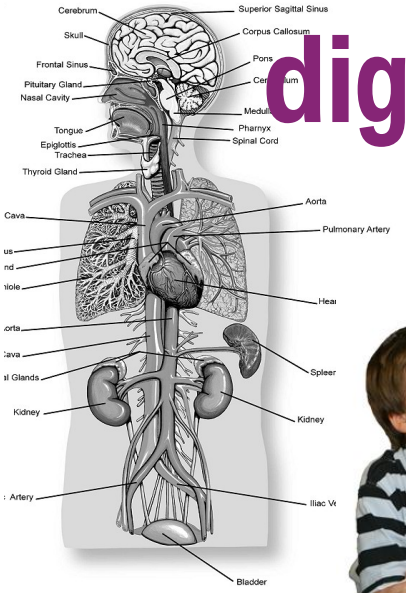



Berry components inhibit digestive enzymes: A source of health benefits?



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The James Hutton Institute

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1st International Conference on Food Digestion, Cesena, 19th March 2012

Berry research at the James Hutton

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We breed market-leading varieties

- *Blackcurrants – the "Ben" series*
- *Raspberries – the "Glen" series*
- *Strawberry and Blueberries*
- Research into Health Benefits of Berries
- Feedback to direct breeding of new varieties

Outline of talk

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Introduction

Berry polyphenols and digestive enzymes

- MODEL *IN VITRO* SYSTEMS

- Polyphenol-enriched extracts
- Inhibition of enzymes relevant to
  - **Diabetes & Obesity**

- Correlate bioactivities with polyphenol composition using LC-MS techniques

healthier scotland
SCOTTISH GOVERNMENT

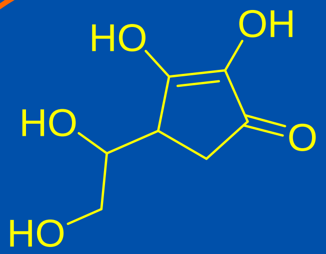
- "Insufficient intake of fruit and vegetables increases the chances of developing cancers, cardiovascular disease and strokes" - World Health Organisation (2003)
- The 3 main causes of premature death in Scotland

Led to the "5 a day" programme - Government led Mass Intervention to alter o

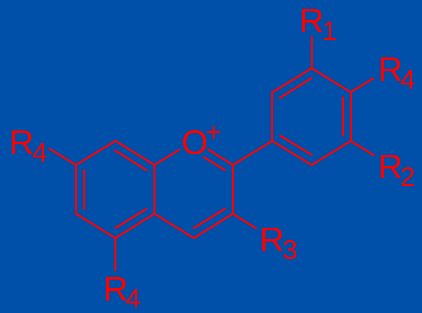
How do FAV affect health?

