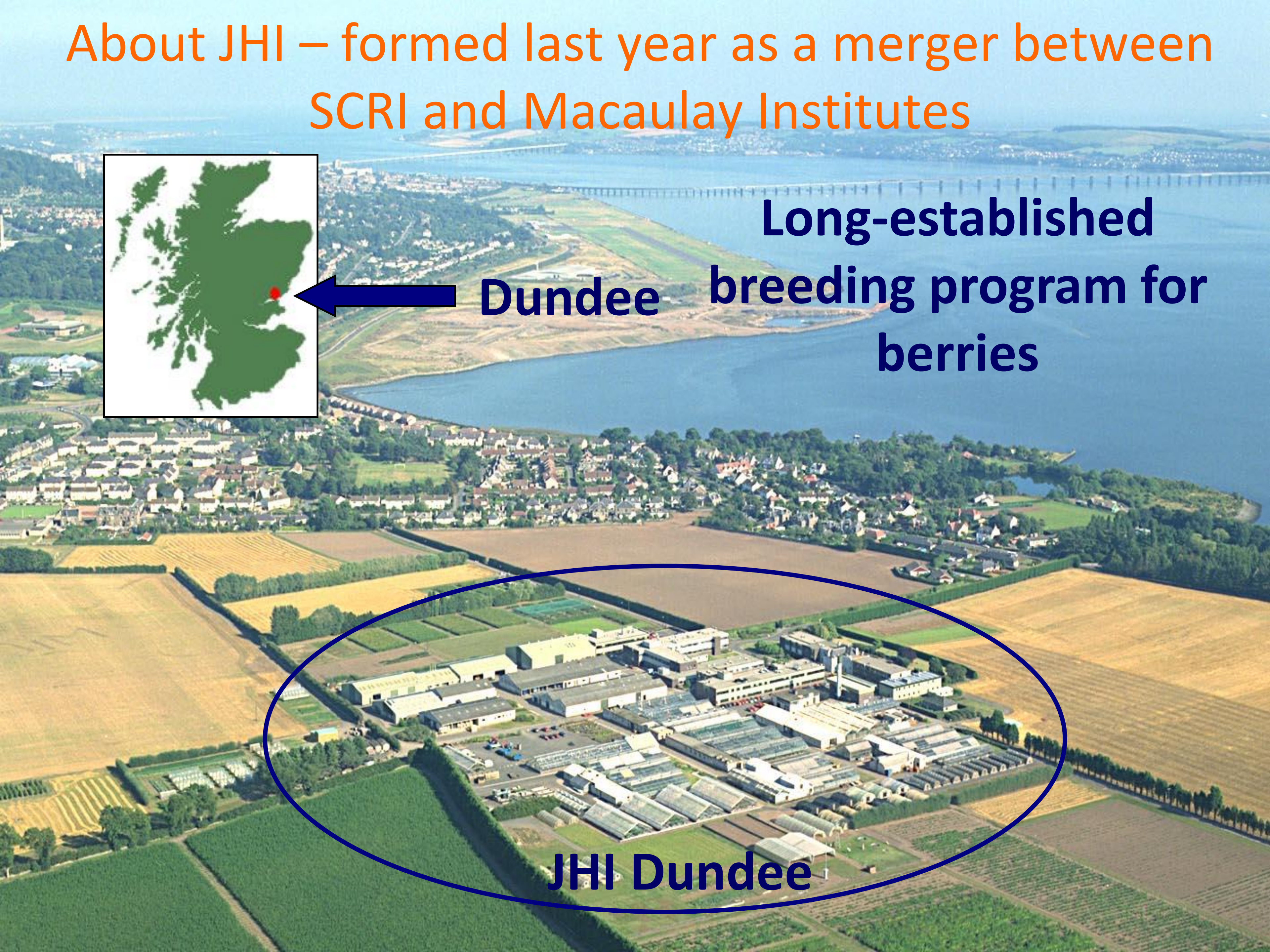
MRC Human Nutrition Research labs, Cambridge, 14th May 2012


About JHI – formed last year as a merger between SCRI and Macaulay Institutes
Dundee
Long-established breeding program for berries
JHI Dundee

Berry research at JHI

We breed market-leading varieties

- *Blackcurrants – the "Ben" series*
- *Raspberries – the "Glen" series*
- *Strawberry – "Symphony, Rhapsody…."*
- *Blueberries for UK conditions*
- Research into Health Benefits of Berries
- Feedback and direct breeding of new varieties

Scheme of talk

Background

Biological activities of berry components

- MODEL SYSTEMS
- Cardiovascular Health
- Neuroprotection
- Cancer
- Control of nutrient availability
  - **Diabetes & Obesity**

Analytical studies

- **Correlate bioactivity with polyphenol composition**
- **Examine bioavailability of components**

- “Insufficient intake of fruit and vegetables increases the chances of developing cancers, cardiovascular disease and strokes” - World Health Organisation (2003)
- The 3 main causes of premature death in Scotland

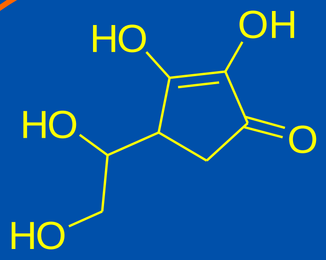
Led to the “5 a day” programme - Govern … to alter our die …

How do FAV affect health?

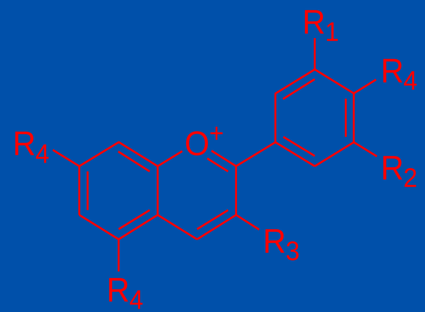
Minerals (Zinc)? Vitamins (C and E)? Fibre? Displacement? Lower Fat? **Antioxidants?** **Phytochemicals?**

healthier scotland
SCOTTISH GOVERNMENT

Berries contain a diverse and species specific mixture of antioxidants – the two main types are Polyphenols and Vitamin C

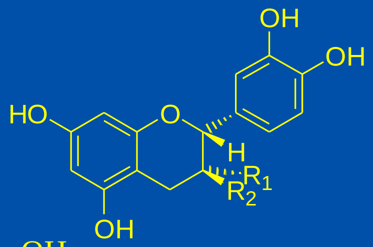


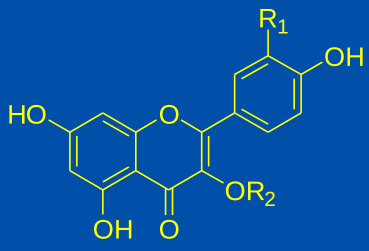
Vitamin C



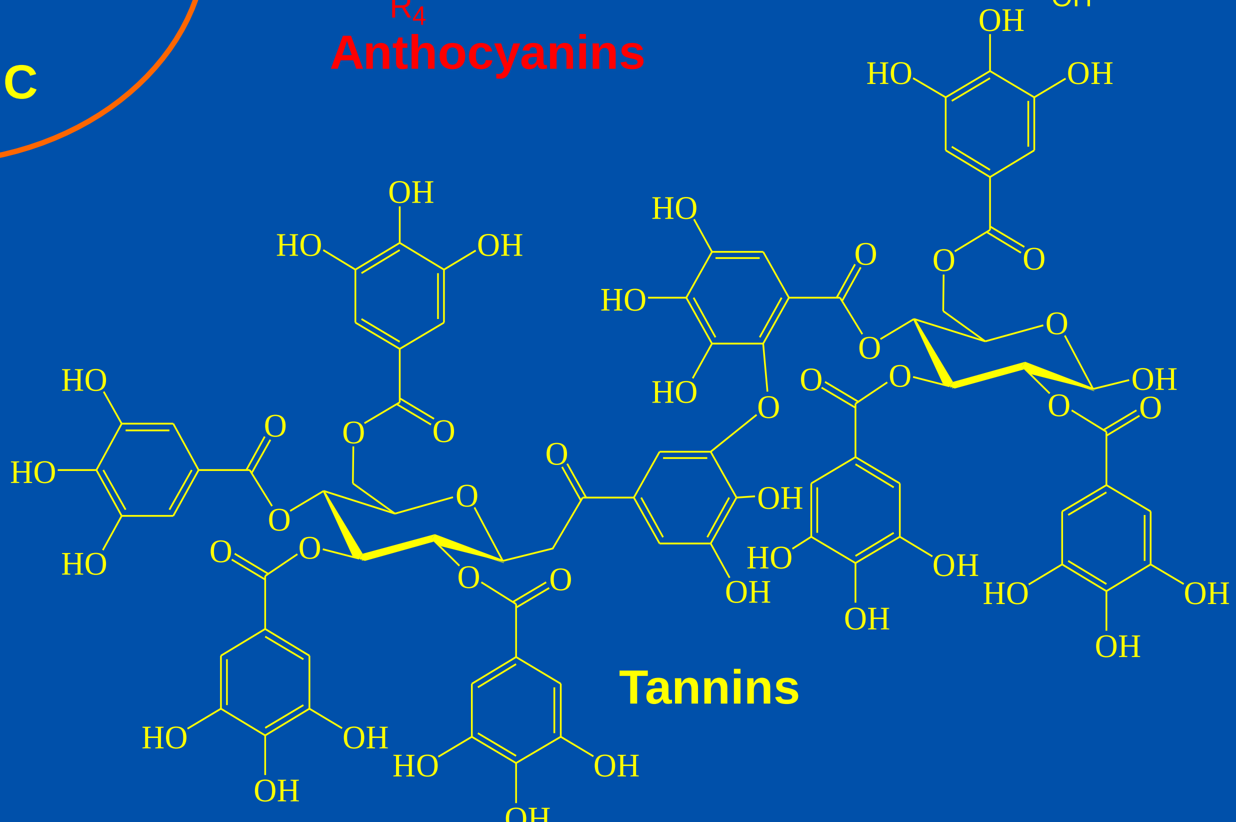
Anthocyanins

flavanols/PACs





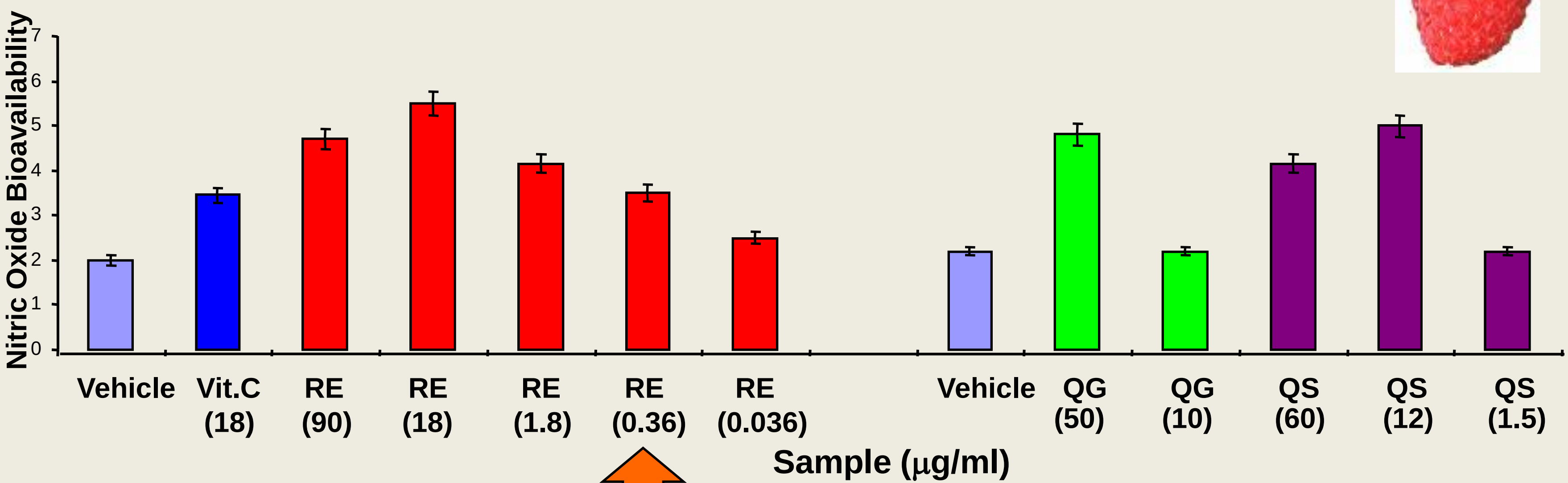
Flavonols



Tannins

Availability of nitric oxide (NO) in *ex vitro* rat carotid arteries

Nitric oxide protection by Raspberry extracts



Raspberry extract effective at 50-fold less than Vitamin C or Quercetin derivatives – known effectors of CV performance

Protection of NO bio-availability maintains blood vessel flexibility

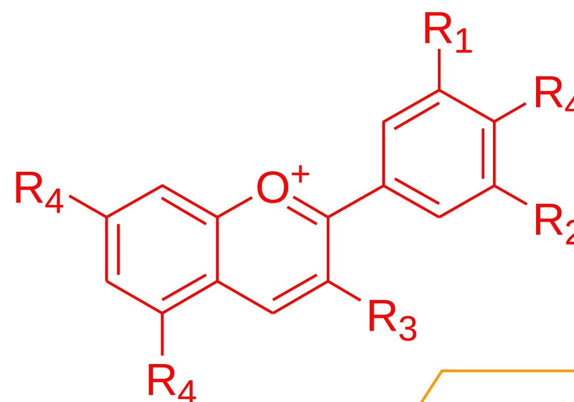
Prof. Carlene Hamilton, BHF, University of Glasgow

Whitson et al. Functional Plant Science and Biotechnology (2010)

Cardiovascular disease (CVD)

- Protection of vasodilatory responses of rat aorta against inflicted oxidative damage

- Protective effect of polyphenols not predicted by *in vitro* antioxidant activity



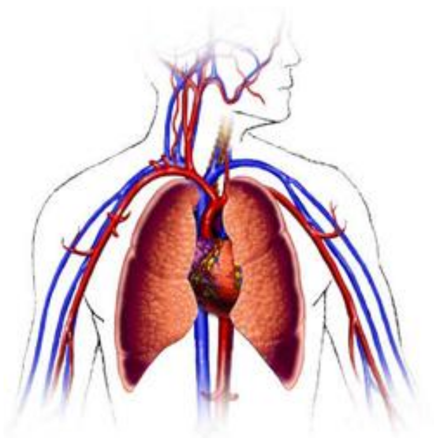
- Anthocyanins particularly effective
- Modelled breakdown products also effective

Prof. Ian Megson, Univ. Highland and Islands

Cardiovascular function and intake of soft fruit: Effects of qualitative and quantitative variation in berry antioxidant status

Intervention trial – assess effects of six week ingestion of

- **blackcurrant berries with low vitamin C content**
- **blackcurrant berries with high vitamin C content**
- **blueberries (No vitamin C)**
- **coloured flavoured water (control)**



Effects on cardiovascular function

Positive effects on intima media thickness and i*n vivo* markers for endothelial cell function and oxidative stress

Effect on Alzheimer's?

Oxidative stress, Alzheimer's and the Brain

Brain = 2 % adult body mass but uses <u>20 %</u> oxygen inhaled

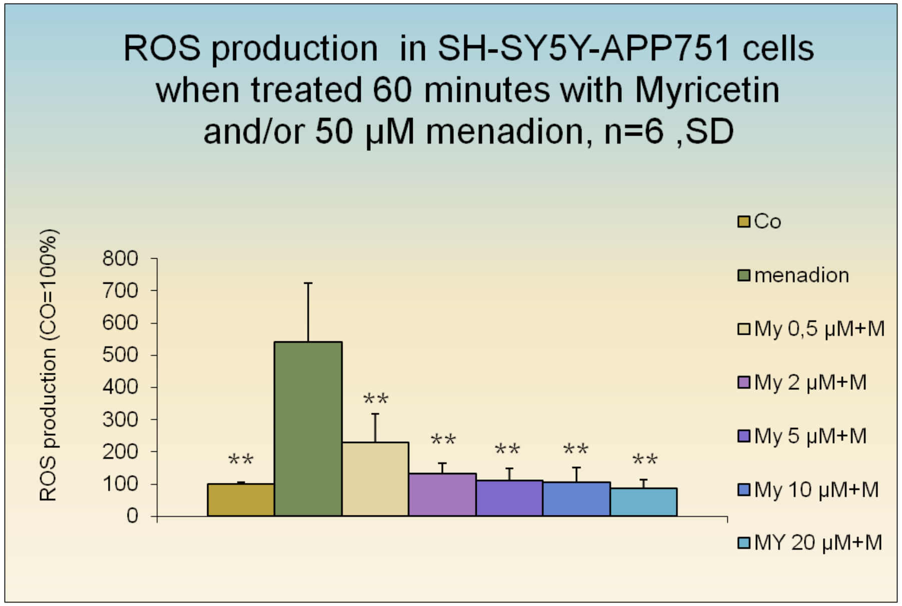
Poorer antioxidant mechanisms

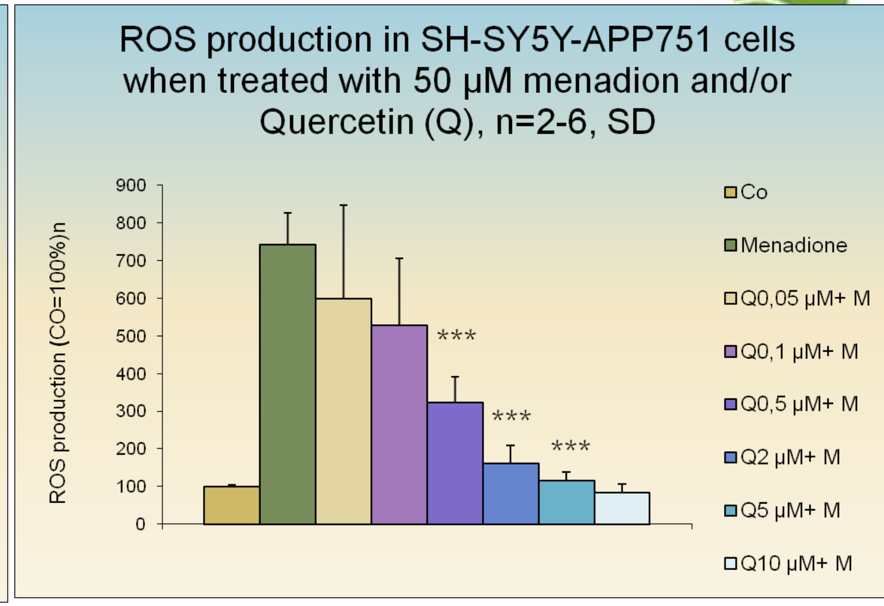
High levels of PUFAs, minerals and neuro-transmitters – good targets for free radicals

Brain cells don't renew by cell division - accumulate FR-induced damage with age & FR damage implicated in AD

- *EU project* **BrainHealthFood**
- **Bioactive compounds from blackcurrant processing waste for brain health**

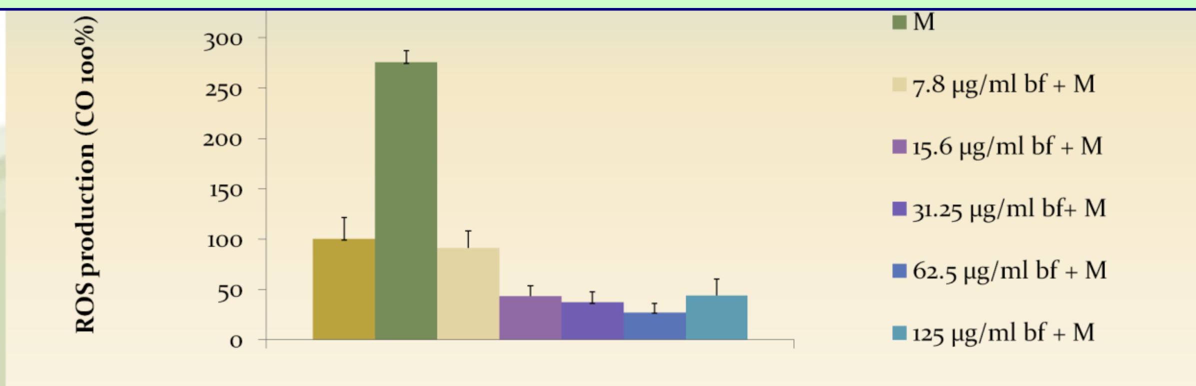
MTT Agrifood
JHI
TTZ
Univ. Kuopio
& SME partners





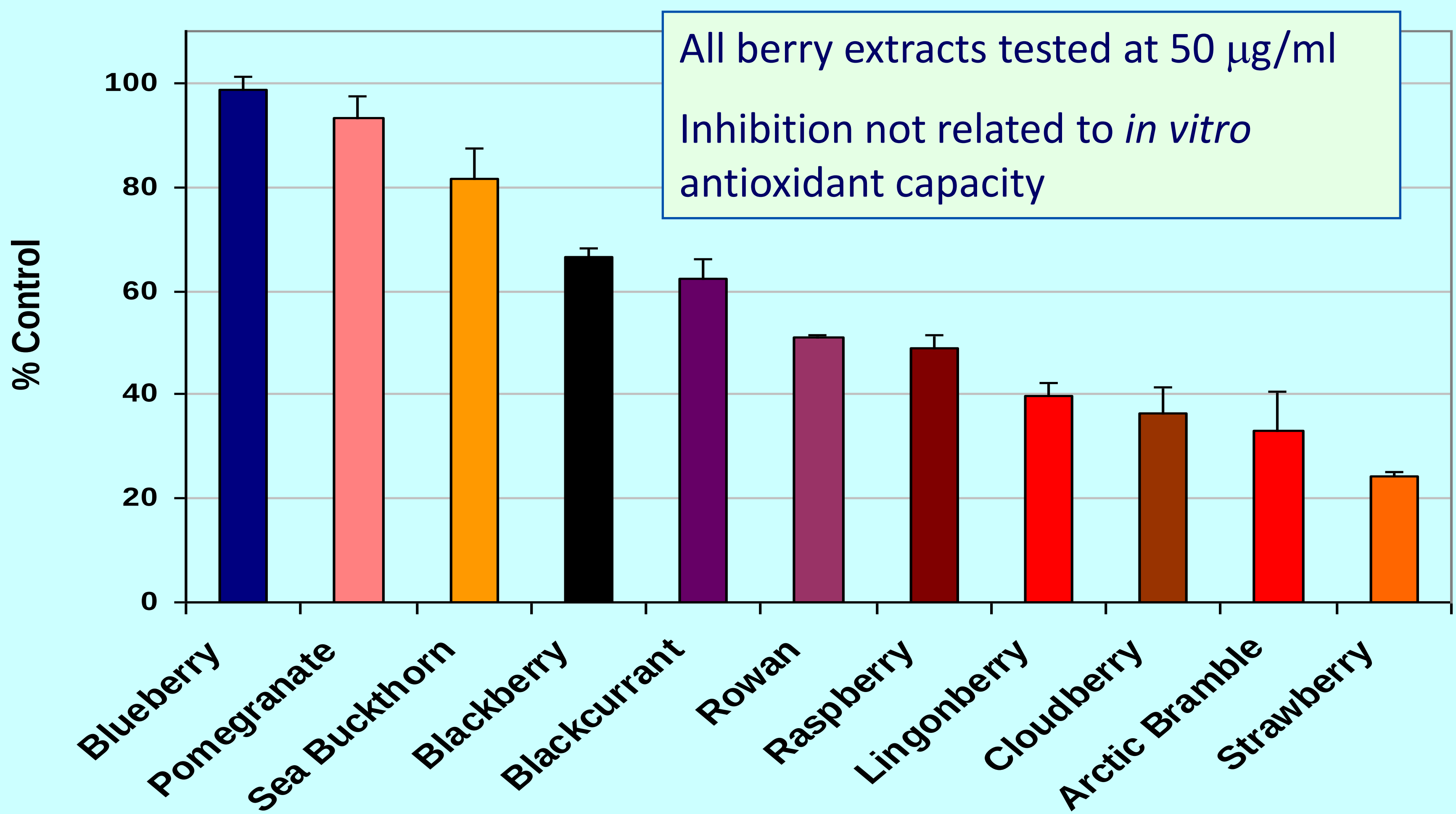
Protective effect of anthocyanins in Alzheimer's model system

Further studies with berry extracts suggest positive results in behavourial studies in mice

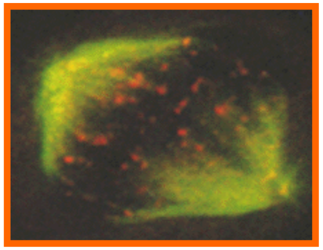


Vepsäläinen et al. in press. Univ. Kuopio

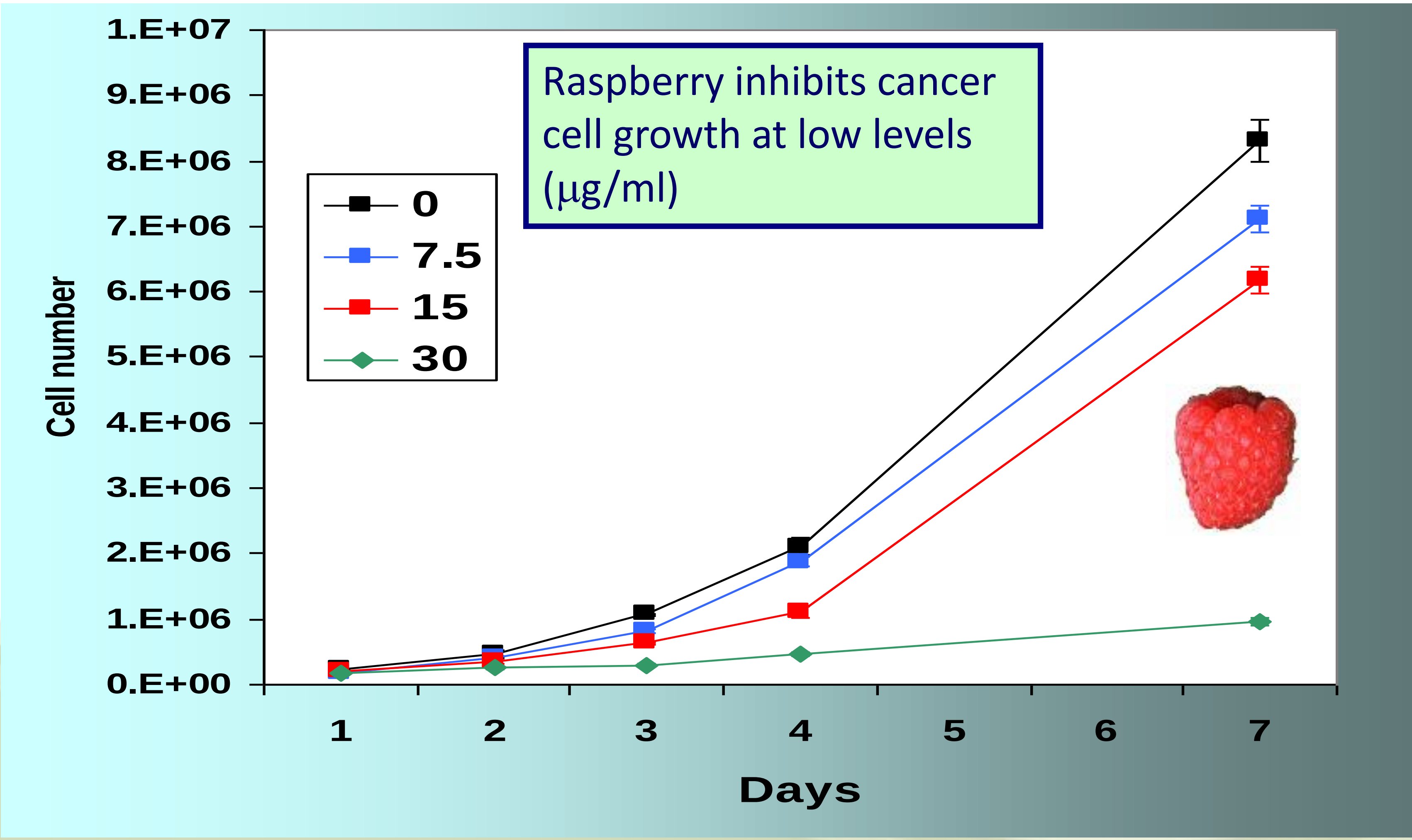
Effects on cancer cells



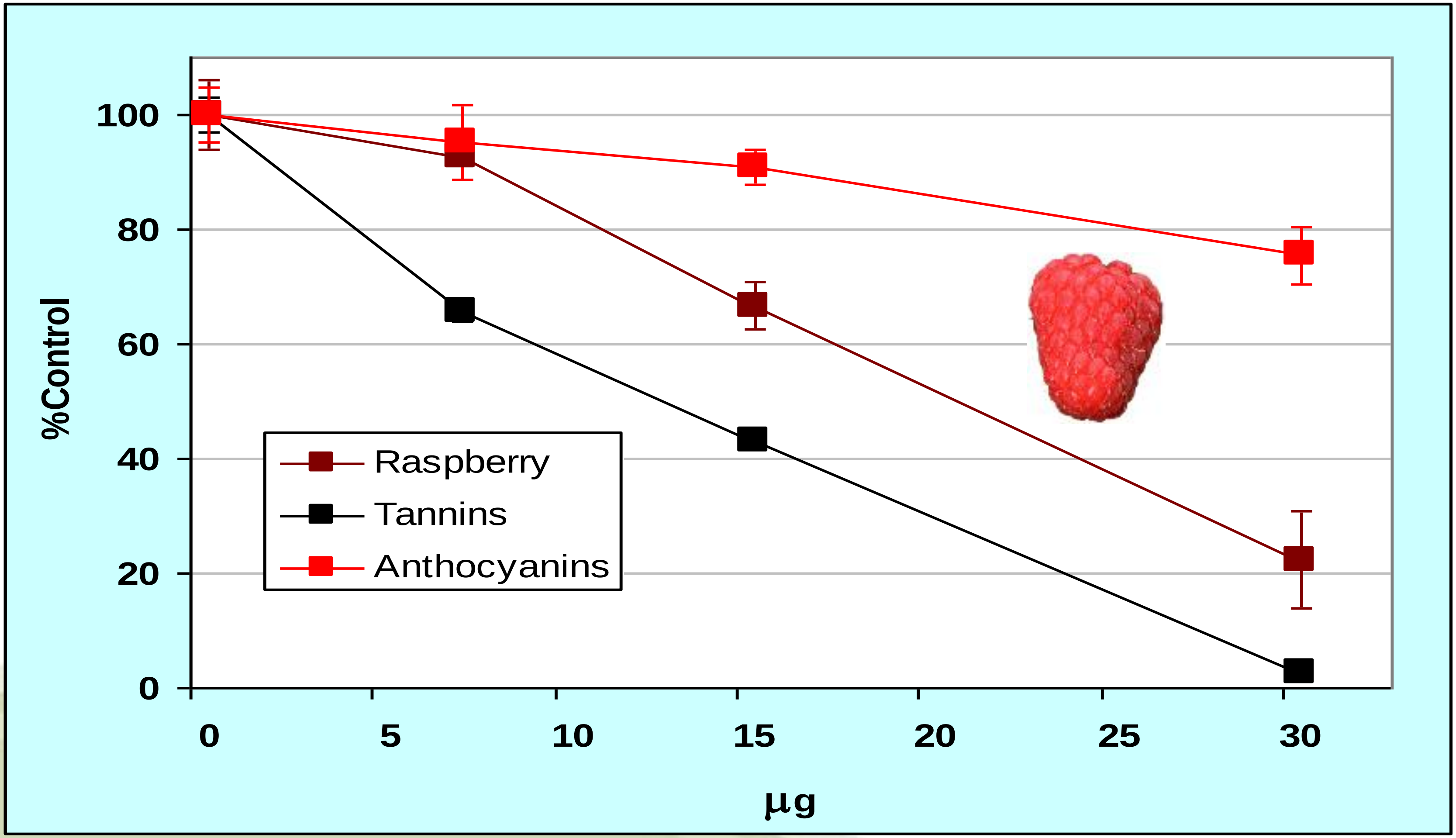
McDougall et al. (2008) JAFC 56; 3016-3023



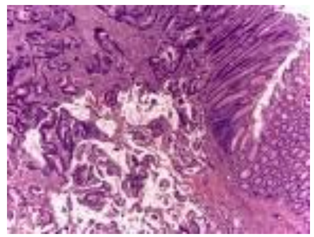
Anti-cancer effects

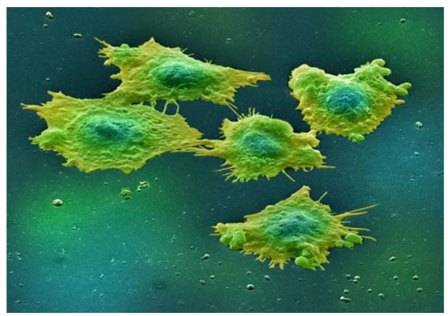


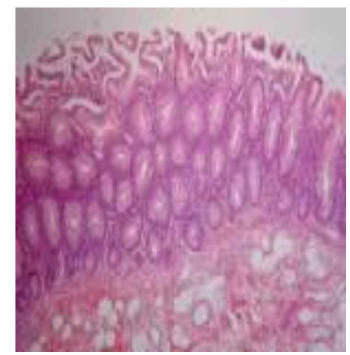
Most effective components are tannins



Joint project on berry polyphenols & colon cancer

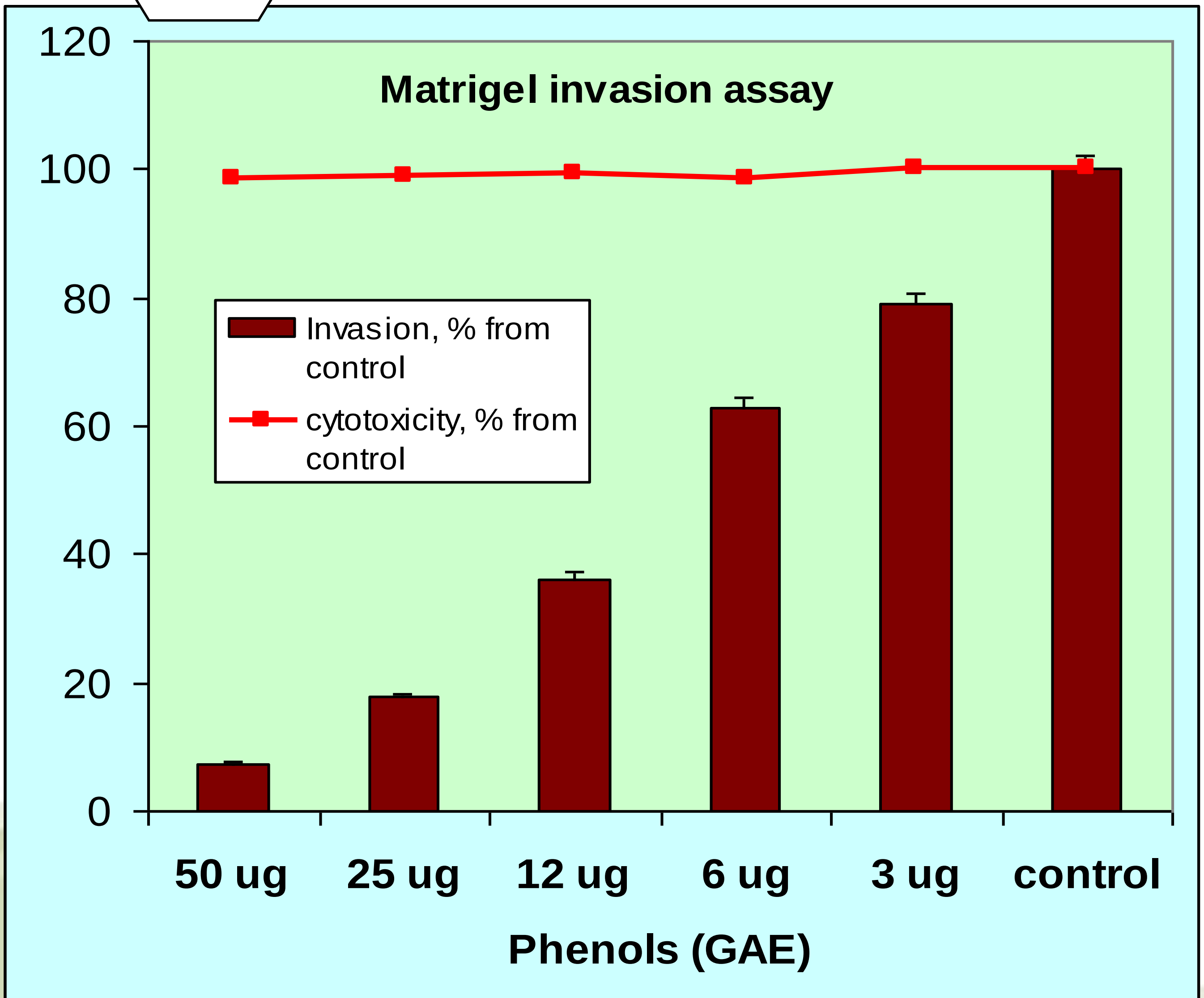






Emma Brown and Dr Chris Gill, Biomedical Sciences, University of Ulster, Coleraine