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

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

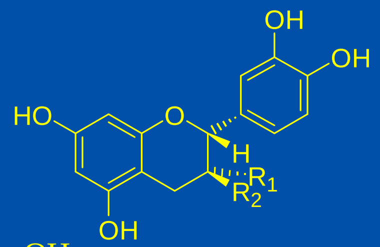


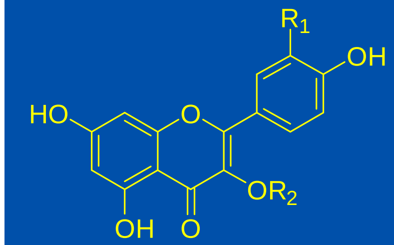
Vitamin C



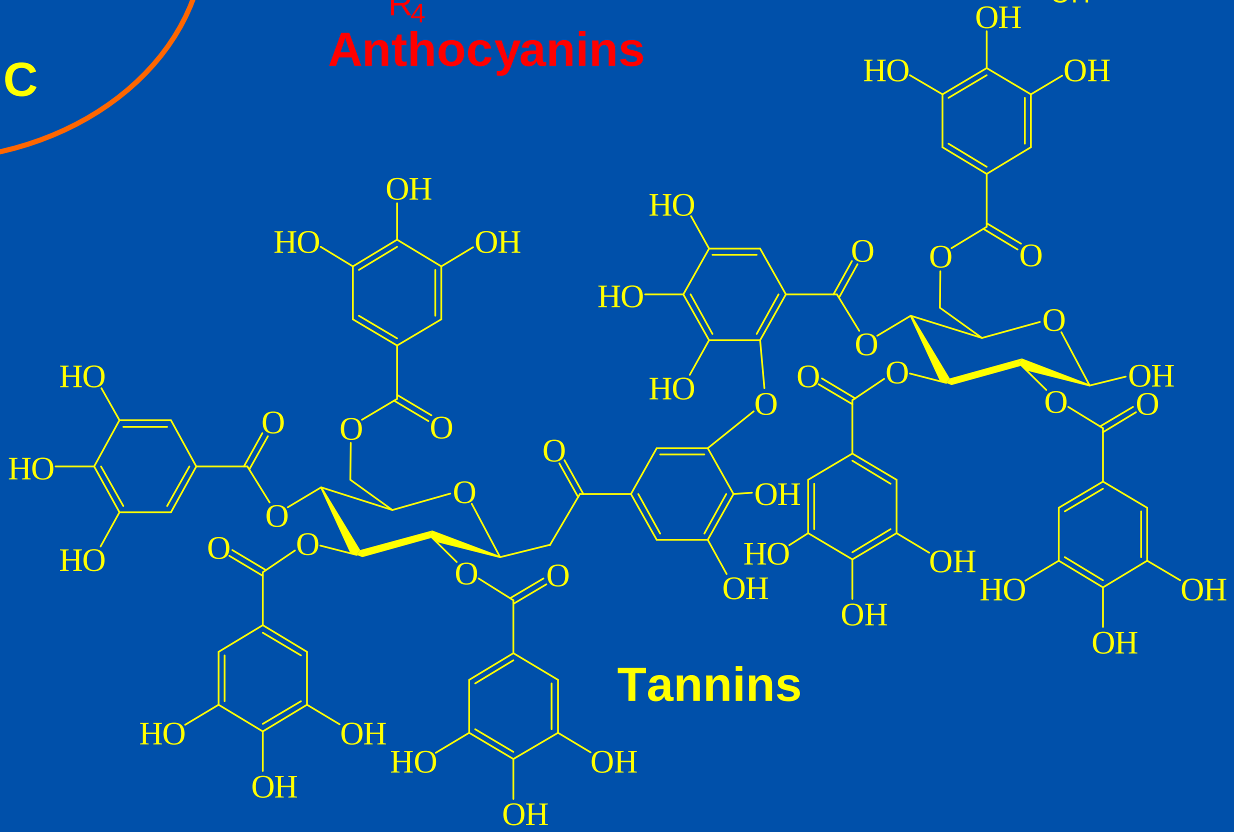
Anthocyanins

Flavanols/PACs





Flavonols



Tannins

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

Control of nutrient availability

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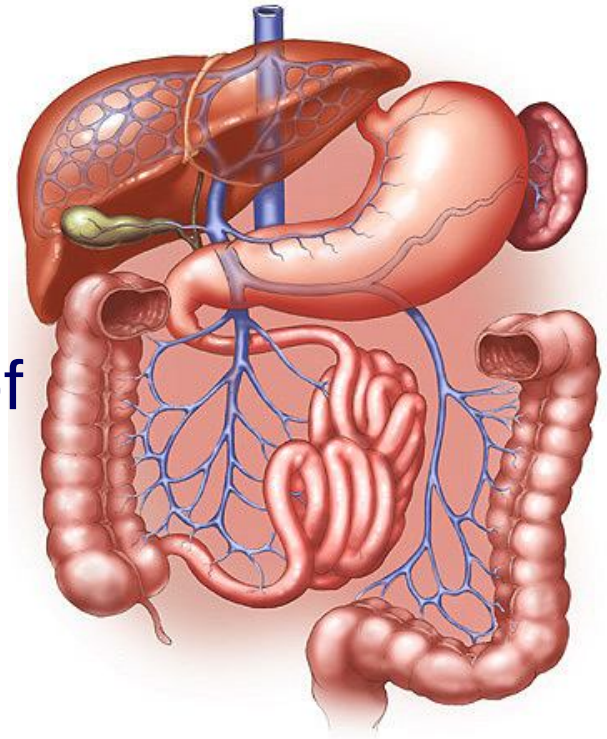
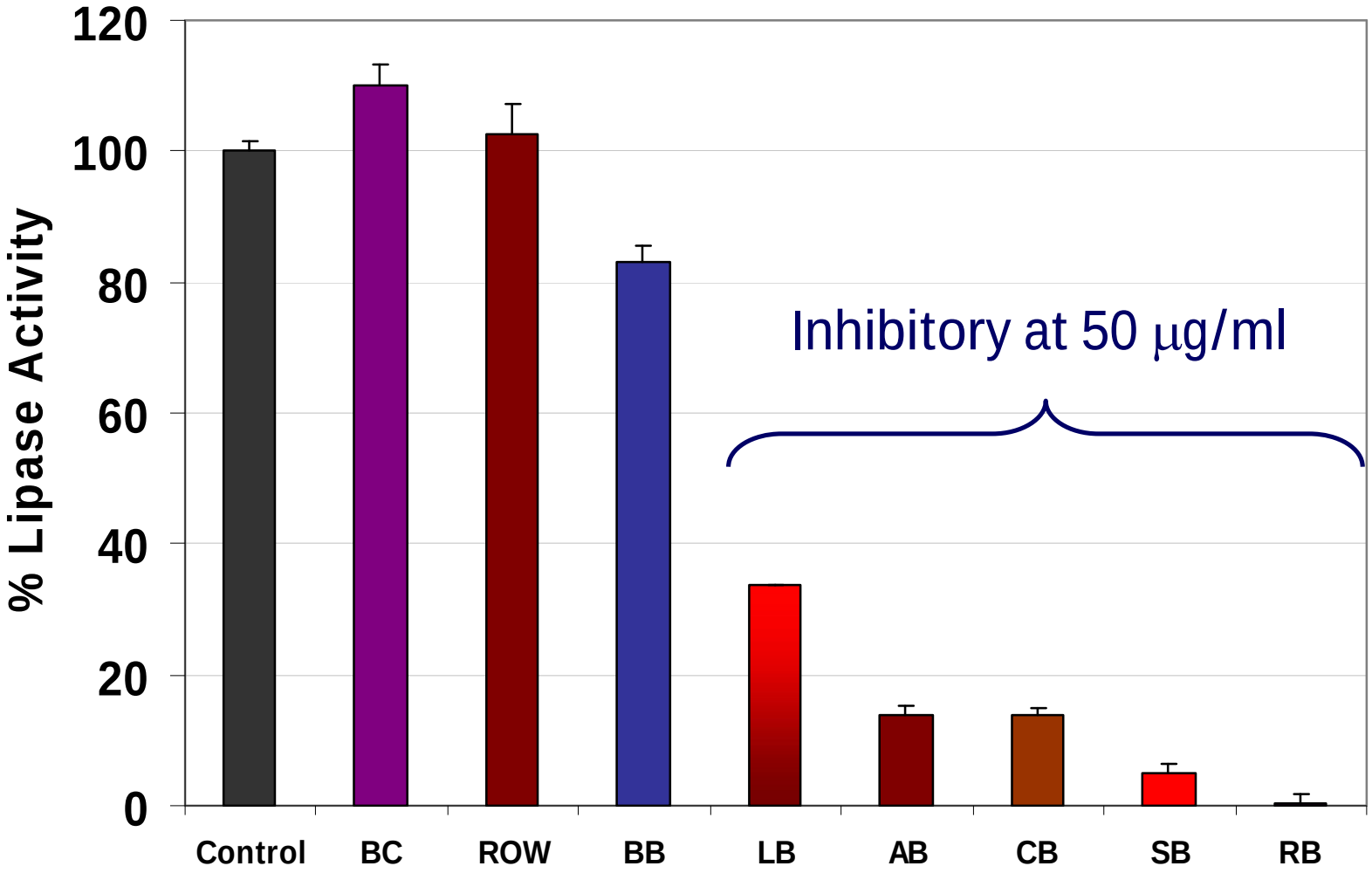
- Polyphenols can inhibit digestive processes and slow or modulate nutrient release from food



- Inhibition of lipid digestion – control of hyperlipidemia, CVD, diabetes and obesity
- Inhibition of starch digestion – blood glucose control and type 2 diabetes

Lipid digestion and lipase



Lipase inhibition

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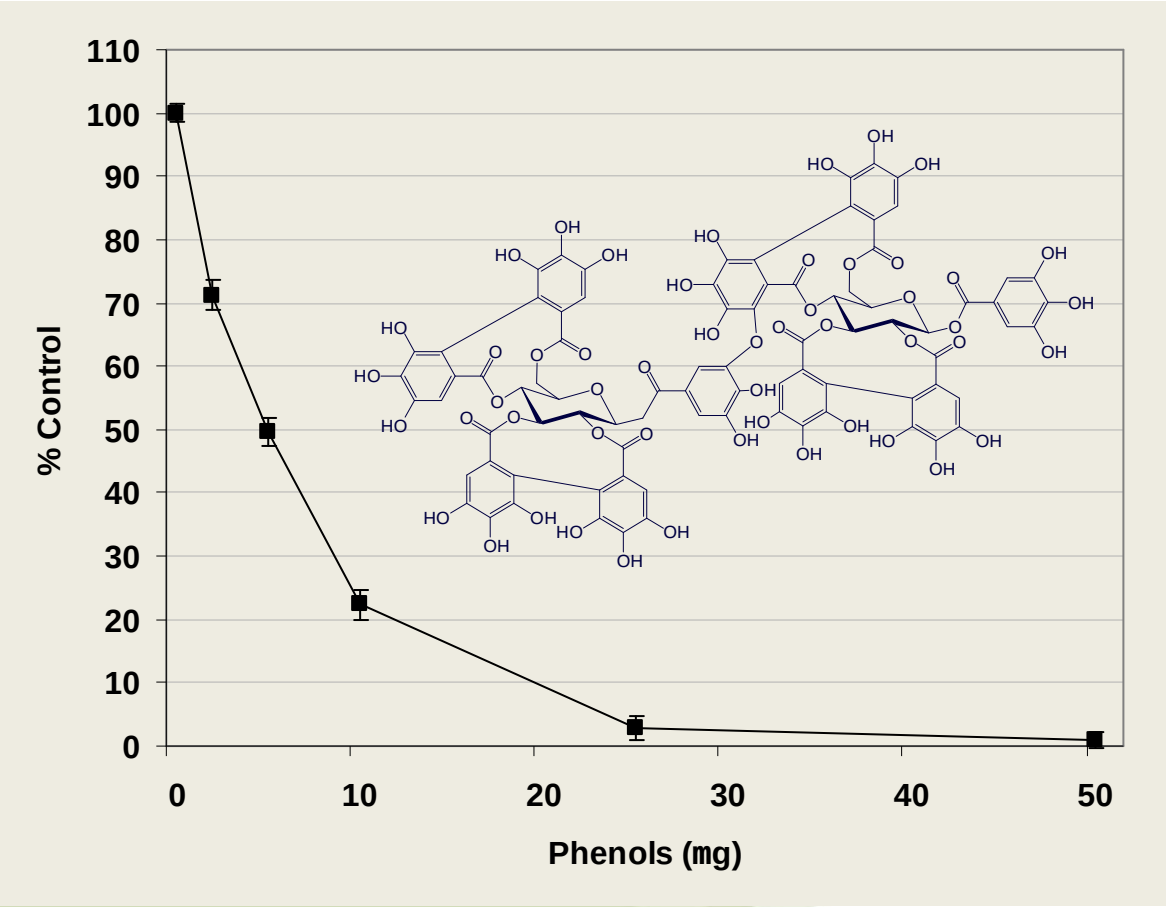


Inhibition by cloudberry extracts is saturable

Caused by ellagitannins (ETs) in cloudberry, arctic bramble and raspberry and

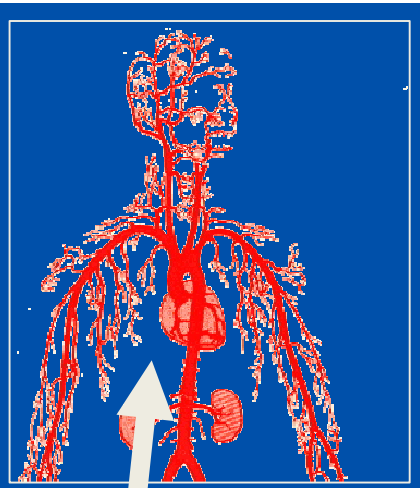
procyanidins and ETs in strawberry

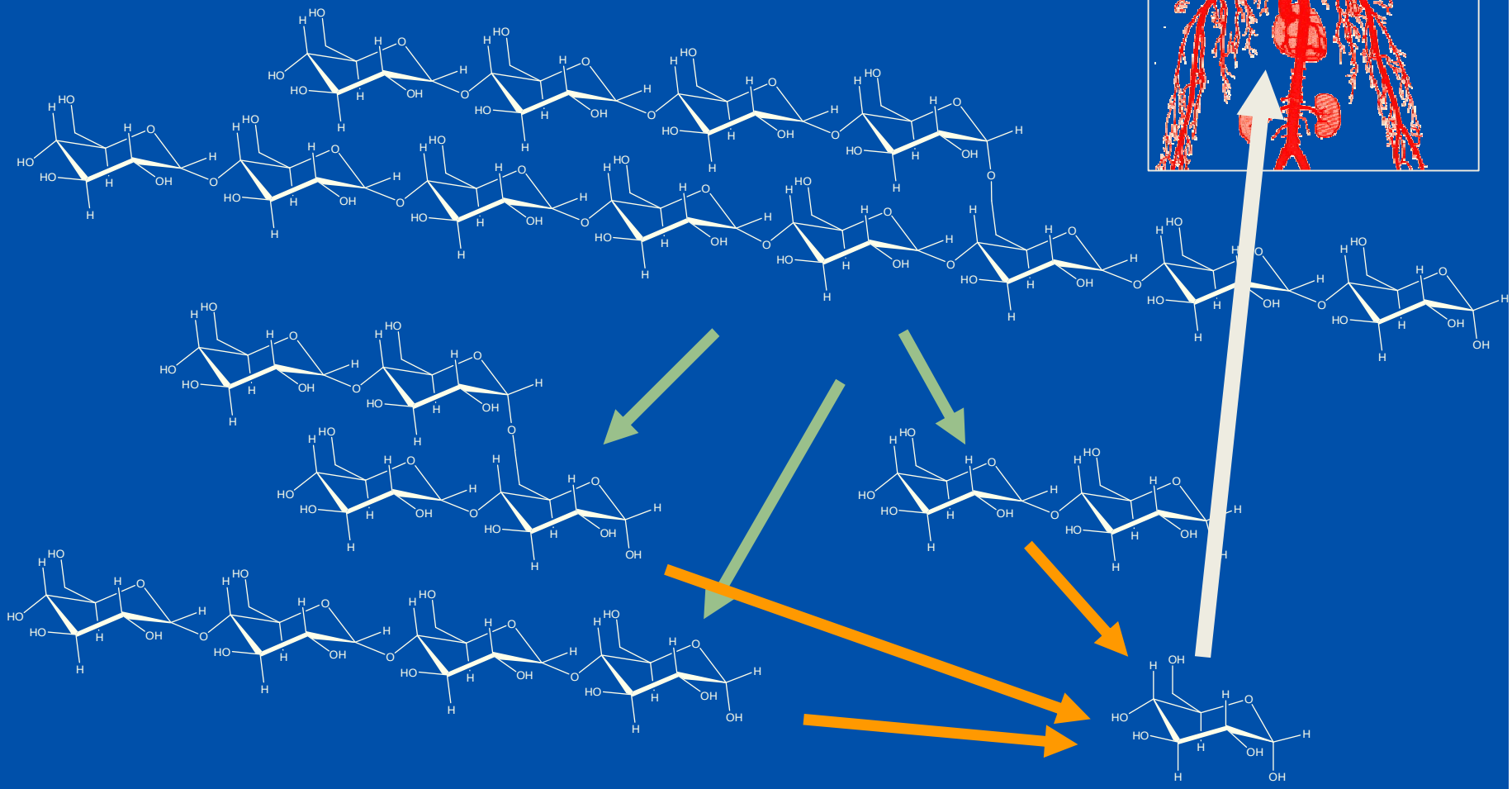
Mainly procyanidins in lingonberry

Ties in with animal studies on obesity

McDougall, Kulkarni & Stewart (2009) Food Chemistry 115, 93–199

Inhibition of starch digestion