Colon cancer and polyphenols

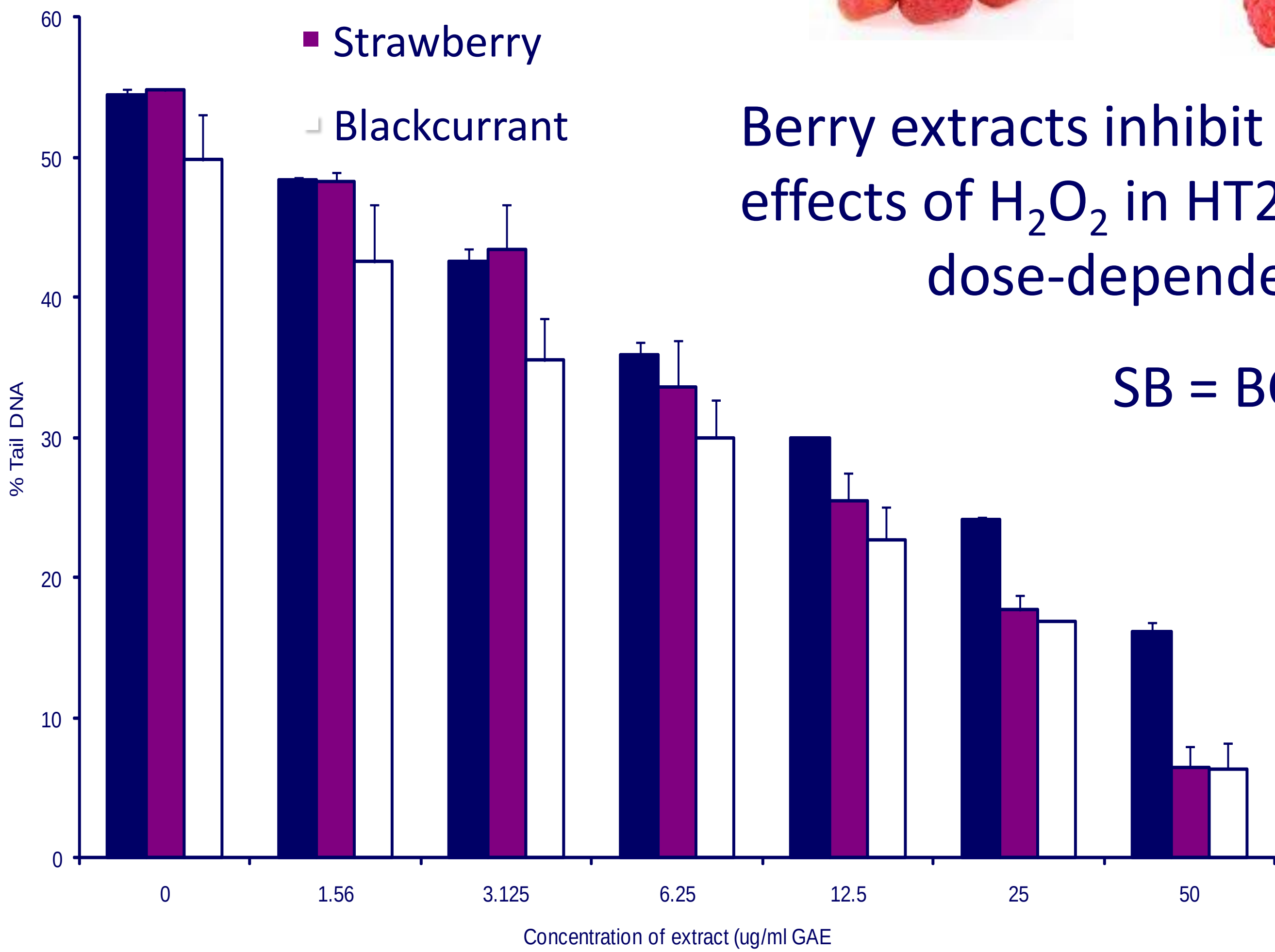


Matrigel invasion by HT115 colon cancer cells was inhibited by raspberry polyphenols in the µg range

Invasion related to ability to spread from initial site

Coates et al. *J. Carcinogenesis* (2007) 6, 1-13

Colon cancer and polyphenols



Berry extracts inhibit genotoxic effects of H_2O_2 in HT29 cells in a dose-dependent fashion;

SB = BC > RB

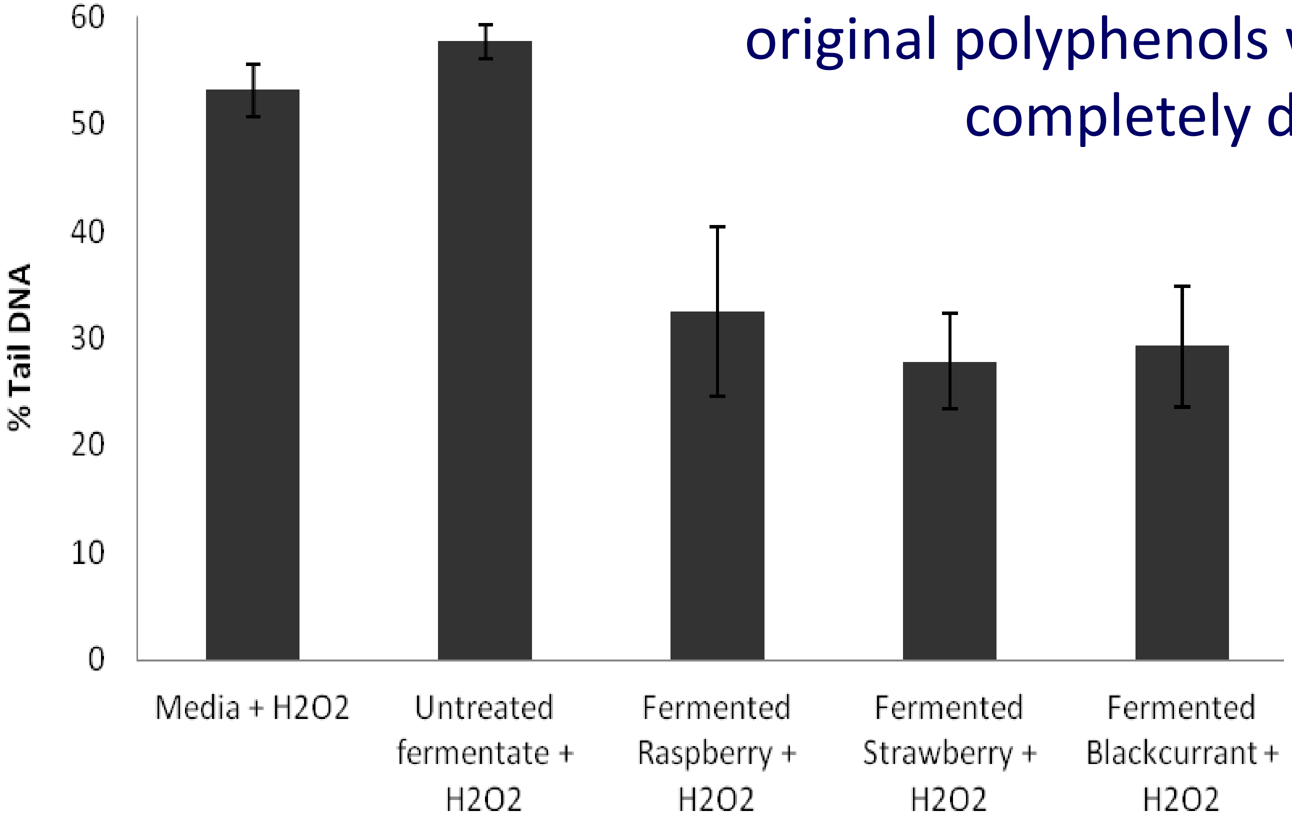
Colon cancer and polyphenols

Fermentation with faecal bacteria produces berry-specific polyphenol products

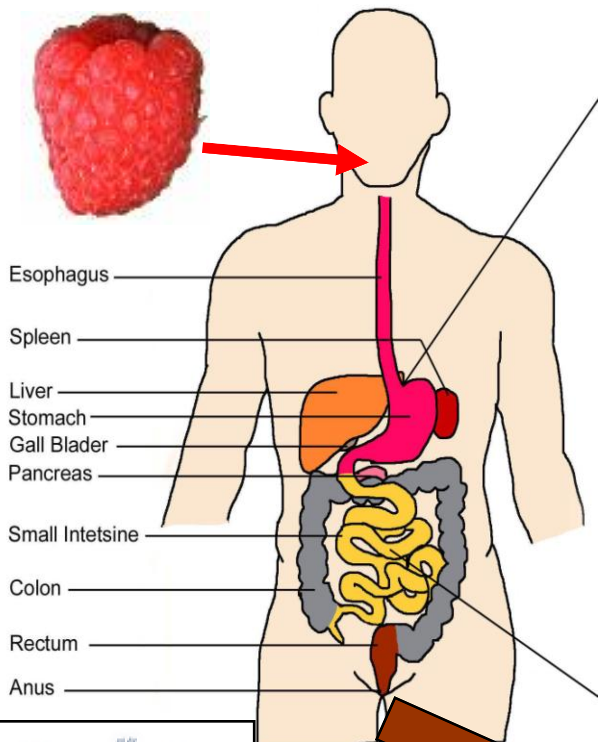
Compound	Control	Raspberry	Strawberry	Blackcurrant
benzoic acid	2.44 ± 0.50	2.32 ± 0.41	2.32 ± 0.08	1.71 ± 0.10
4-hydroxybenzoic acid	n.d.	0.26 ± 0.01*	0.52 ± 0.07*	0.25 ± 0.01*
3, 4-dihydroxybenzoic acid	n.d.	n.d.	n.d.	0.49 ± 0.08*
tyrosol	0.15 ± 0.03	0.32 ± 0.02*	0.32 ± 0.07*	0.17 ± 0.20
phenylacetic acid	16.34 ± 4.20	34.05 ± 3.5*	20.57 ± 1.4	7.35 ± 0.11
4’-hydroxyphenylacetic acid	1.52 ± 0.20	3.65 ± 0.5*	3.04 ± 0.3*	5.33 ± 0.30*
3-(phenyl)propionic acid	0.99 ± 0.30	2.10 ± 0.30	3.60 ± 0.21*	20.73 ± 0.51*
3-(4’-hydroxyphenyl)propionic acid	n.d.	n.d.	n.d.	1.66 ± 0.21*

Colon cancer and polyphenols

After fermentation with faecal bacteria, the berry-derived components were still genoprotective even though the original polyphenols were completely degraded



Faecal metabolism of berry polyphenols



- Profiling of faecal water metabolites in 10 free-living students after intake of raspberry puree (200 g/d for 14 d) by gas-chromatography mass spectrometry (GC-MS)
- Substantial ingestion of polyphenols (anthocyanins, ellagitannins etc)
- Focus on major phenolic metabolites

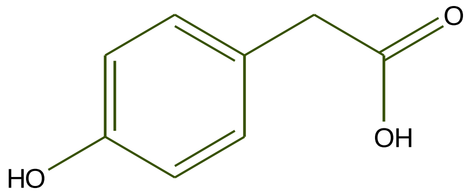
Faecal metabolism of berry polyphenols

But not the same subjects

- Phenylacetic acid increased in **7/10** subjects

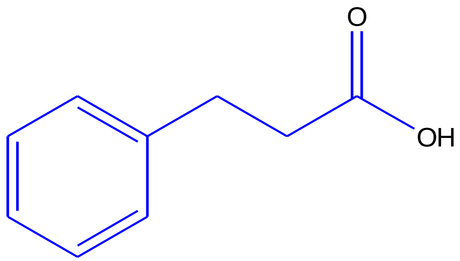
4-hydroxy phenylacetic acid increased in 6/10 subjects

3-hydroxy phenylacetic acid increased in 5/10 subjects



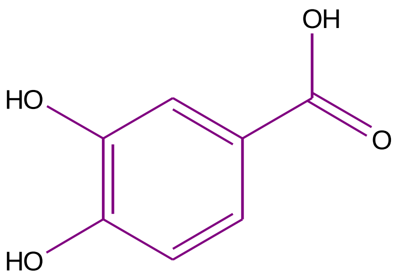
3-Phenylpropionic acid increased in 6/10 subjects

3(4-hydroxy) phenylpropionic acid increased in 5/10 subjects



3, 4-dihydroxy benzoic acid increased in **7/10** subjects

- 4-hydroxy benzoic acid increased in 2/10 subjects



- Fits evidence from model studies with faecal inoculates but shows large inter-individual variation - Due to differences in diet or microflora?

Gill et al, JAFC (2010) 58, 10389–10395

DGGE analysis of faceal microbiota revealed that this supplementation did not alter the composition

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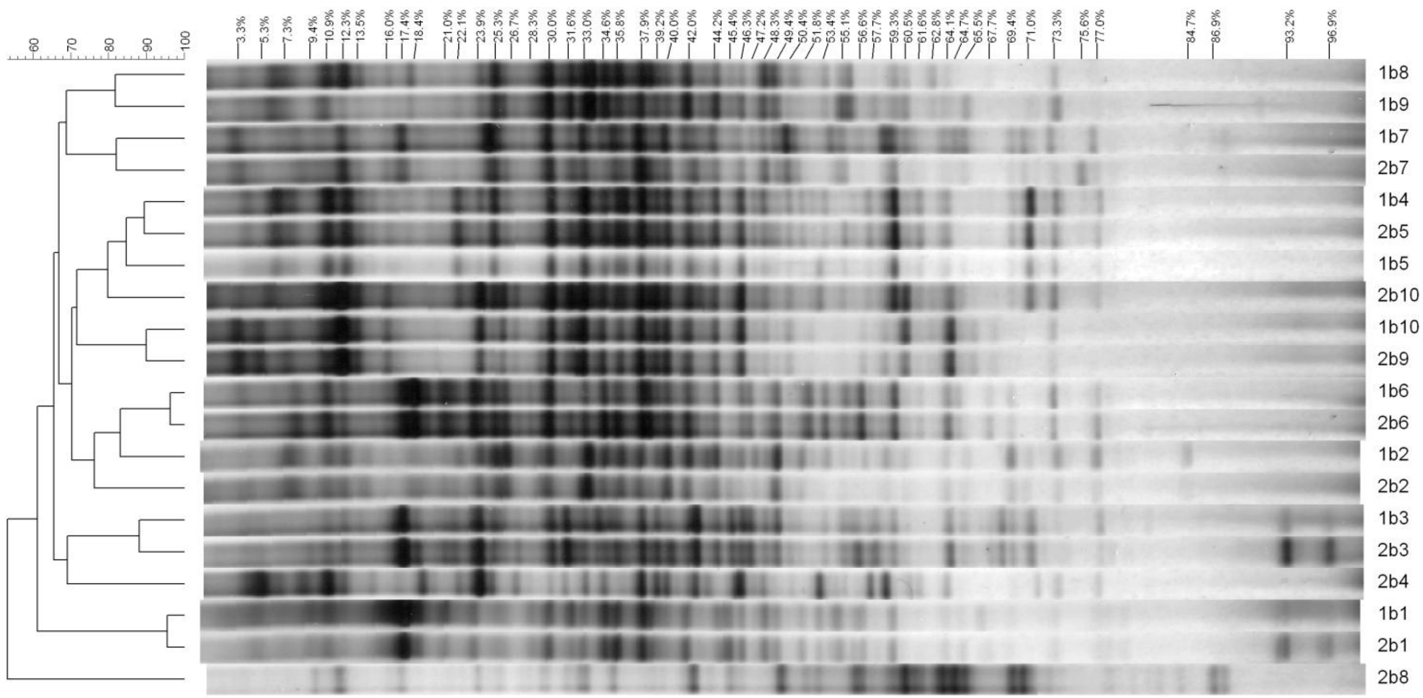
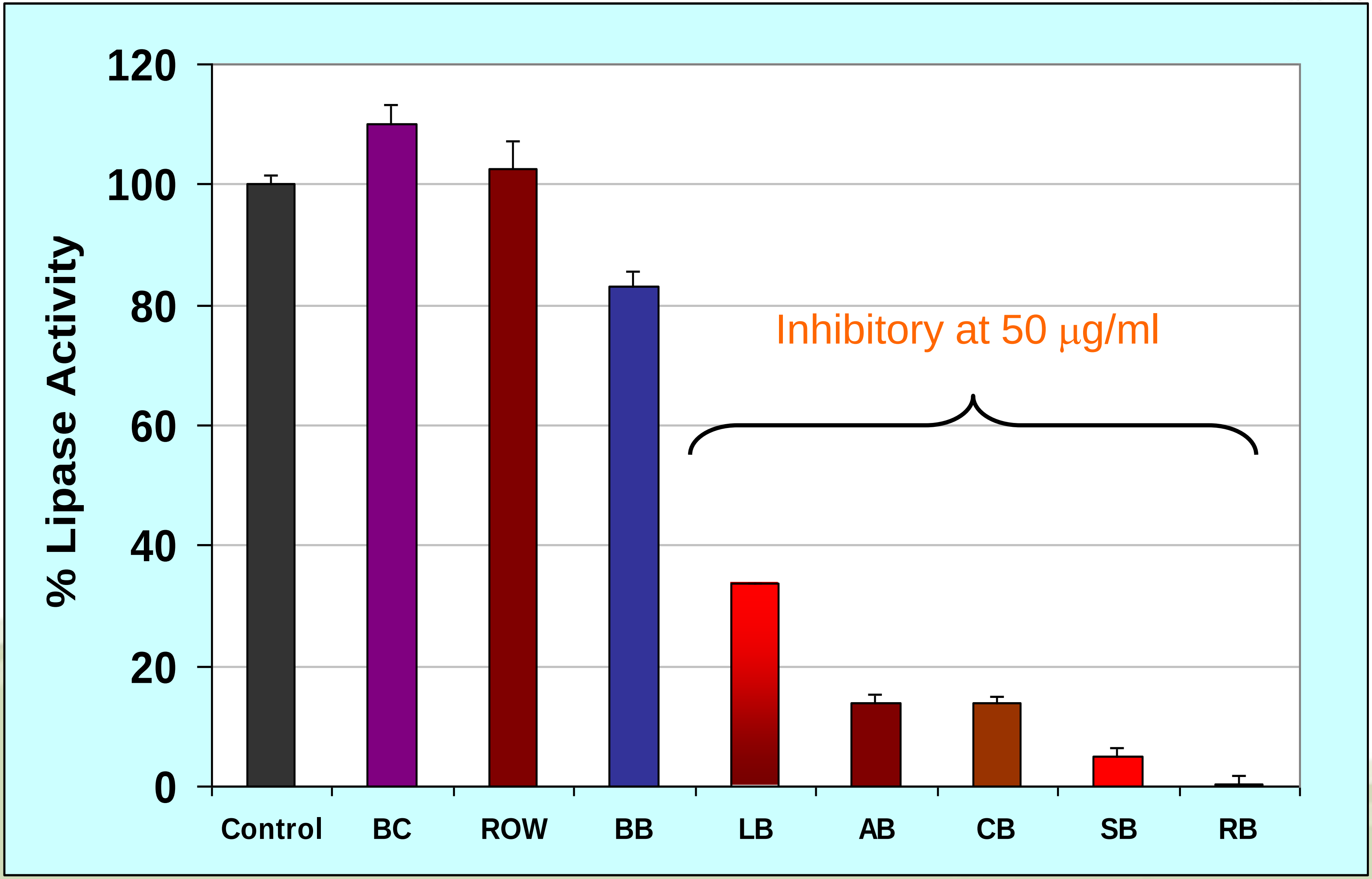


Samples before and after supplementation are more alike than between individuals.