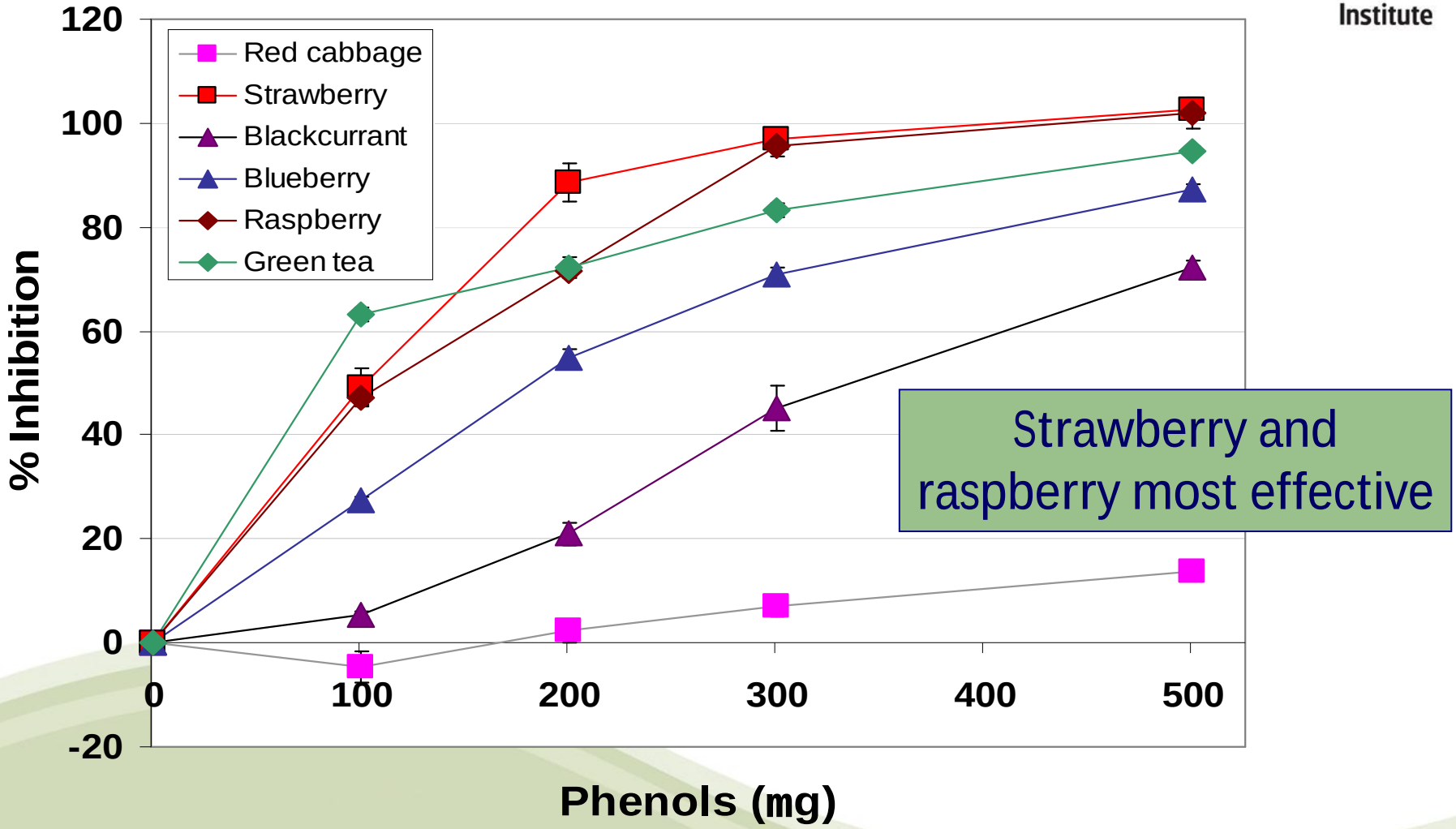




Amylase chops into fragments

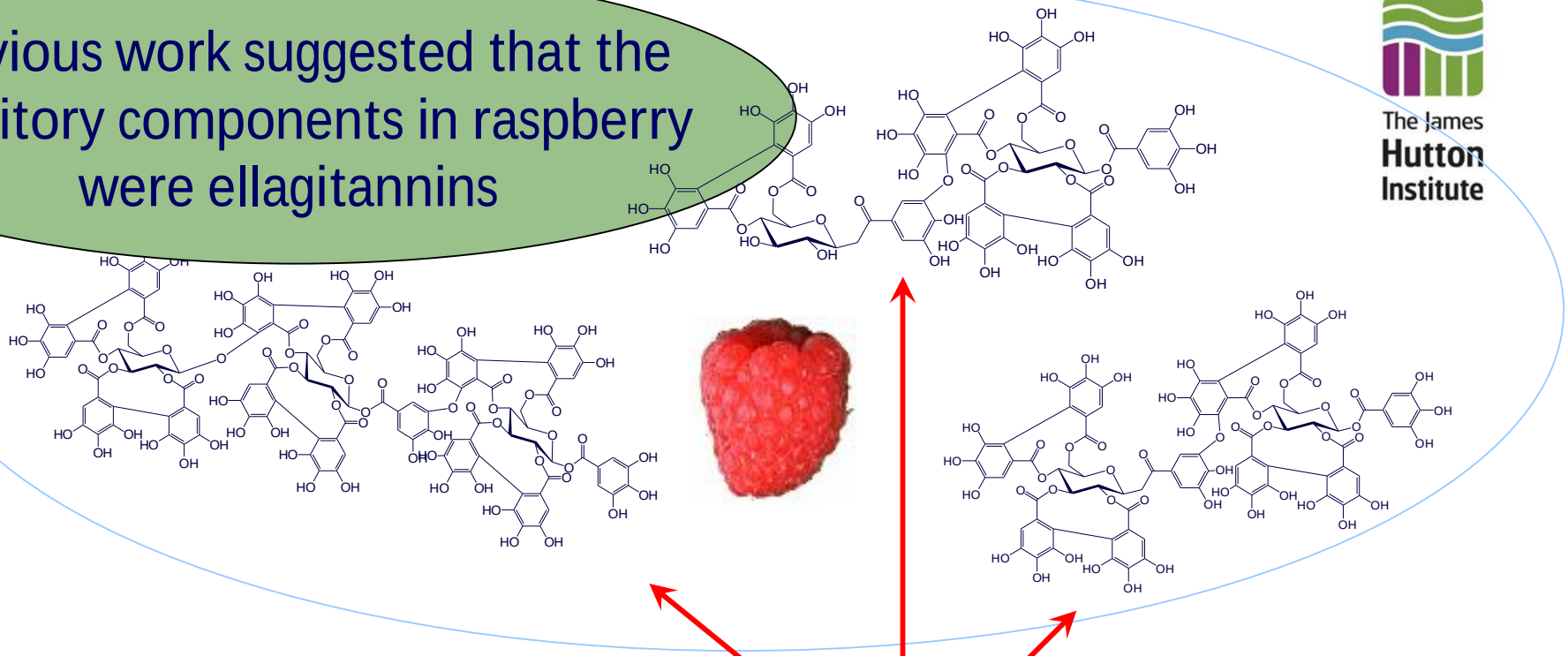
α-glucosidase nibbles off glucose

α-amylase inhibition

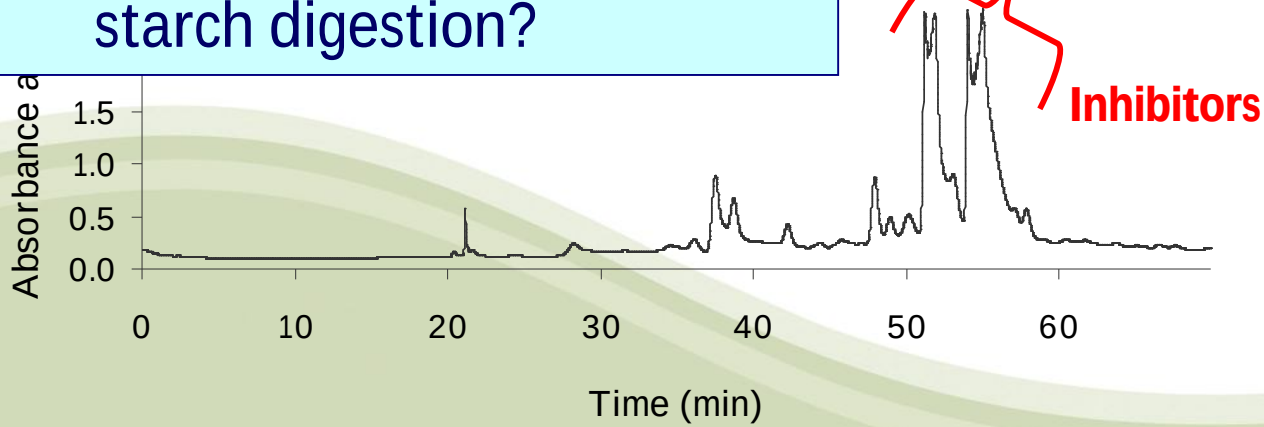


McDougall et al *(2005) JAFC 53, 2760-2766*

Previous work suggested that the inhibitory components in raspberry were ellagitannins

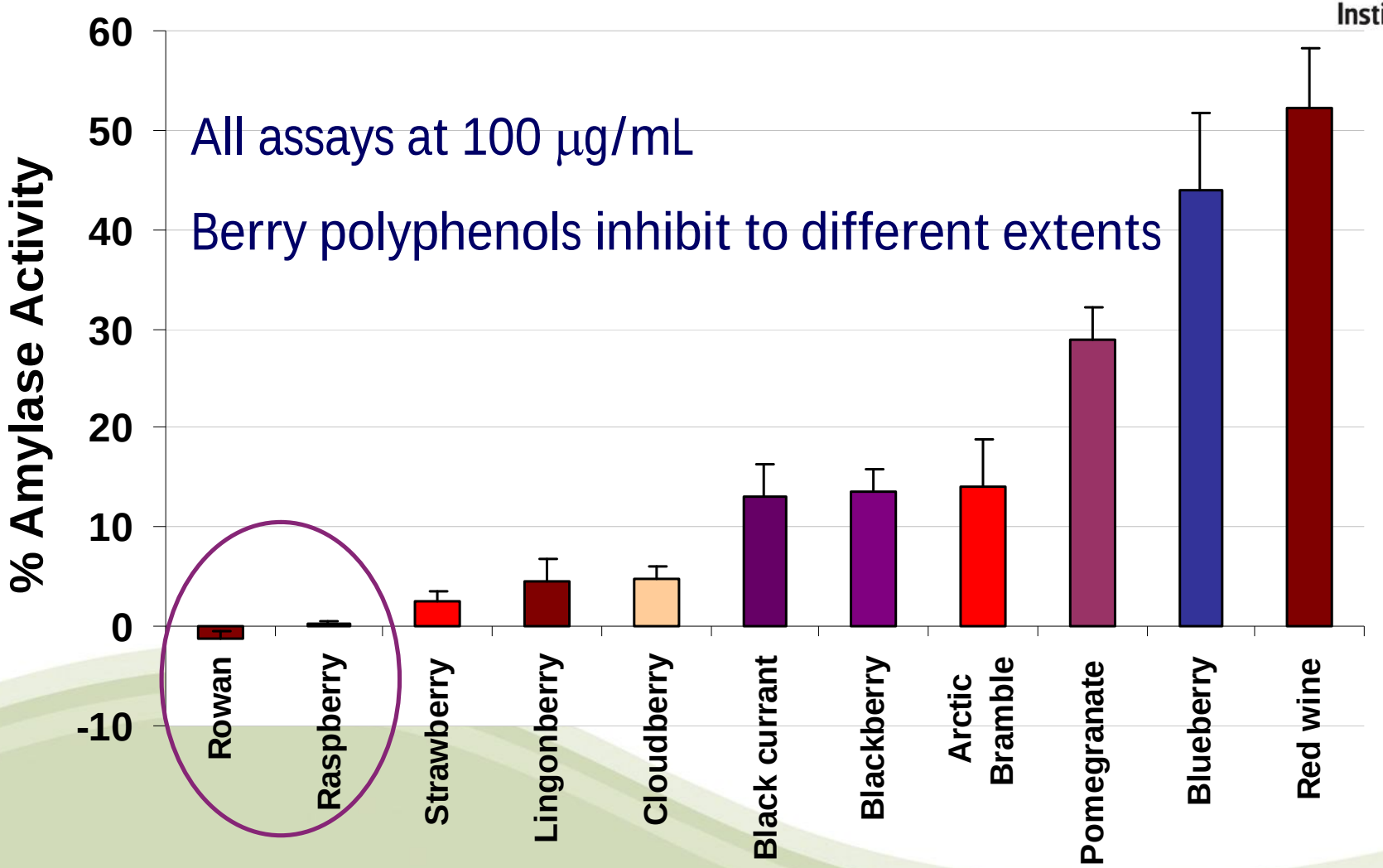


Tannins bind to amylase and prevent starch digestion?