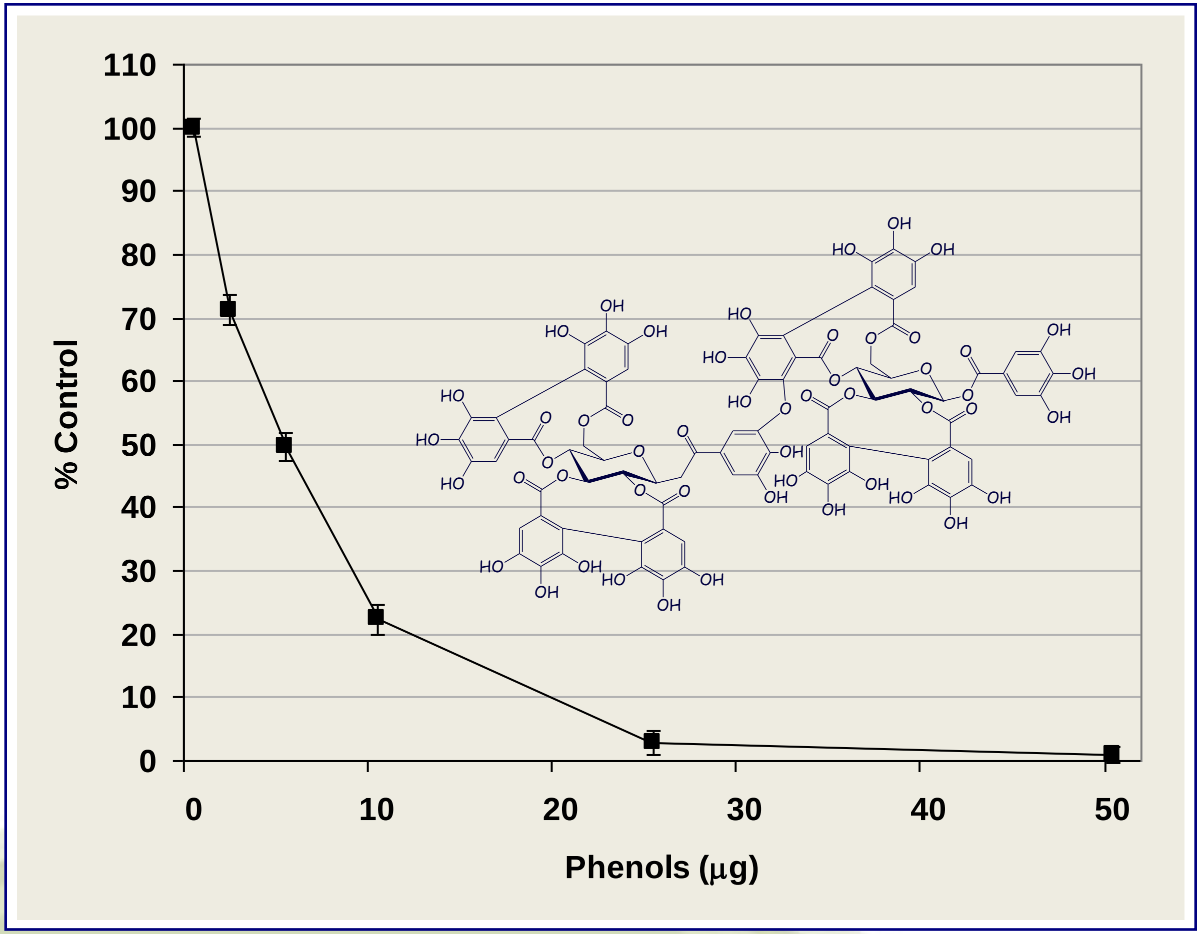
Control of nutrient availability

- Polyphenols can inhibit digestive processes and slow or modulate nutrient release from food
- Inhibition of starch digestion – blood glucose control and type 2 diabetes
- Inhibition of lipid digestion – control of hyperlipidemia, CVD and obesity

Lipid digestion and lipase



Lipase inhibition



Inhibition by cloudberry extracts is saturable

Due to ellagitannins (ETs) in cloudberry, arctic bramble and raspberry and

procyanidins and ETs in strawberry

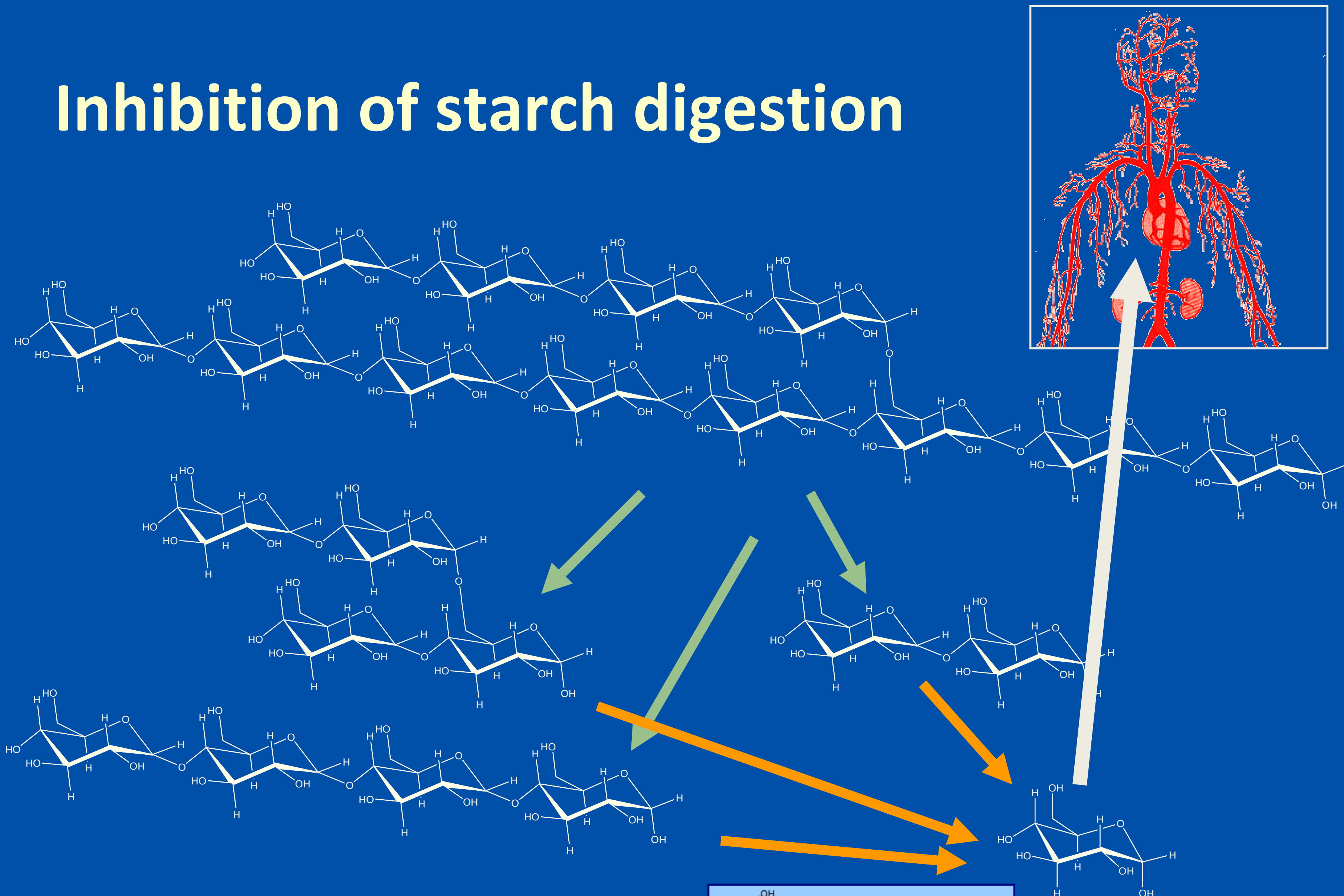
Mainly procyanidins in lingonberry

ASTRIGENT EFFECT?

Ties in with animal studies on obesity

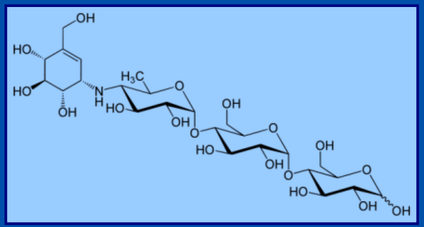
McDougall et al. (2009) Food Chemistry 115, 93–199