McDougall et al *(2005) JAFC 53, 2760-2766*

α-amylase inhibition



Grussu et al (2010) JAFC 53, 2760-2766

Yellow vs. Red Raspberries

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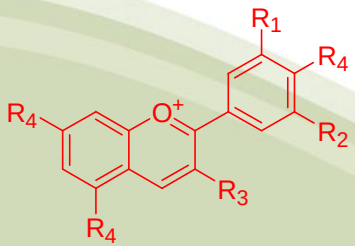
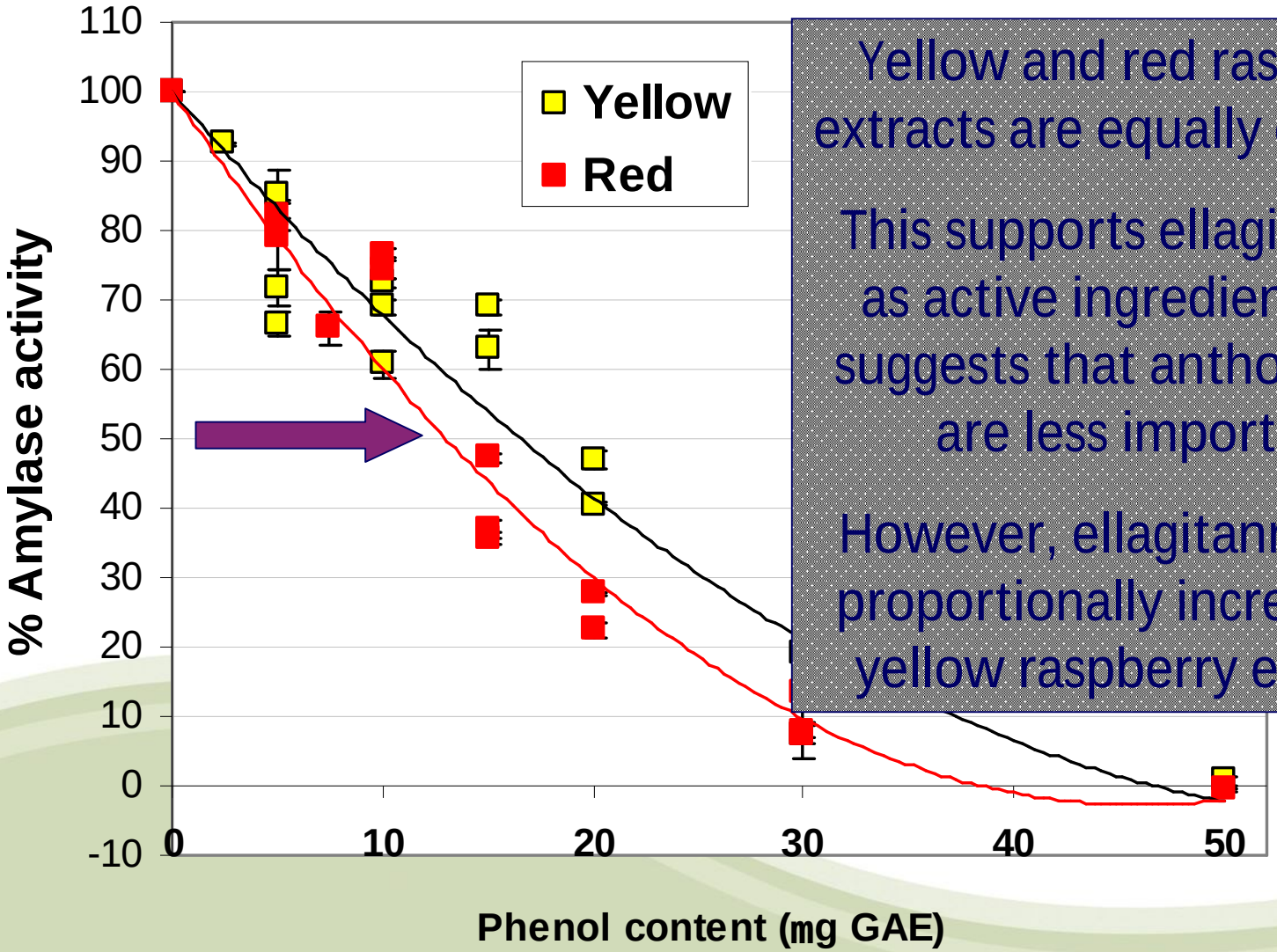


Re-examine inhibition by raspberry by comparing extracts of red raspberry (Glen Ample) with yellow raspberry (selection 97134B1)

These have similar polyphenol profiles but yellow raspberries effectively lack anthocyanins

α-amylase inhibition



Yellow and red raspberry extracts are equally effective

This supports ellagitannins as active ingredients and suggests that anthocyanins are less important