Inhibition of starch digestion

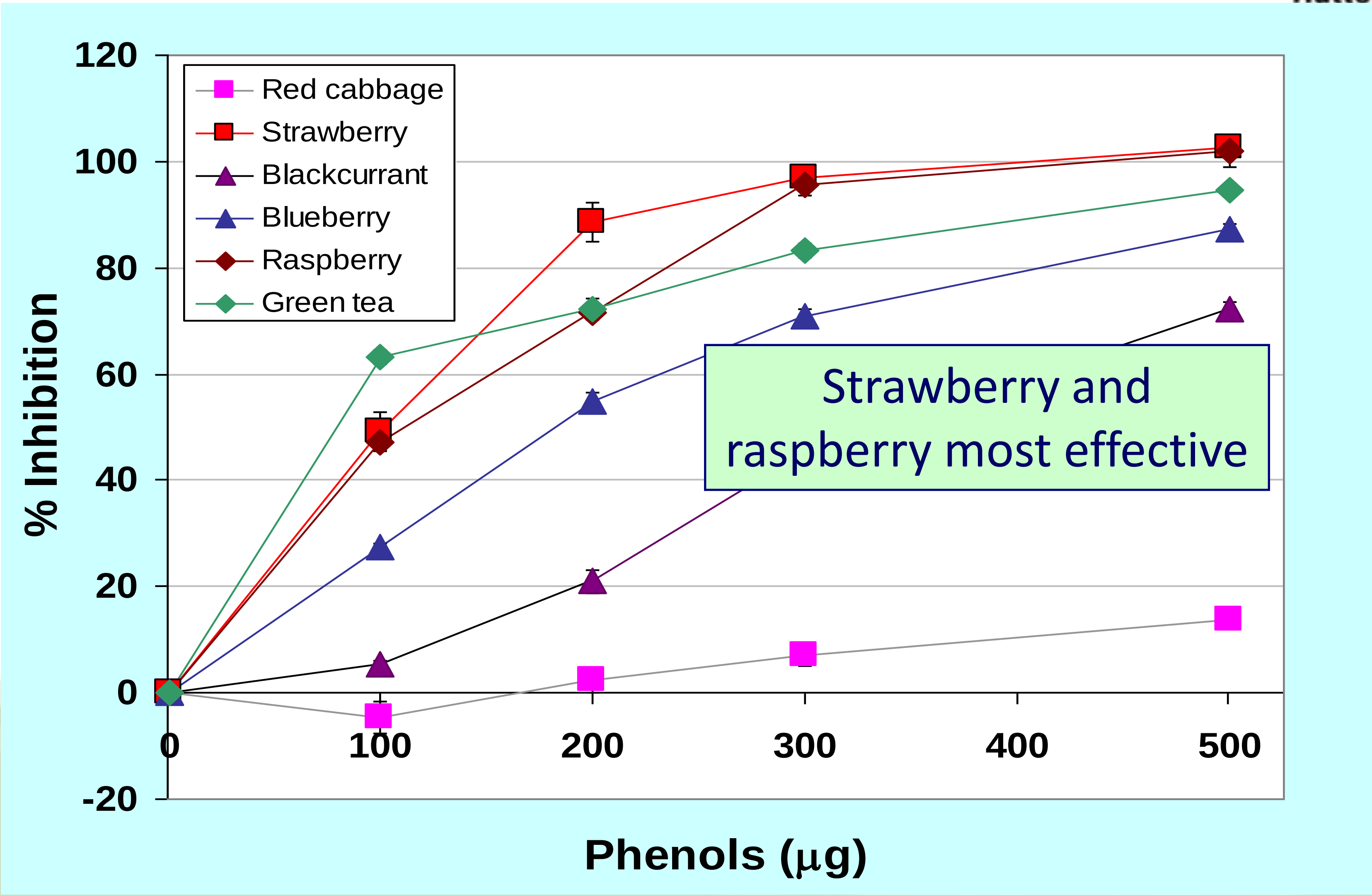


Amylase chops into fragments

Glucosidase nibbles off glucose



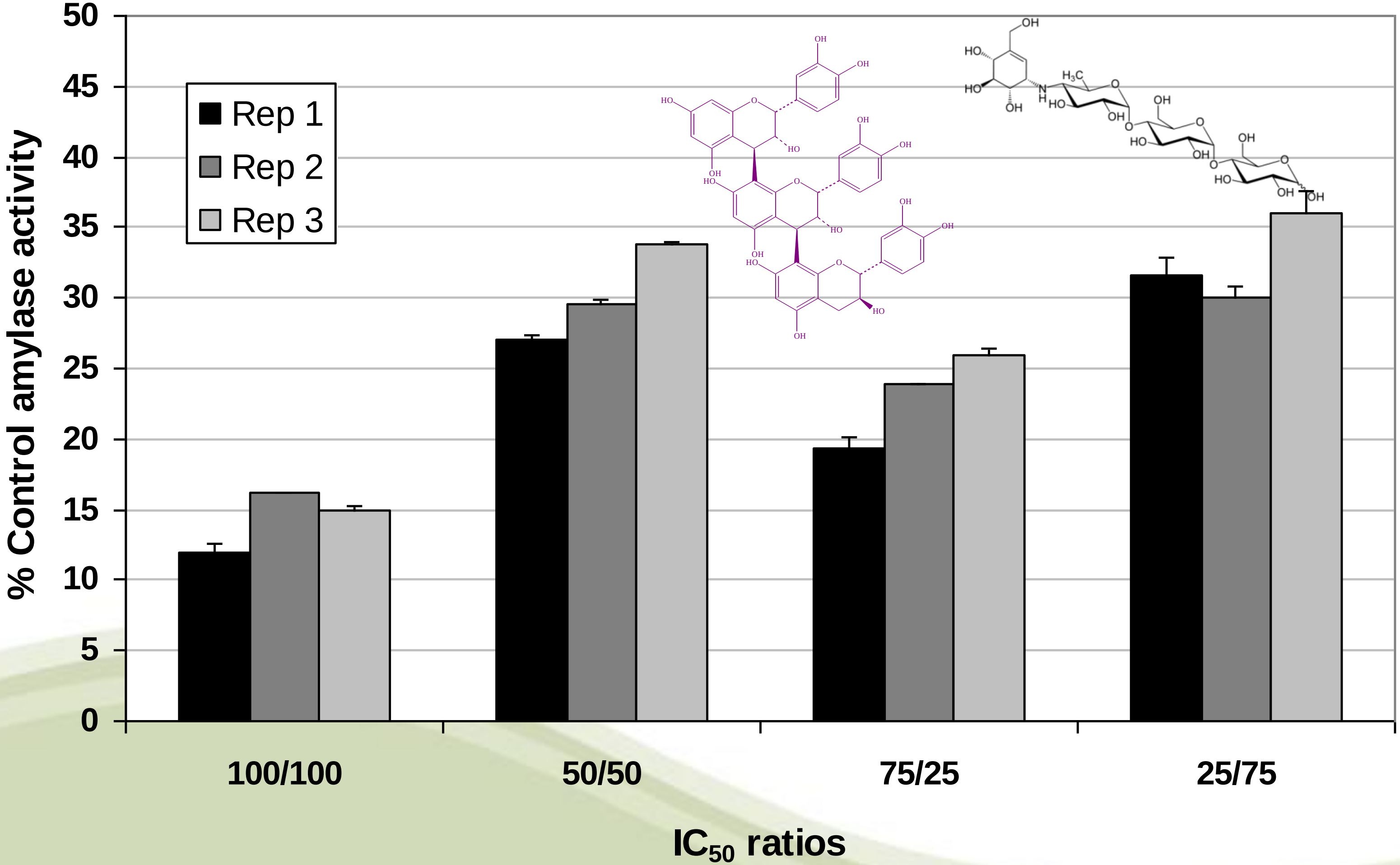
α-amylase inhibition



McDougall et al (2005) JAFC 53, 2760-2766; Grussu et al., 2011

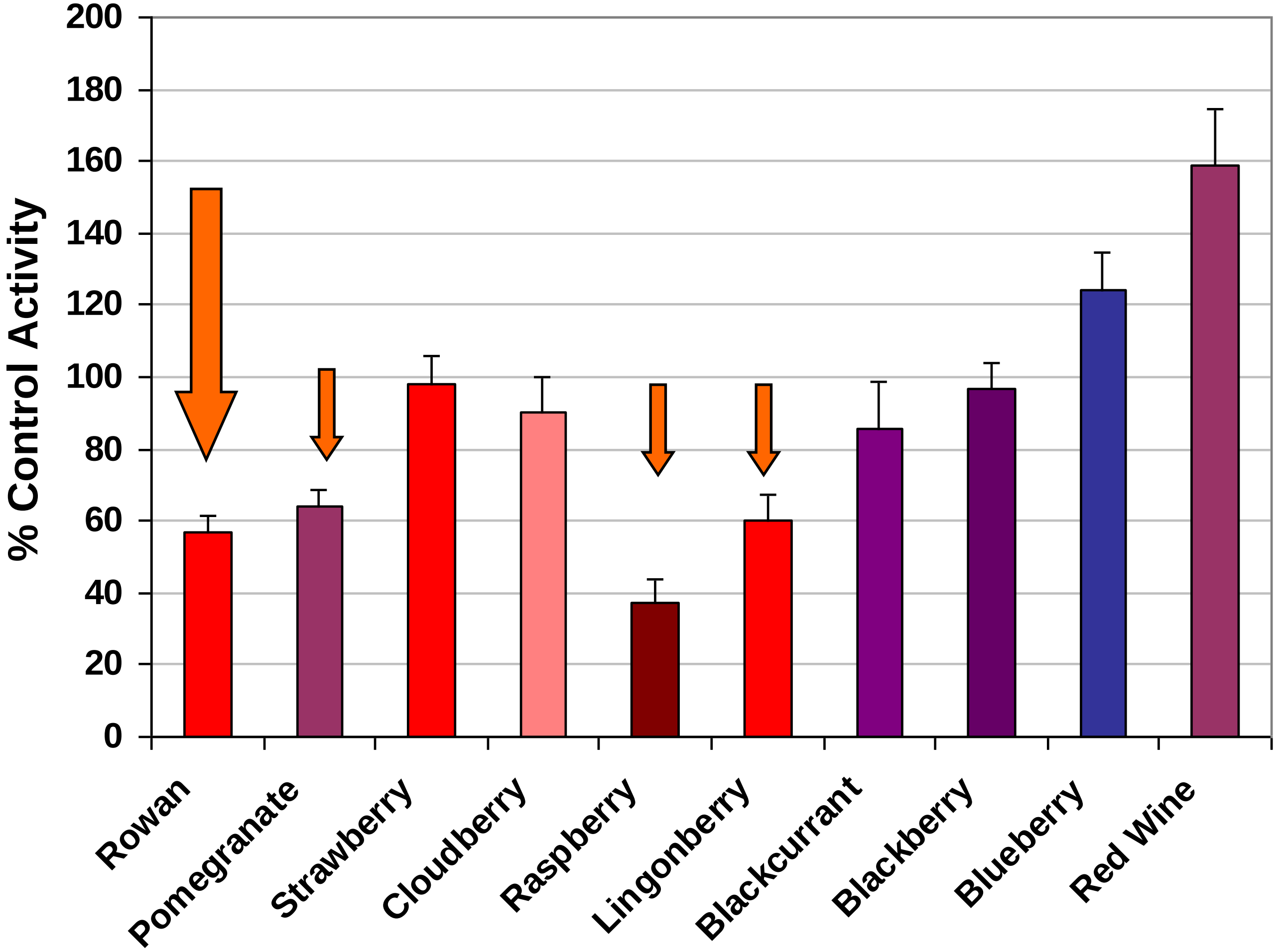
Co-incubation with acarbose

Co-incubations at ratios of IC_{50} – rowanberry PACs first



Each at IC_{50} | At half IC_{50} | 75/25 | 25/75

α-glucosidase inhibition by berries

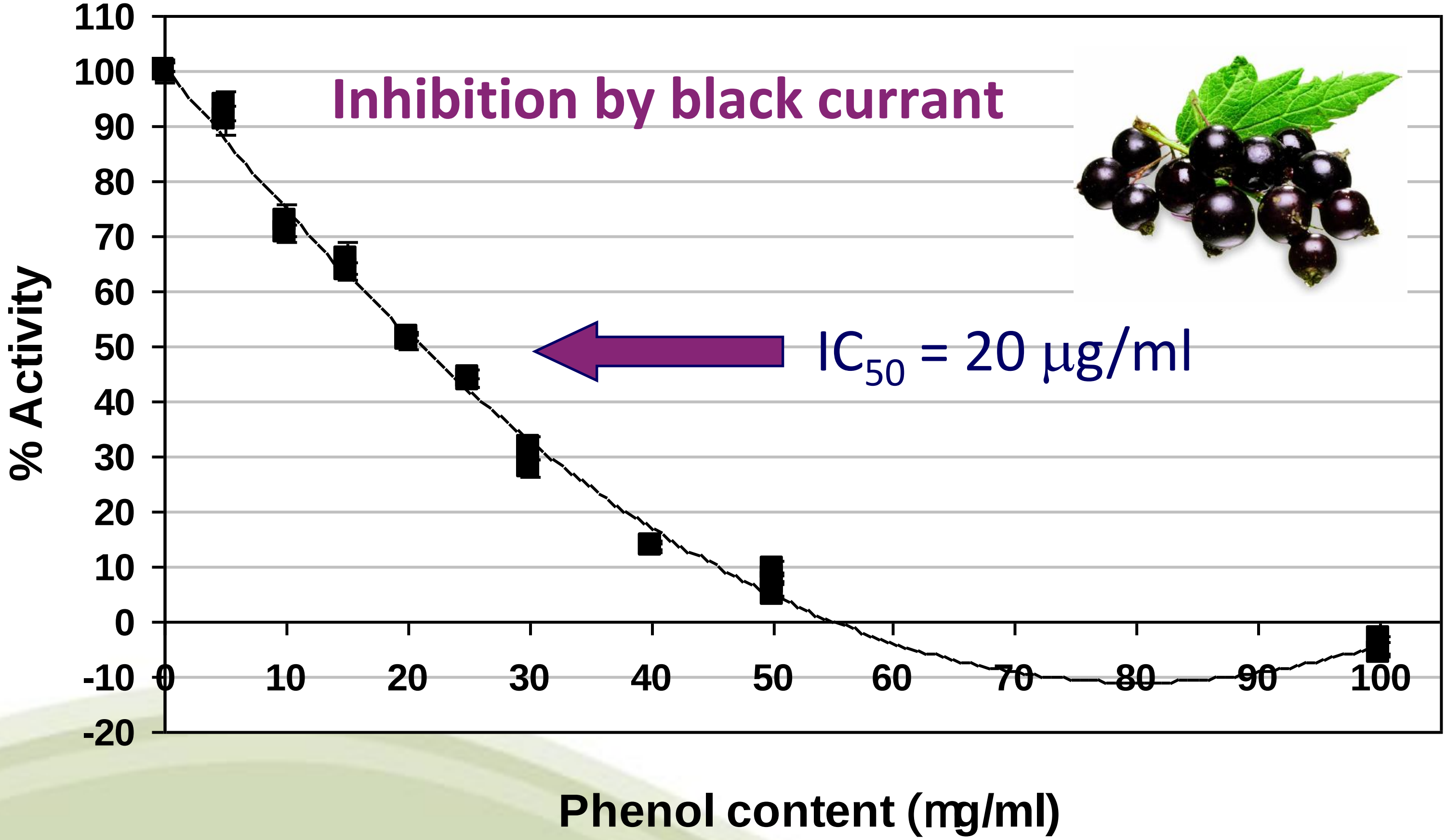


Not all berries equal?

Akin to pharmaceutical inhibition with acarbose ?

Whitson et al. Functional Plant Science and Biotechnology (2010)

α-glucosidase inhibition by berries



Boath et al. submitted; & Whitson et al. Funct. Plant Sci. & Biotech. 4, 34-38 (2010)

Co-incubation with acarbose

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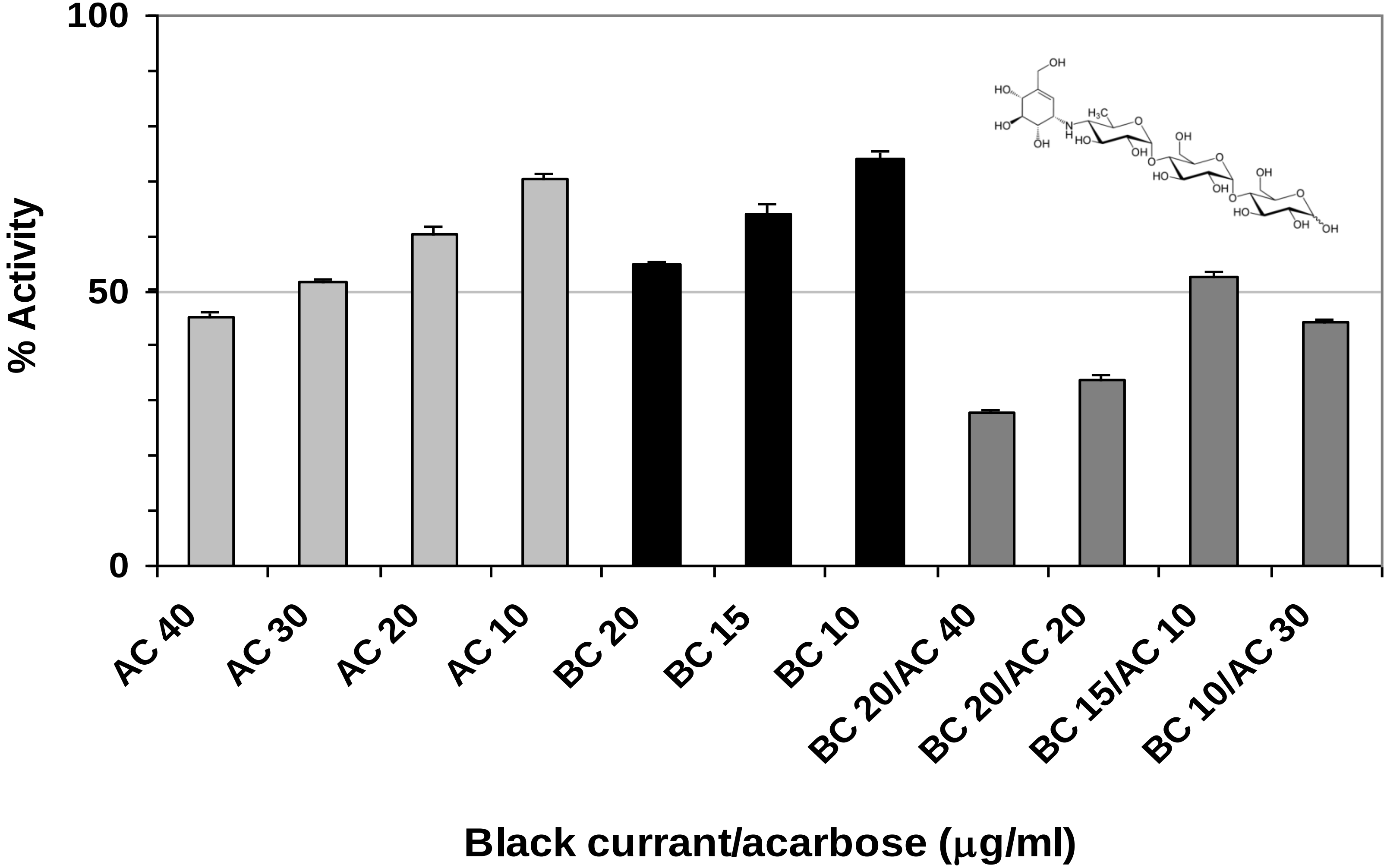
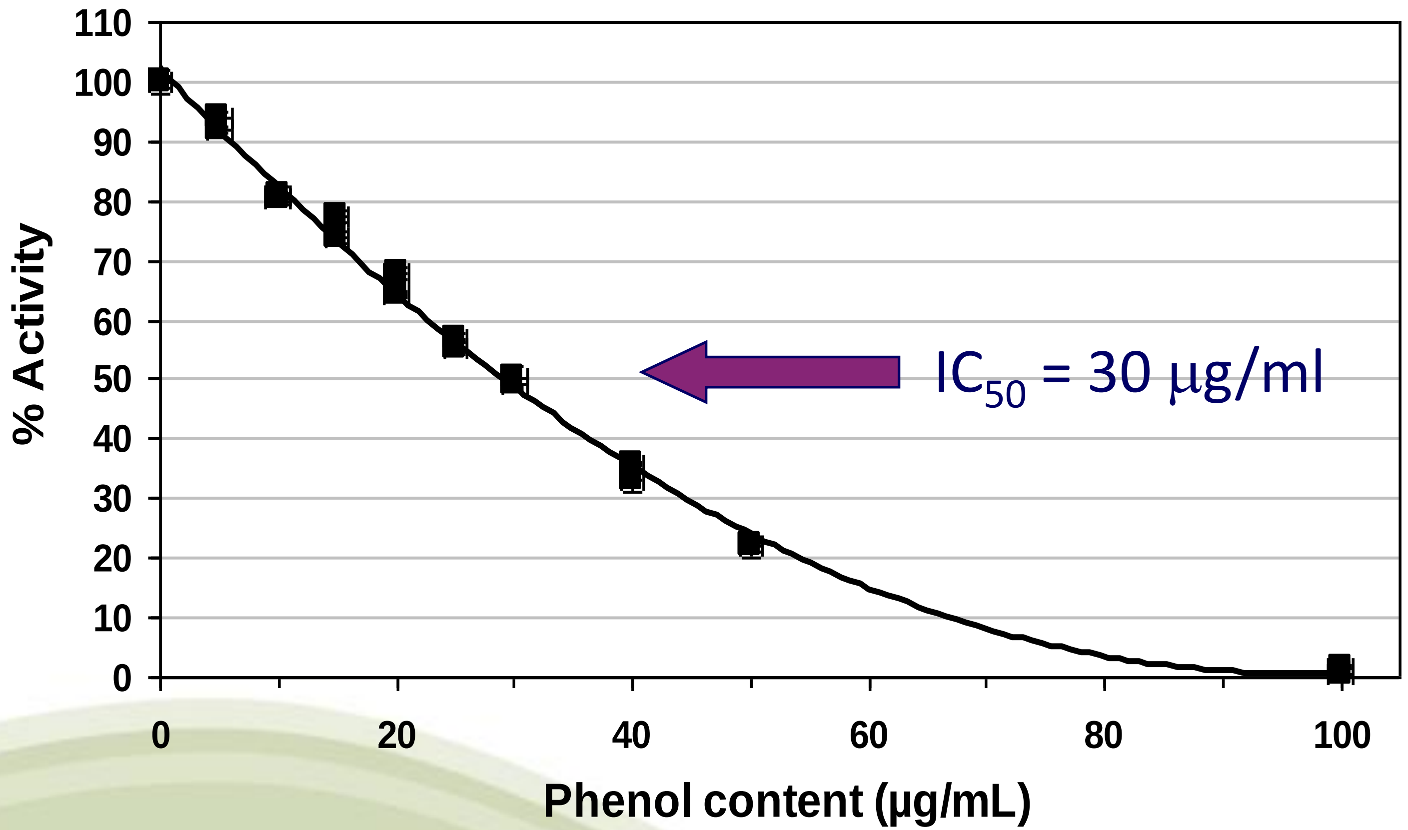
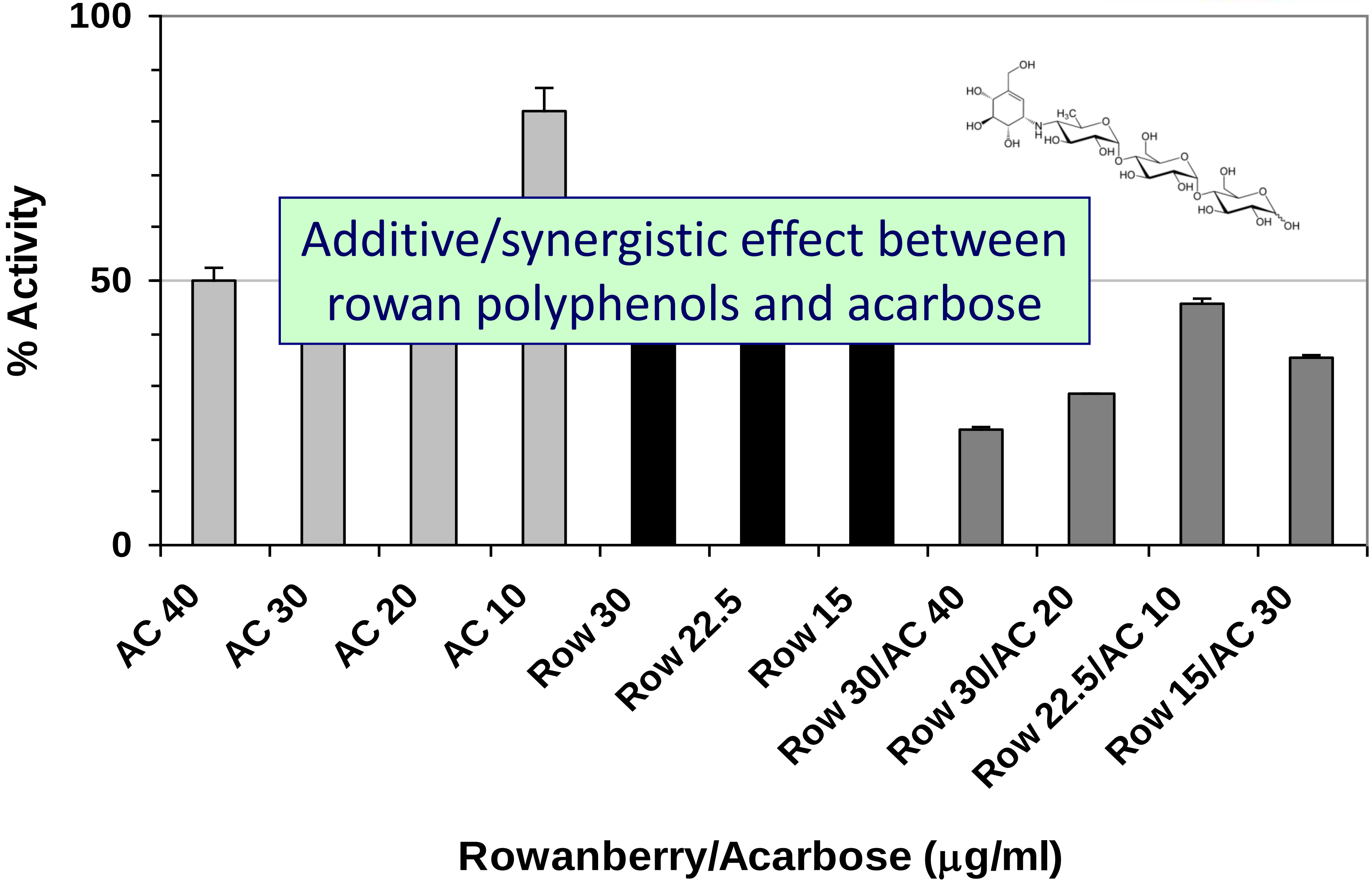