However, ellagitannins are proportionally increased in yellow raspberry extracts

Rowan fractionation & amylase inhibition

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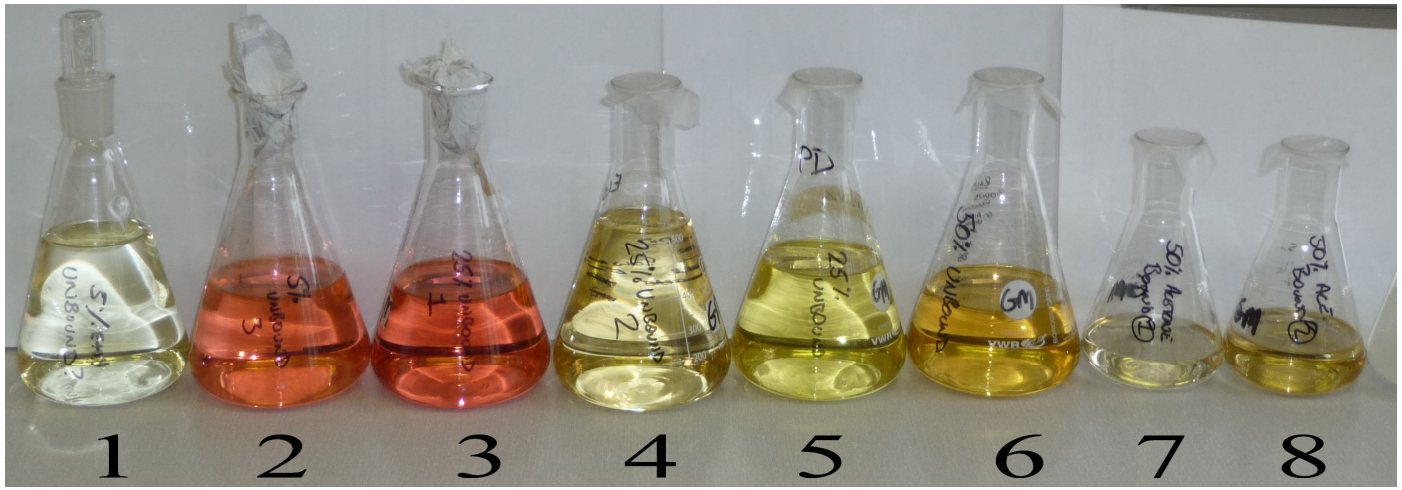
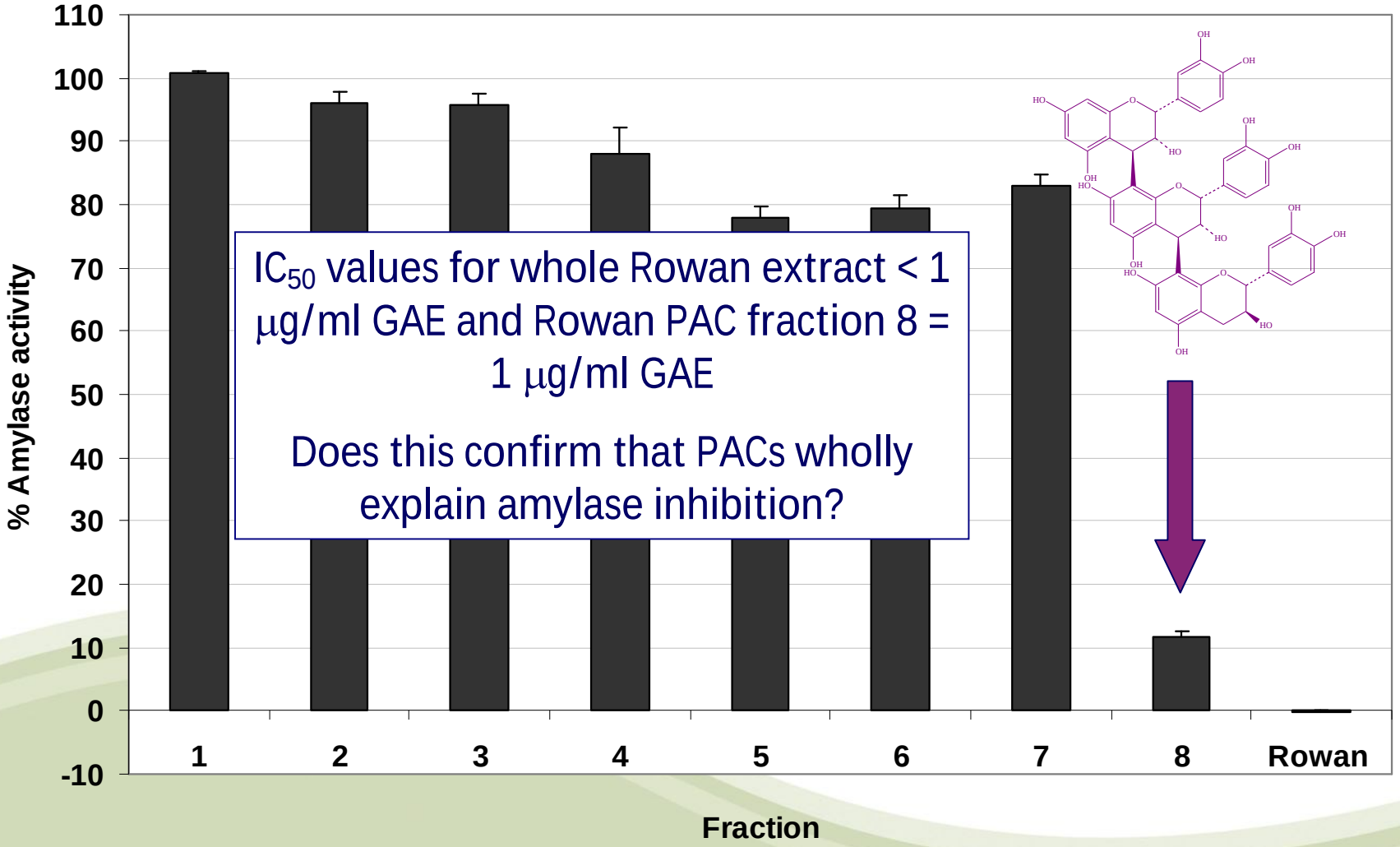
Sephadex LH-20 – step elution with decreasing polarity



1. Chlorogenic acids
2. Mainly chlorogenic acid (CGA)
3. Anthocyanins + CGA
4. Quercetin hexoses
5. Undefined Flavonols
6. Unknowns
7. Quercetin coumaroyl hexoses
8. Procyanidins