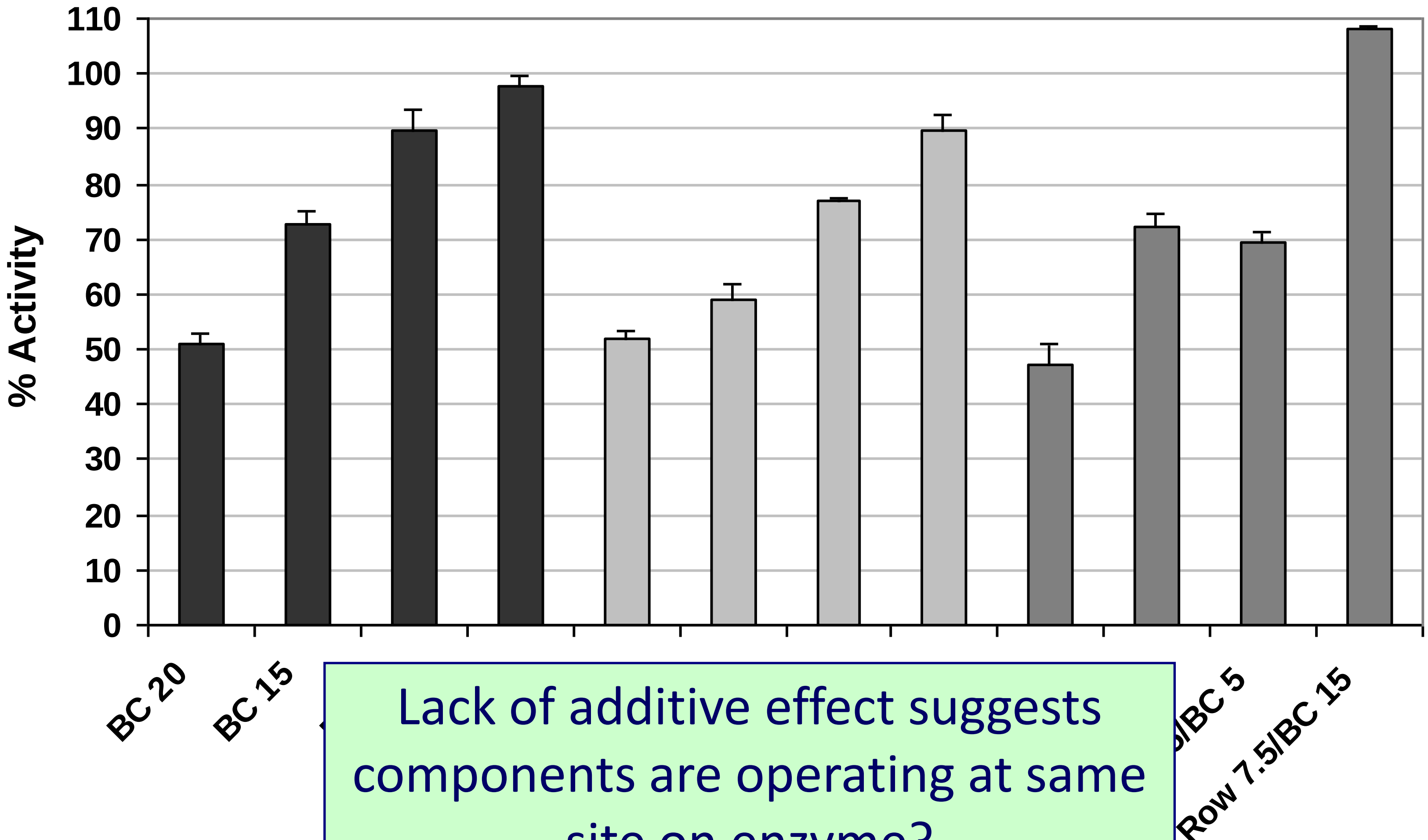
Inhibition by rowanberry



Co-incubation with acarbose



Mixing of berry extracts



Lack of additive effect suggests components are operating at same site on enzyme?

Summary – α-glucosidase inhibition

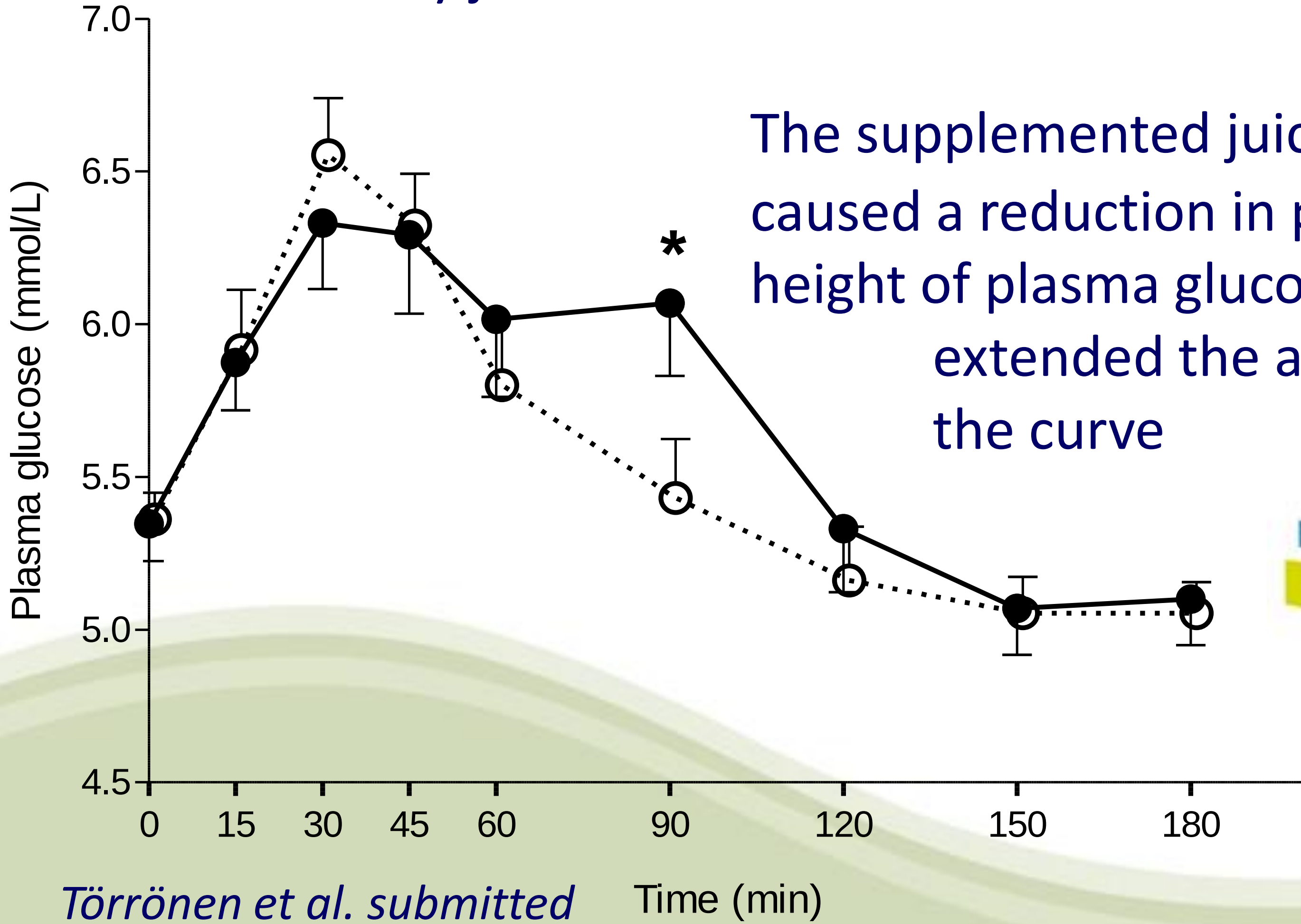
- Berry polyphenols inhibit glucosidase activity *in vitro* at low levels

- Inhibition depends on polyphenol composition
- Tannins are not important and astringency is probably not the main mechanism
- Anthocyanin-rich and chlorogenic acid-rich black currant and rowanberry are similarly effective

- The active components potentiate effect of acarbose but different berries do not act additively – sites of action?

Human trial – modified glycemic response

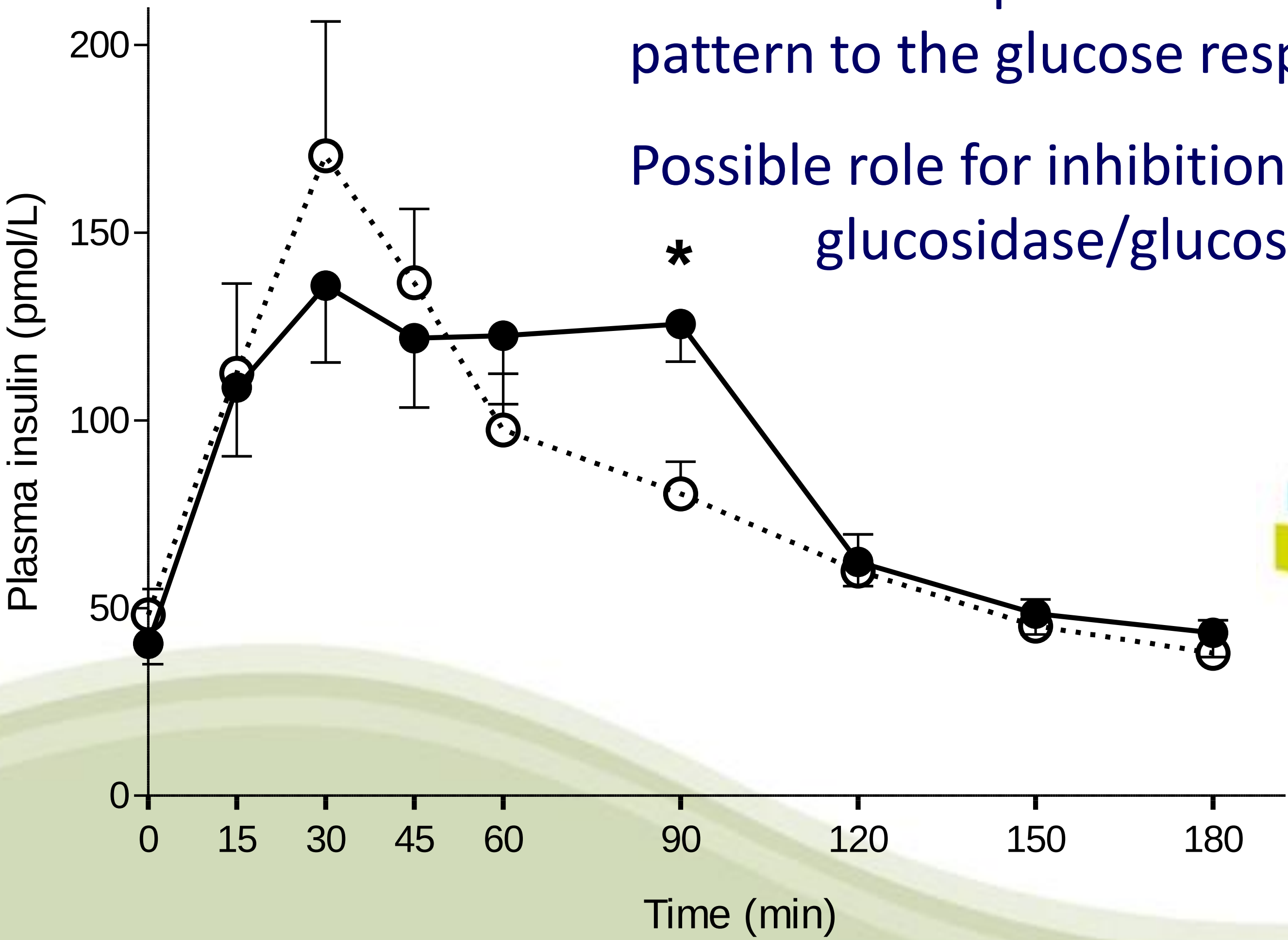
Volunteers given sucrose-loaded black currant (BC) juice or sucrose-loaded BC juice supplemented with crowberry juice



The supplemented juice (•) caused a reduction in peak height of plasma glucose and extended the area under the curve

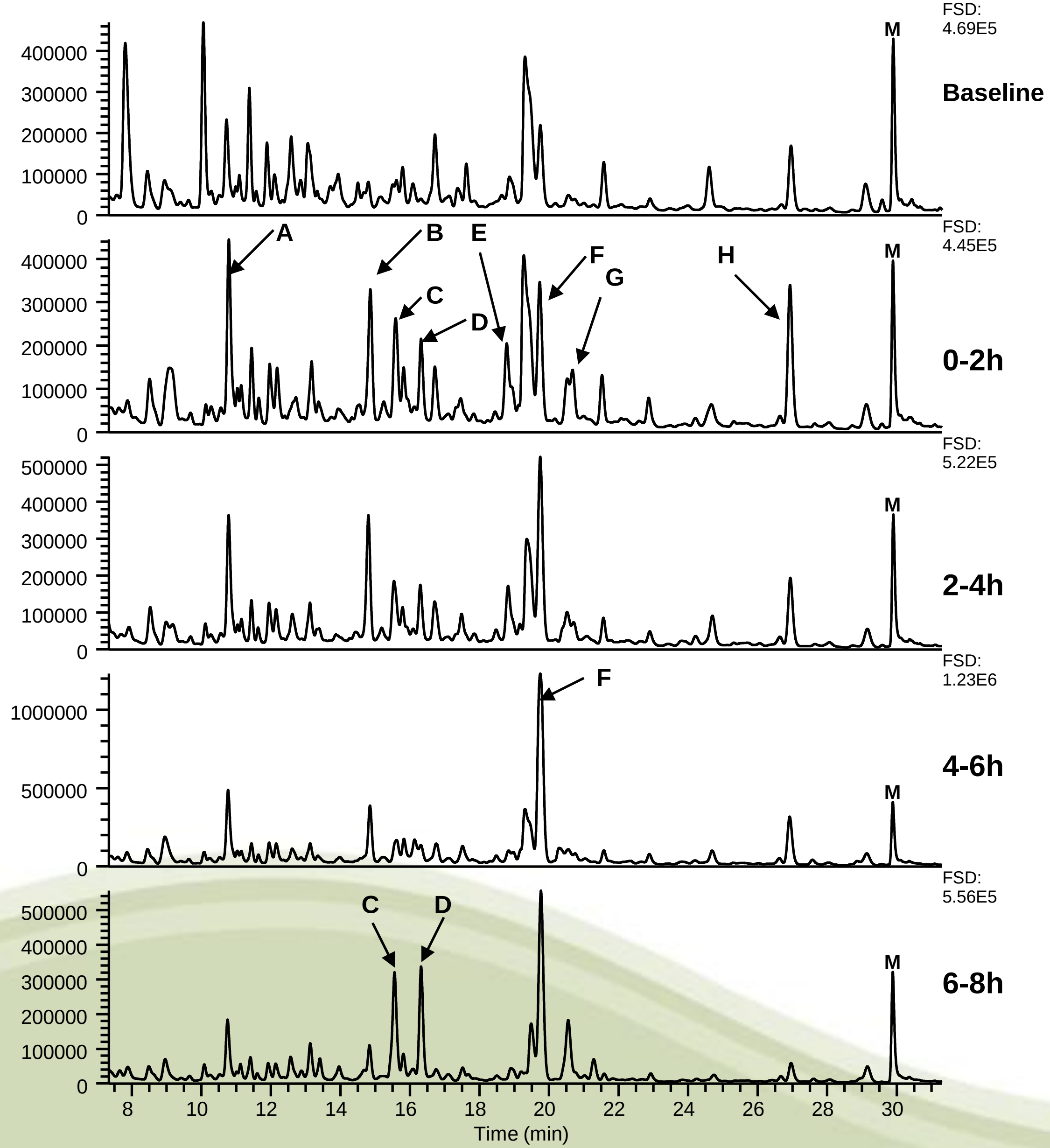
Törrönen et al. submitted

Human trial – insulin response



The insulin responses showed a similar pattern to the glucose response

Possible role for inhibition of glucosidase/glucose transport?

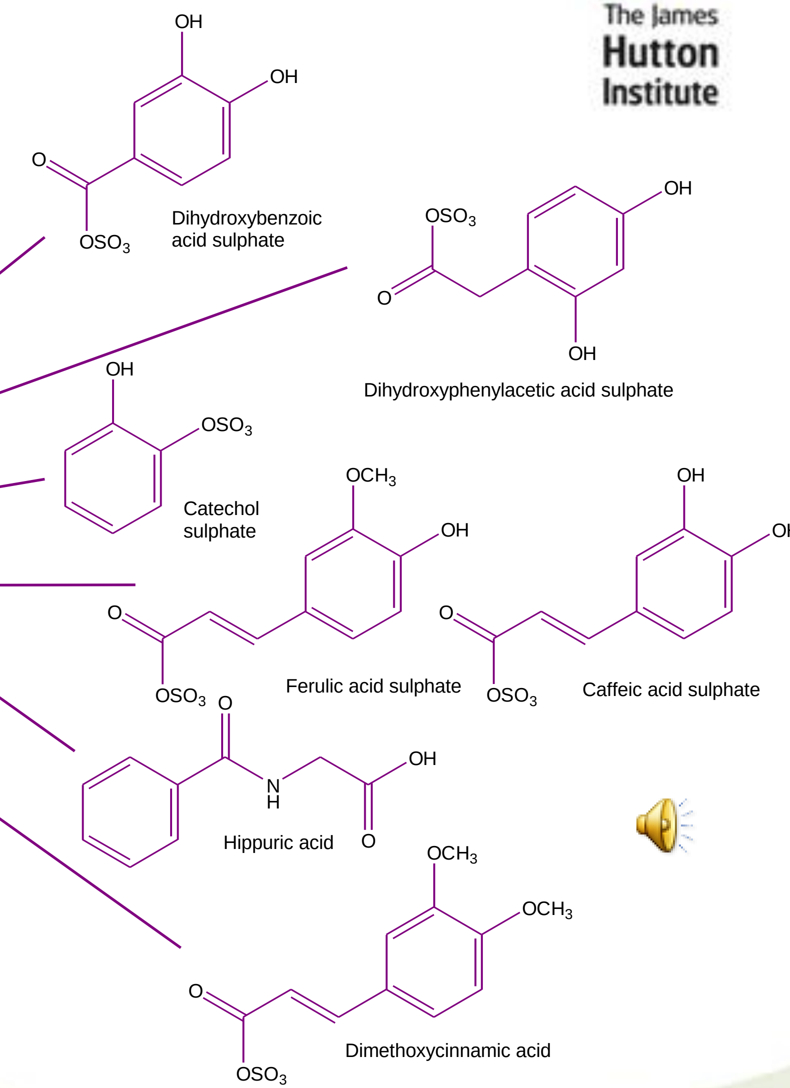


Urinary polyphenol metabolites after intake of fortified juice quantified by LCMS

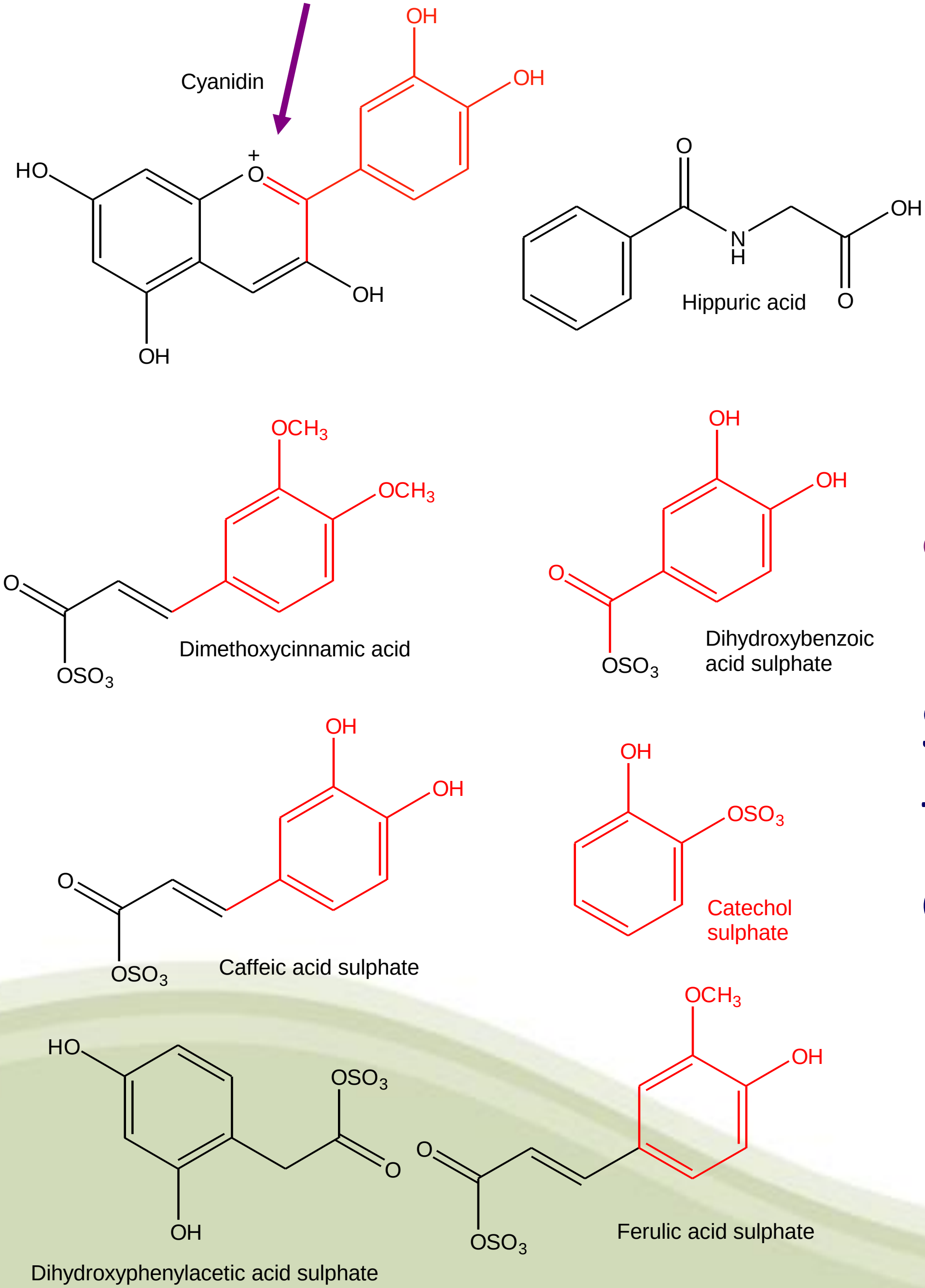
Increases noted within 2hrs

Human trial – urinary metabolites

	[M-H] *m/z*	MS²	Putative Identity
A	**244**	**164**, 162, 80	Phenolic acid sulphate
B	**233**, 153, 109	189, **153**, 97	Dihydroxybenzoic acid sulphate
C	**247**, 167	203, **167**, 123	Dihydroxyphenylacetic acid sulphate
D	**189**, 109	**109**	Catechol sulphate
E	**259, 273 mix**	**179, 193 resp.**	Mix of ferulic & caffeic acid sulphates
F	**178**	**134**	Hippuric acid
G	**287**	**207**, 163	Dimethoxy cinnamic acid sulphate
H	Multiple	-	Unknown



Identities were confirmed by exact mass determination at 4 decimal places



Breakdown products of anthocyanins?

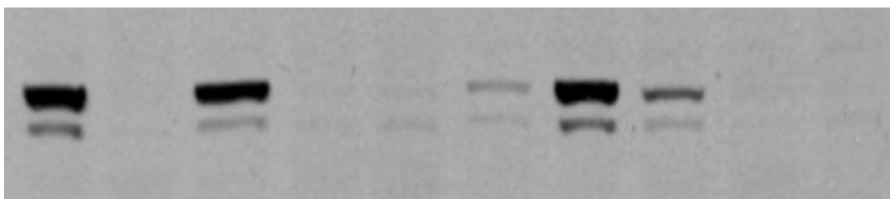
Suggestive of ring fission as reported in other studies

Nutrient Digestion

- Berry polyphenols inhibit enzymes involved in starch and lipid digestion *in vitro*
- The inhibition occurs at concentrations easily reached in the GIT
- The active components are unknown but differ between enzymes and in potential mechanisms (↑ synergy?)
- Berry components can potentiate inhibition by acarbose at low levels
- Initial human studies show promise

Insulin mimicking

Various polyphenols stimulate the phosphorylation and activation of FOXO1A, a transcription factor involved in regulating insulin responses and controlling glucose mobilization



Two different berry extracts (1 & 2) stimulate phosphorylation of FOXO1A but the active ingredients fractionate differently on SPE

Sandra Bacon & Graham Rena, University of Dundee

Summary



- Berry polyphenols have bioactivities that may influence human health
- Their mechanisms of action are not well defined but efficacy not always related to antioxidant capacity
- Structure-activity relationships are beginning to be gleaned
- Their stability and bioavailability *in vivo* is not fully understood but components can be identified in blood, urine and faeces that are characteristic of their uptake and metabolism

Acknowledgements

The James Hutton Institute

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SCOTTISH GOVERNMENT

UNIVERSITY of ULSTER

UHI Millennium Institute
Creating the University of the Highlands and Islands

UNIVERSITY OF DUNDEE

British Heart Foundation

University of Glasgow

Kuopio

Abertay University

UNIVERSITY OF EASTERN FINLAND

MTT

All staff in CPU, JHI

B.Sc and M.Sc students

Questions?

JHI is located in Invergowrie on the north bank of the River Tay

Visit http://www.hutton.ac.uk

Other areas

Effect of tea and coffee polyphenols on neurodegeneration and obesity models resp.

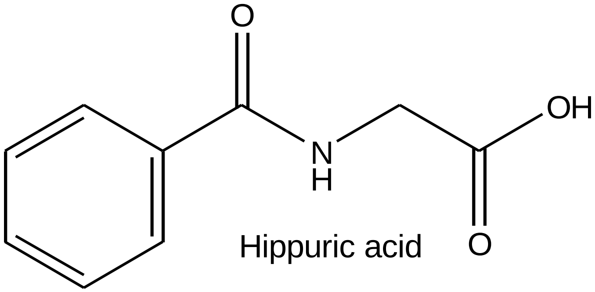
Analysis of carotenoids in sea buckthorn & carrot

Anti-parasitic effects of berry and vegetable extracts

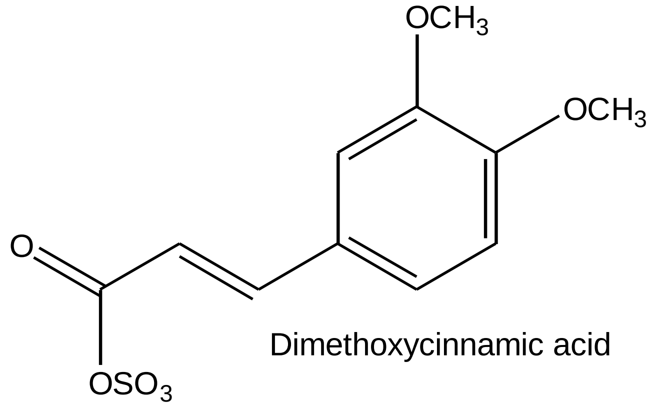
Natural products as anti-inflammatory agents



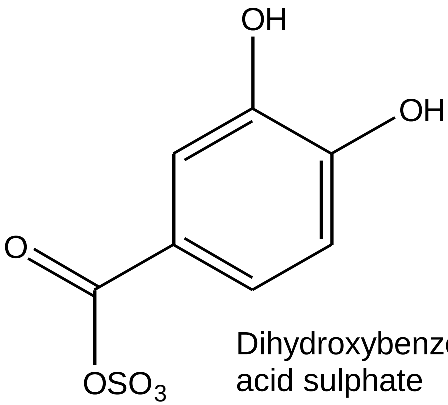




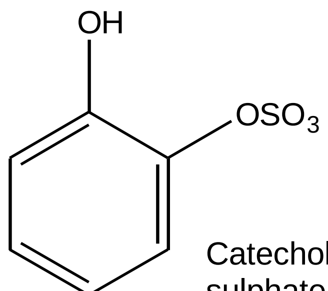
Hippuric acid



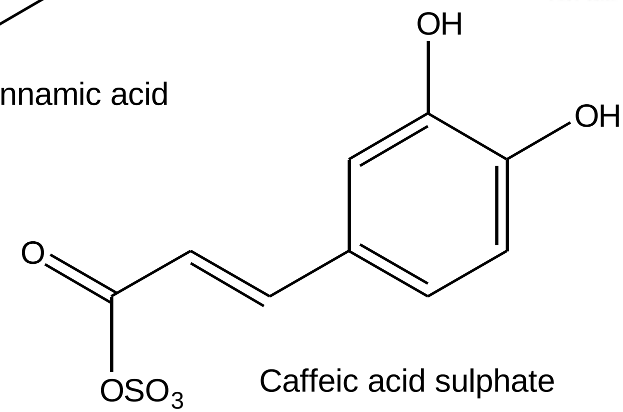
Dimethoxycinnamic acid



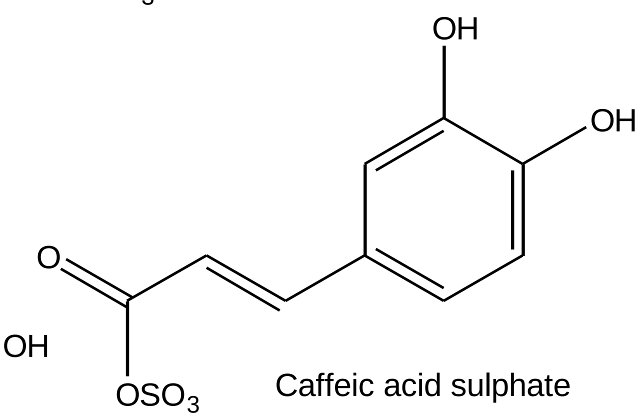
Dihydroxybenzoic acid sulphate



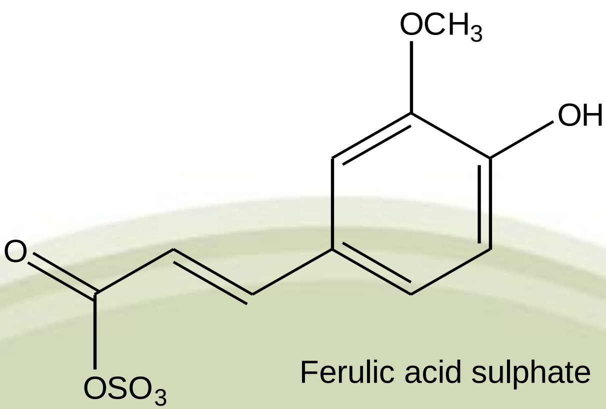
Catechol sulphate



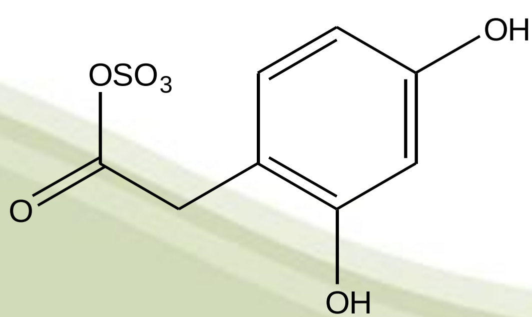
Caffeic acid sulphate



Caffeic acid sulphate



Ferulic acid sulphate



Dihydroxyphenylacetic acid sulphate

Developing high-through-put methods for assessing inheritance of polyphenols

- Link to genetic maps and markers to speed up selection
- Improve on traditional means of assessing polyphenol levels slow
- Develop and validate new methods
- Use power of mass spectroscopic and metabolic profiling methods

Stewart et al (2007) Mol.Nutr.Food Res. 51, 645–651

McDougall et al (2008) J. Chromatog. B 871, 362–369

How can polyphenols affect human health?

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Antioxidant theory? Low serum bioavailability!

Majority of polyphenols remain in gut

Are these components inactive?

Possible roles

Modulating colonic microbiota?

In-gut antioxidants?
Benefit gut epithelia function / colon cancer

Modulate digestive processes

Latham

x

Moy

Two environments, 5 seasons

Targeted analysis
Yield, flavour, aroma taste, texture, disease resistance, bioactivities, antioxidant capacity, polyphenol content, ascorbate, anthocyanins

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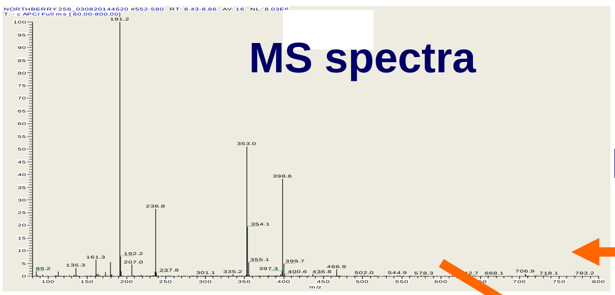
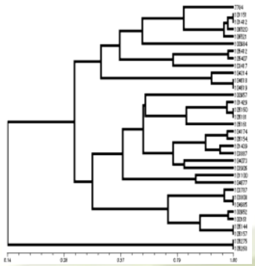
Untargeted analysis **Hi-through-put metabolic profiling**

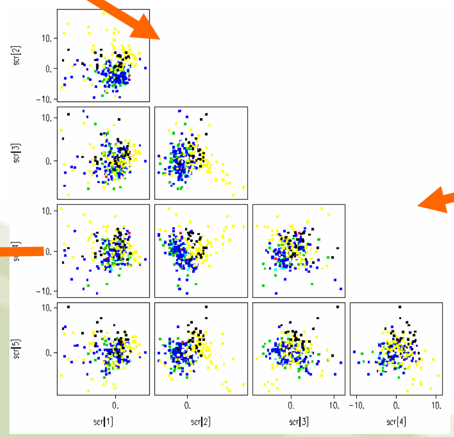


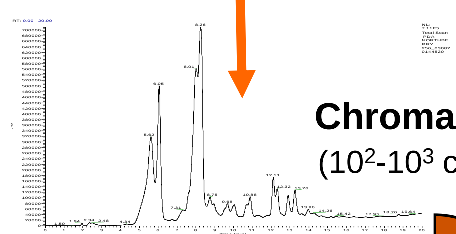
MS spectra

Direct Infusion MS
No chromatography

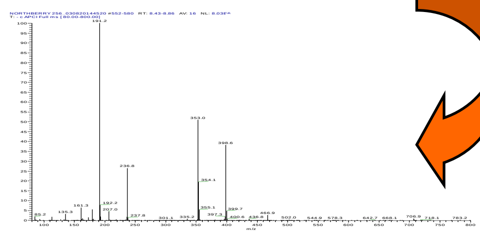


Hierarchical cluster analysis:
Measure of (phytochemical) biodiversity - link to genetic map

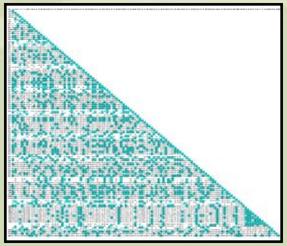




Chromatogram
(10^2-10^3 compounds)



Compounds 1-500

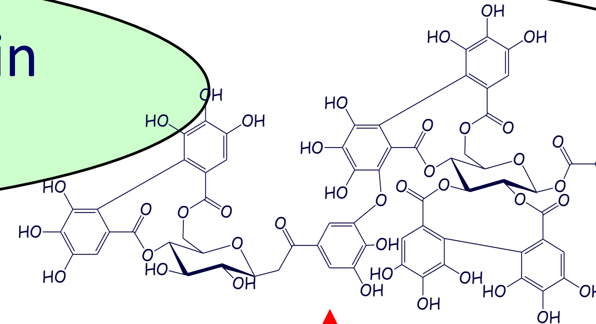


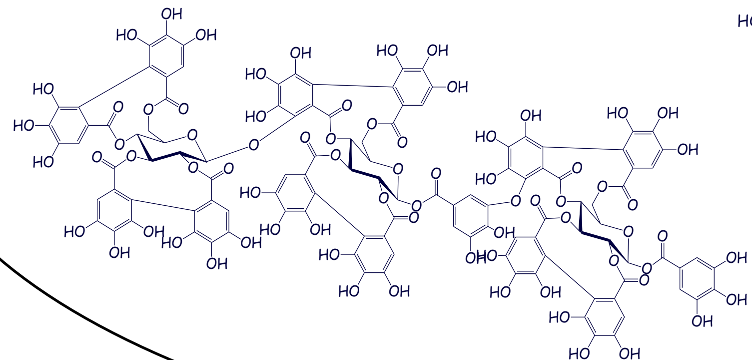
Compounds 1-500

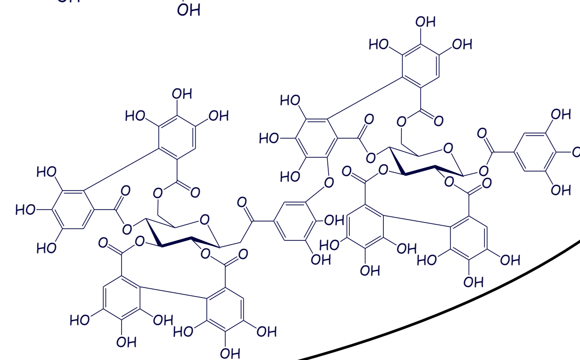
Correlation Network:
Interrelate metabolite changes.