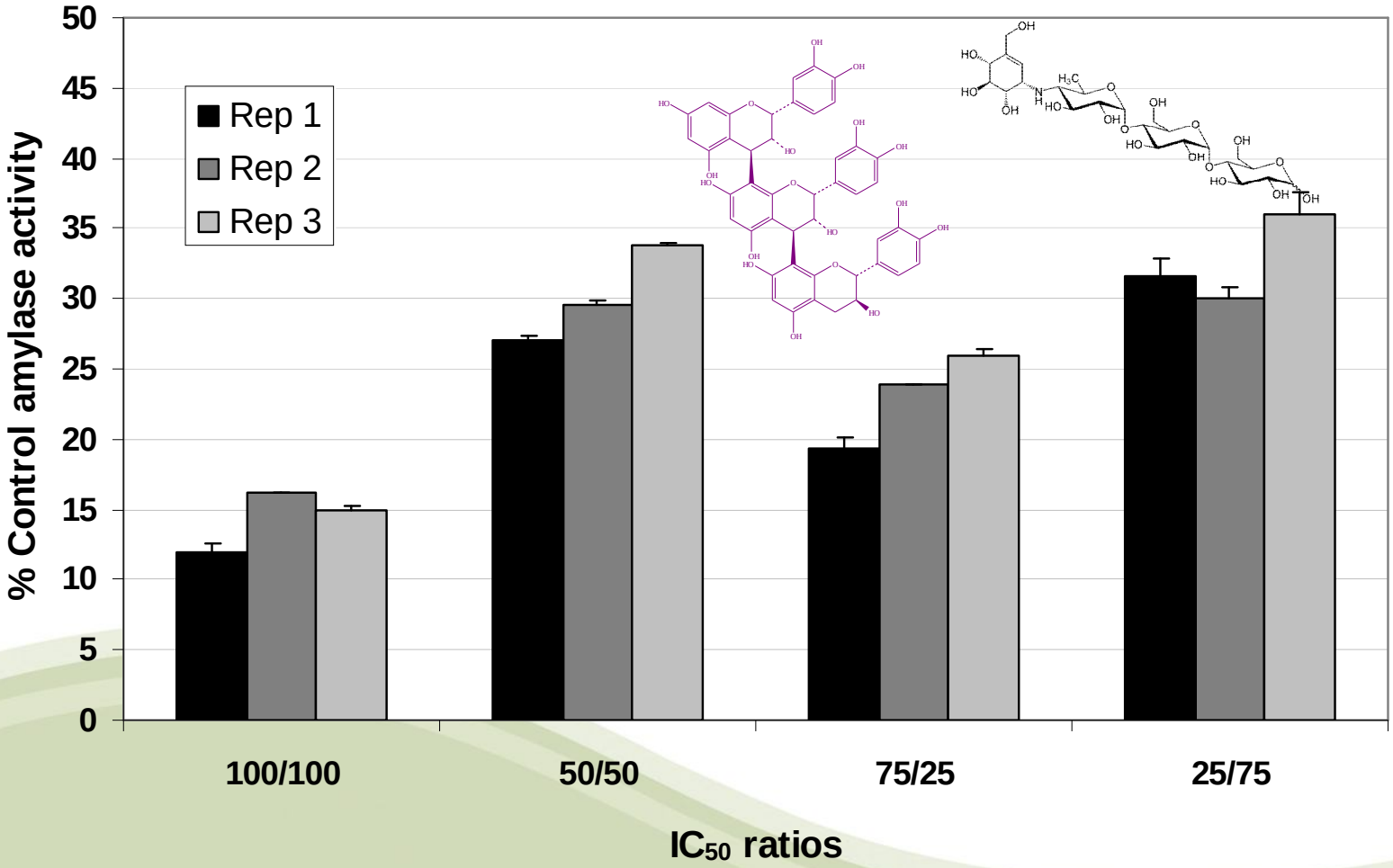
By LC-MS analysis

Inhibition by procyanidin-rich fraction



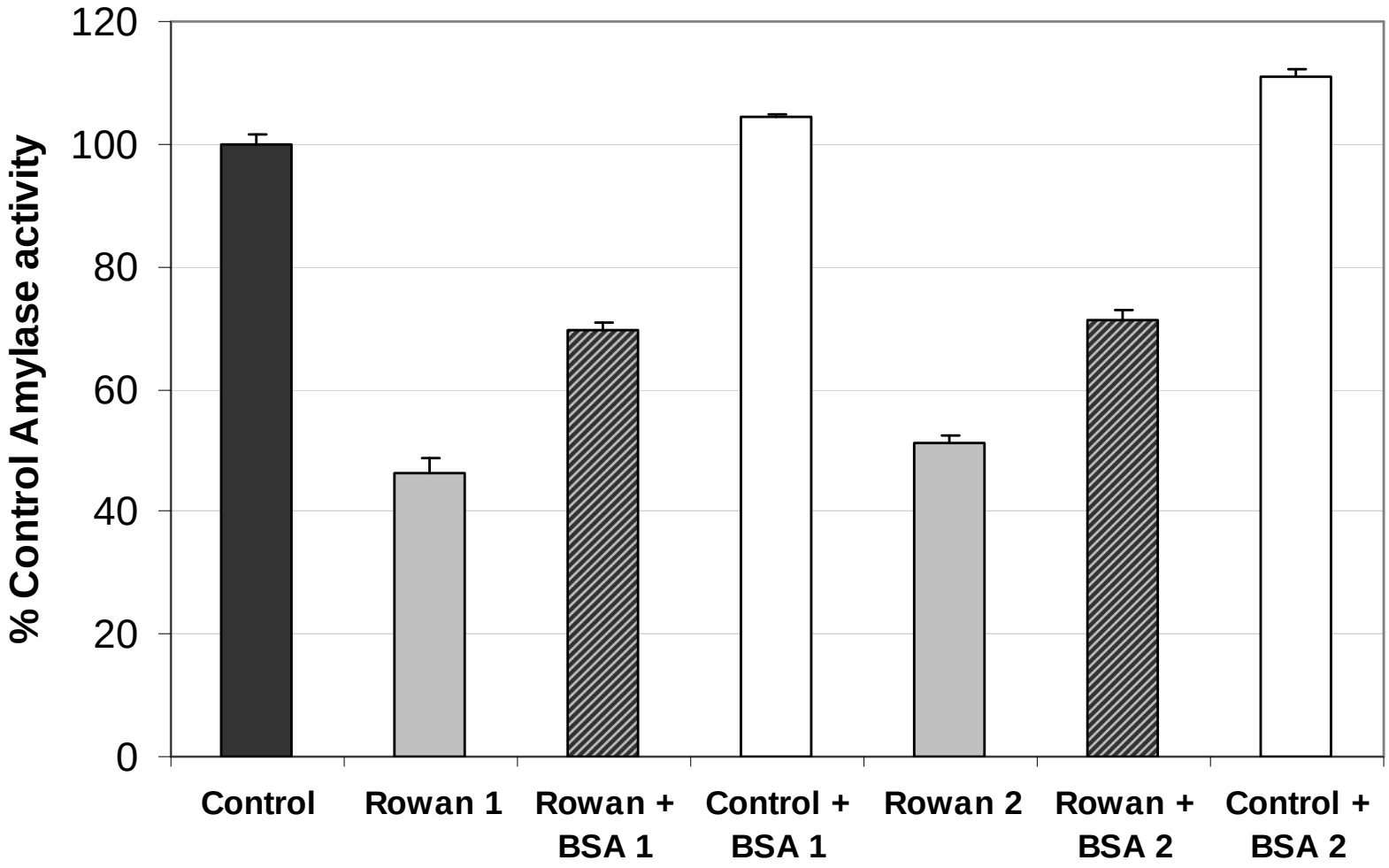
Co-incubation with acarbose

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

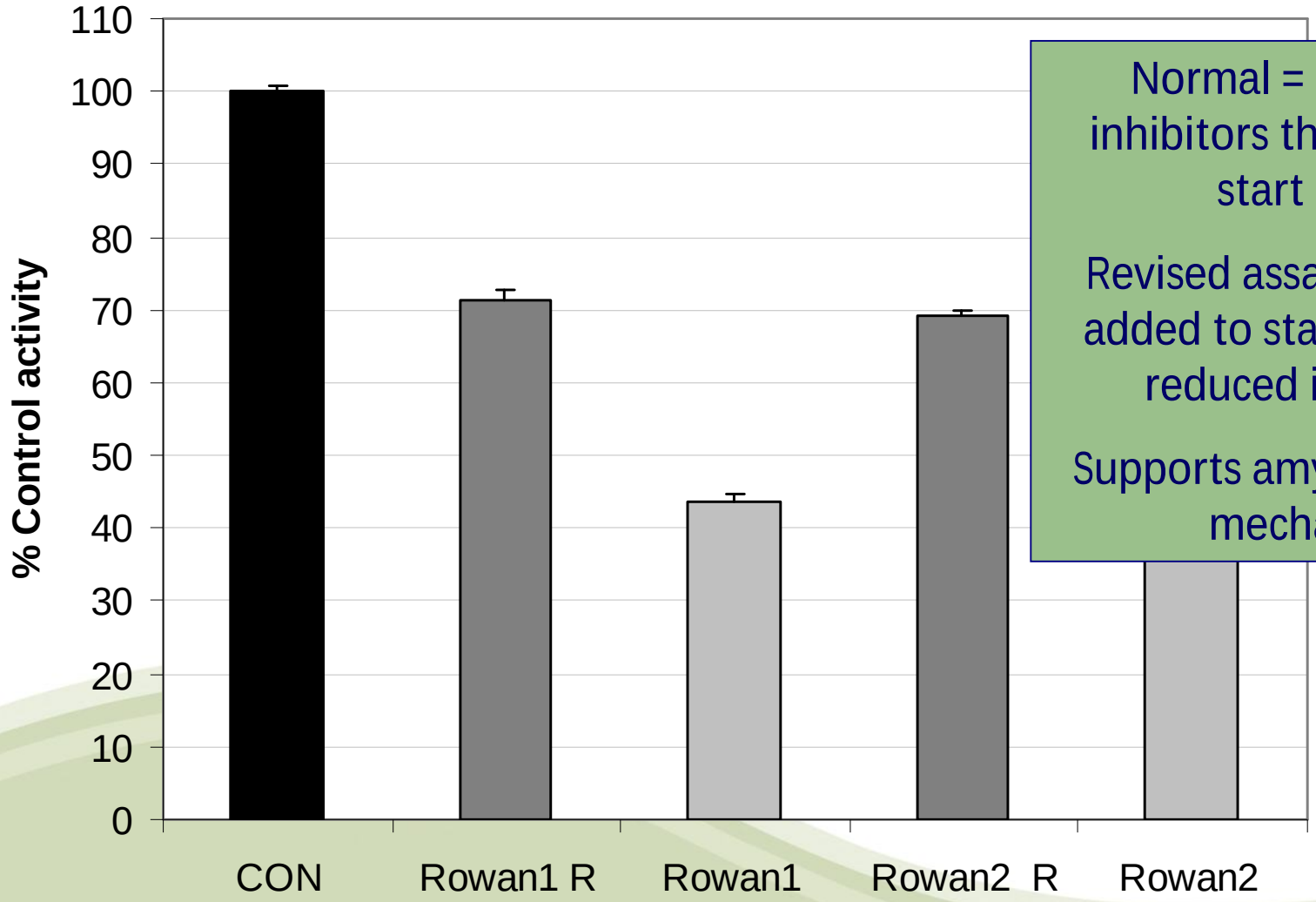


Each at IC_{50} At half IC_{50} 75/25 25/75

Addition of protein reduces inhibition



Order of addition assays



Normal = amylase + inhibitors then starch to start assay

Revised assay = Amylase added to start reaction – reduced inhibition

Supports amylase-binding mechanism

Amylase inhibition

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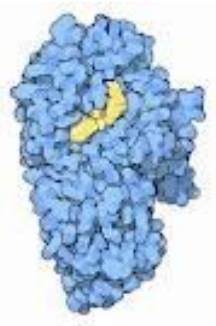