Principal component analysis of MS data

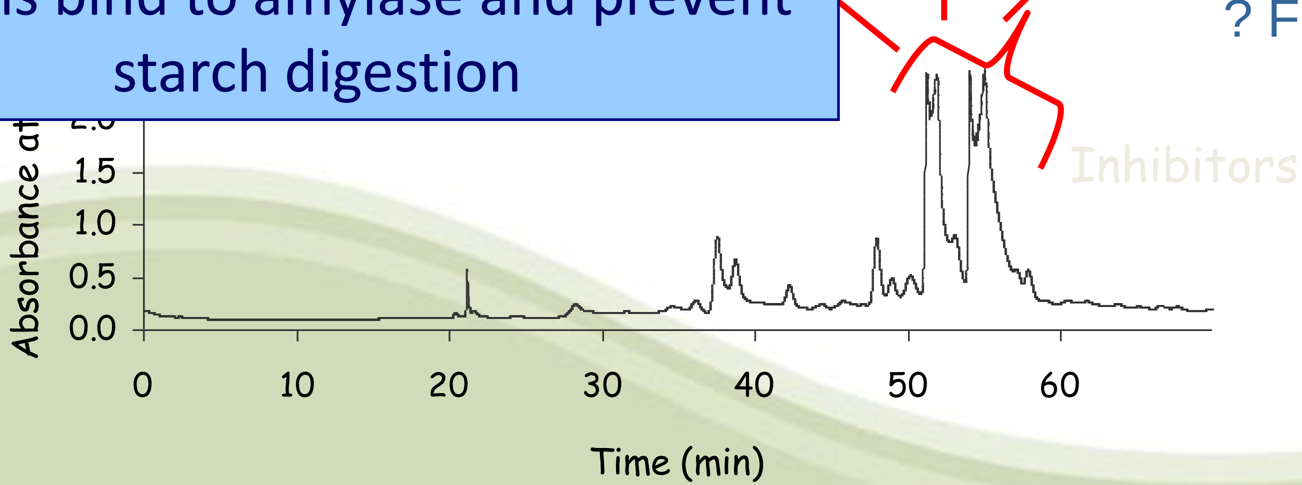
The inhibitory components in raspberry are Ellagitannins







Tannins bind to amylase and prevent starch digestion



? Full story ?

The Rhubarb story

The James Hutton Institute

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Effect of different cooking regimes on rhubarb polyphenols

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A B S T R A C T

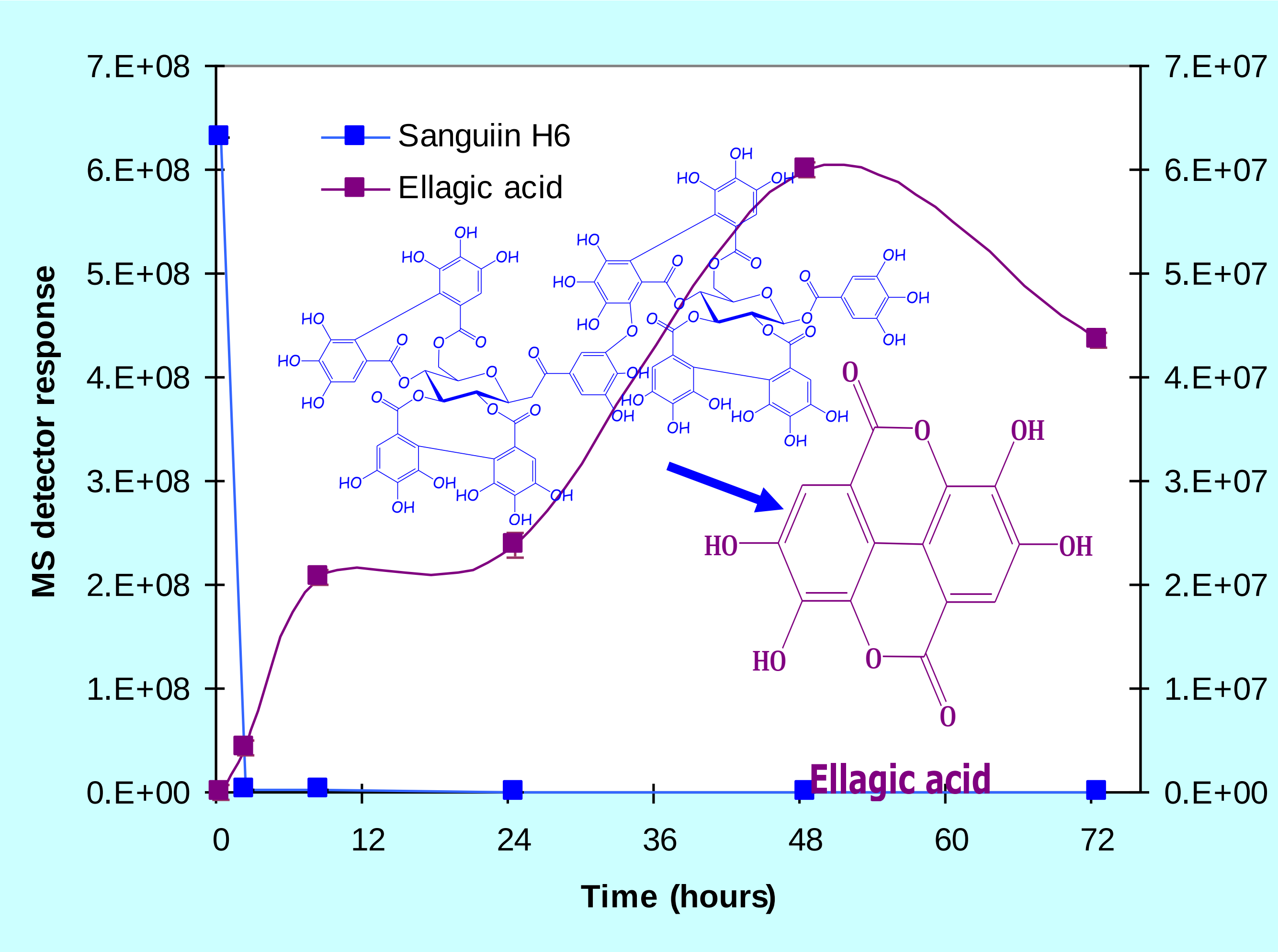
Polyphenolic components, such as anthraquinones and stilbenes, from species of the genus *Rheum* have been shown to have a range of bioactivities relevant to human health. This paper outlines the polyphenolic composition of edible petioles of garden rhubarb (*Rheum rhapontigen*) and describes the effects of common cooking methods on total polyphenolic content, anthocyanin content and total antioxidant capacity.

Most cooking regimes (fast stewing, slow stewing and baking) except blanching increased total polyphenol content and overall antioxidant capacity, compared to the raw material. The patterns of anthocyanin content and total polyphenol content between the different cooking regimes suggested a balance between two processes; cooking facilitated the release of polyphenol compounds from the rhubarb but also caused breakdown of the released compounds.

Baking and slow stewing offered the best maintenance of colour through preservation of anthocyanin and the highest antioxidant capacity. Baking for 20 min provided well-cooked rhubarb with the highest antioxidant capacity and the highest anthocyanin content, which is important for the aesthetic quality of the dish.

Liquid chromatography–mass spectrometric (LC–MS) analysis putatively identified over 40 polyphenol components in raw rhubarb, including anthraquinone, stilbene, anthocyanin and flavonol derivatives. Baking caused selective effects on the stability of the different polyphenol components. Initially, the yield of all components increased but there was a drastic decline in the relative stability of anthraquinone aglycones with increasing cooking time and initial evidence for the turnover of other anthraquinone derivatives was obtained.

Stability and Bioavailability



Raspberry ellagitannins inhibit cancer cell growth

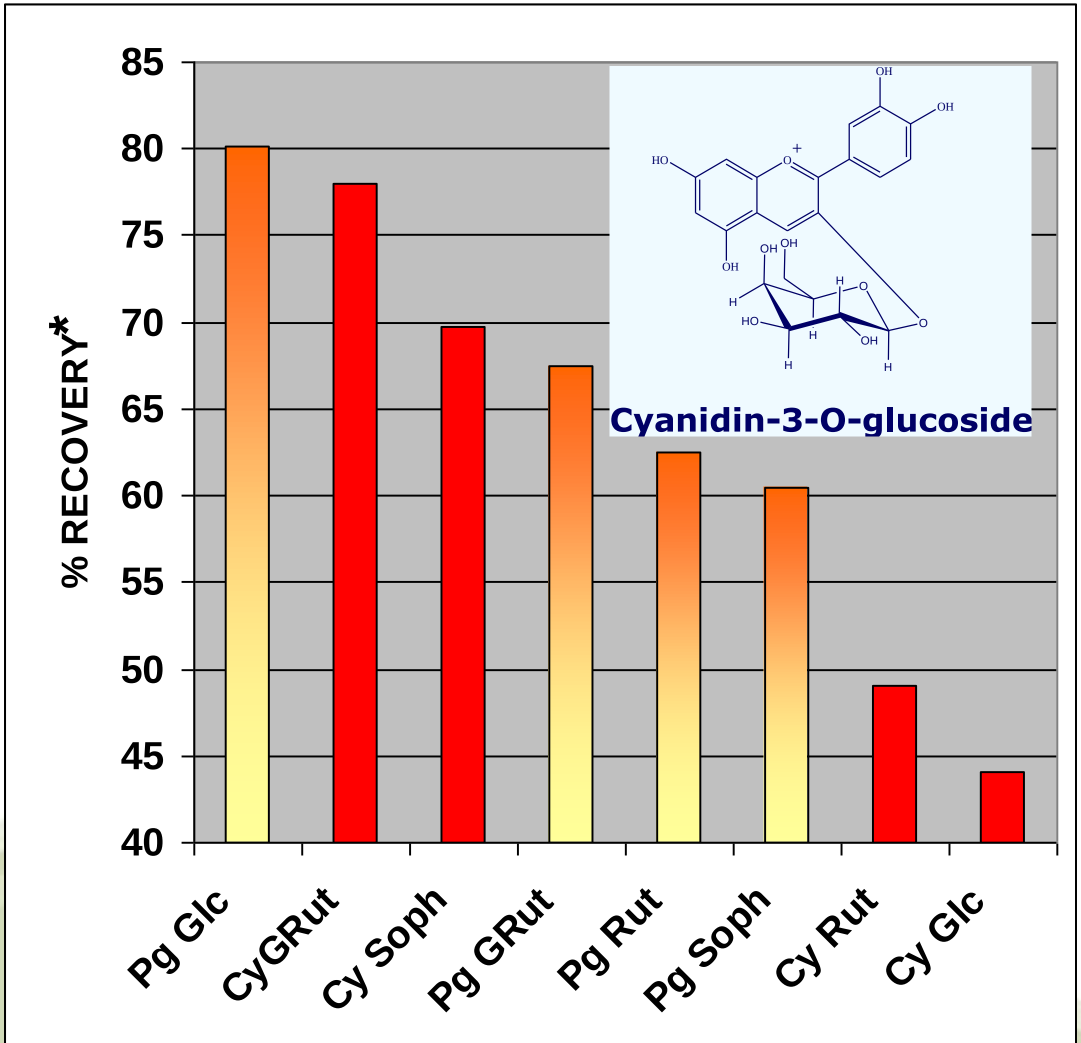
Ellagitannins bind to proteins in media

Not taken up by cells!

Breakdown to release ellagic acid

What is the active anti-cancer component?

In vitro digestion



Stability not related to aglycone or sugar moeity

For Pelargonidin

Glc >GRut >Rut >Soph

For Cyanidin

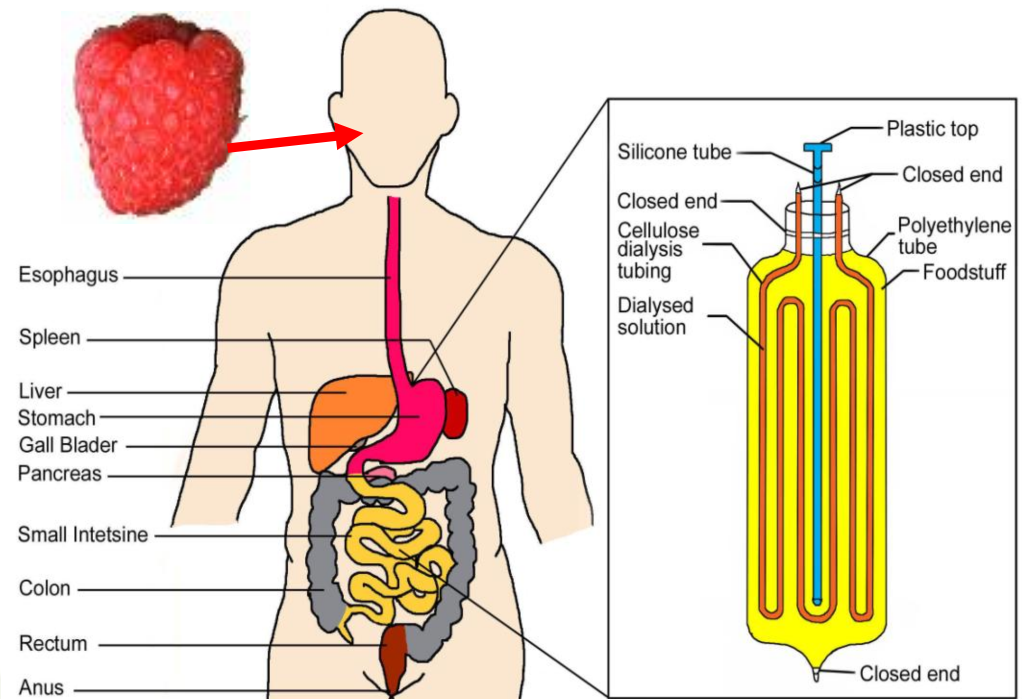
GRut >Soph >Rut >Glc

Stability dependent on components in mixture

*Total Recovery wrt gastric figures - McDougall et al. (2005) JAFC 53 5896-5904

In vitro digestion

Which components stable and bioavailable?



Scheme of the in vitro simulation of the digestion process

Simulation of human digestive system

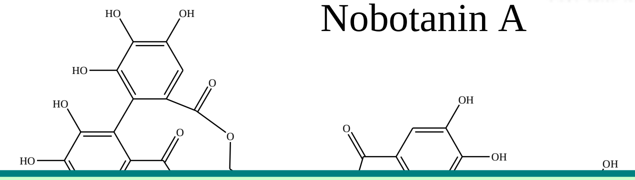
1. Gastric digestion – 2 hrs at 37°C at pH 1.7 with pepsin

2. Pancreatic digestion – 2 hrs at 37°C with digestive enzymes and bile salts

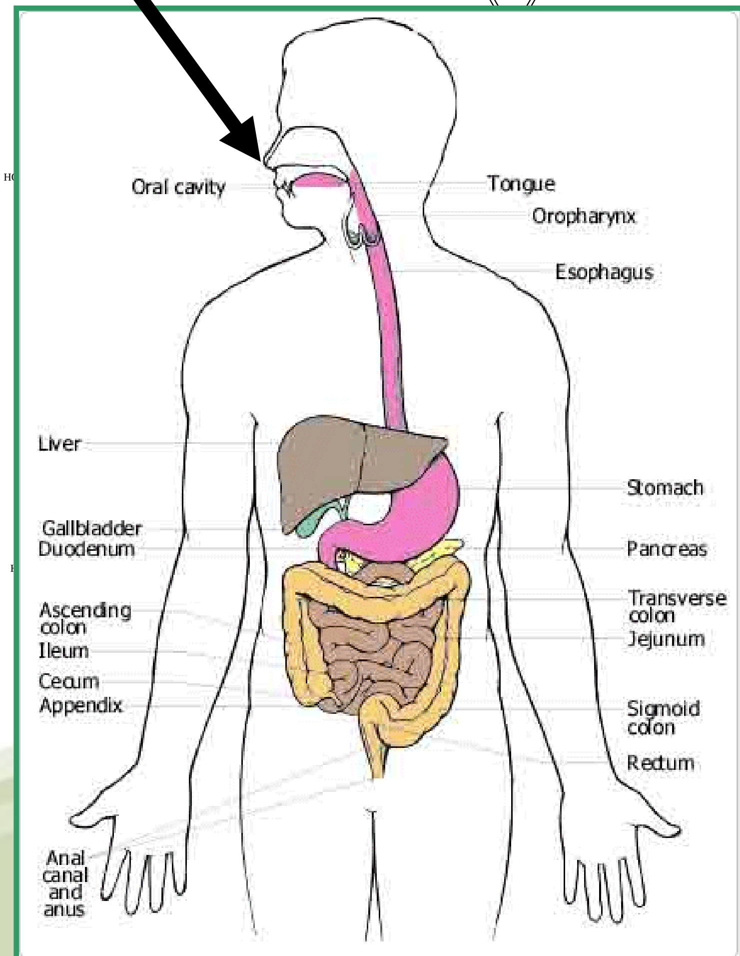
Analyse recovery of components

Ellagitannins – most effective anti-cancer polyphenols in raspberry

Sanguiin H6



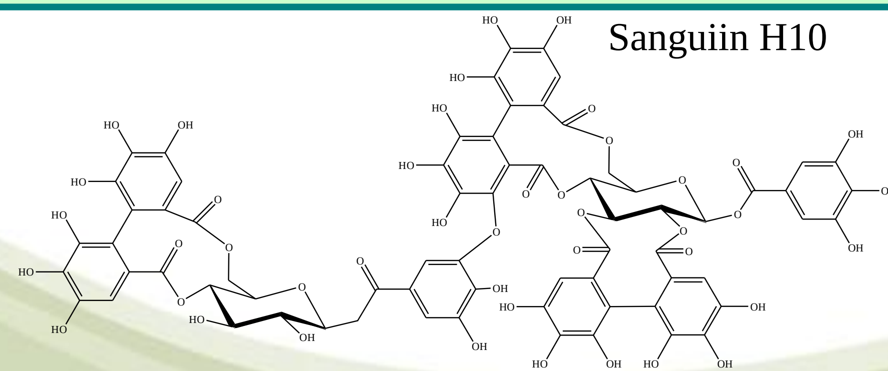
Nobotanin A



Are these components bio-available?

Do they get from berries - gut – site of action?

Sanguiin H10



Ross et al, (2007) Phytochemistry 68, 218

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