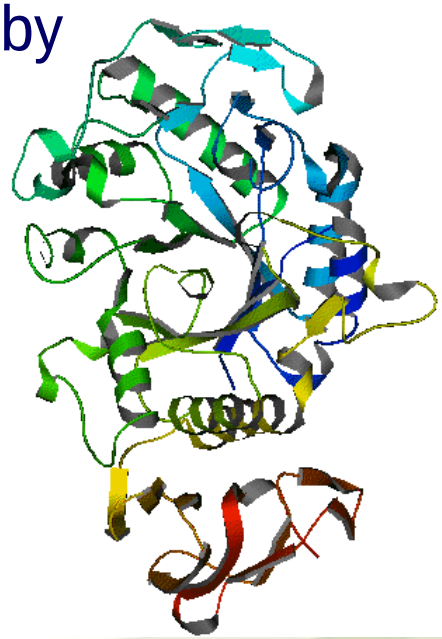
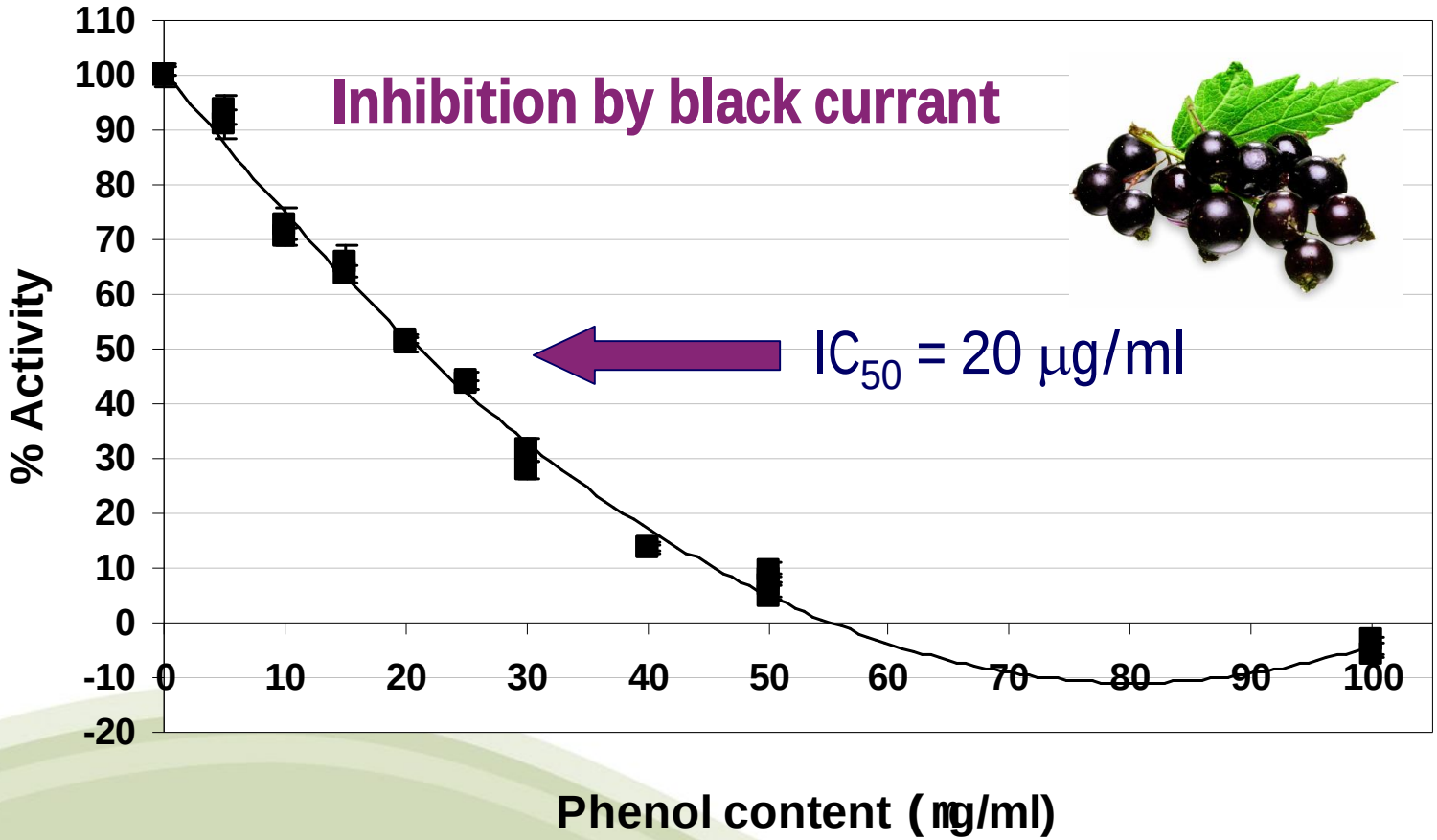
- Berry polyphenols inhibit amylase activity *in vitro* at low levels
- The degree of inhibition depends on the polyphenol composition
- Tannins seem important but inhibition influenced by other components
- Proteins interfere – astringency/enzyme binding mechanism?
- Polyphenols can potentiate inhibition caused by acarbose & could substitute for acarbose and maintain inhibition





α-glucosidase inhibition by berries



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

Inhibition by rowanberry

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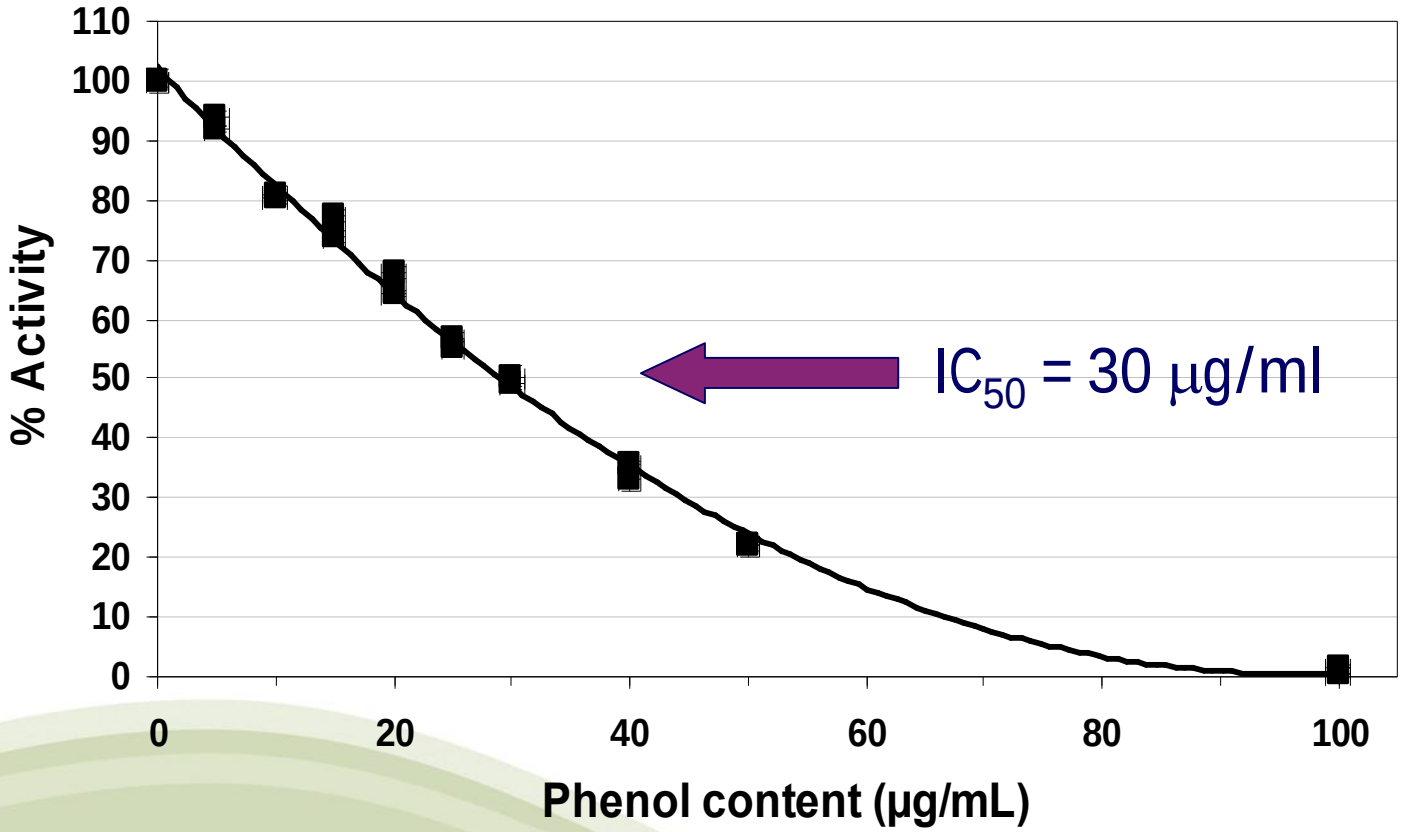
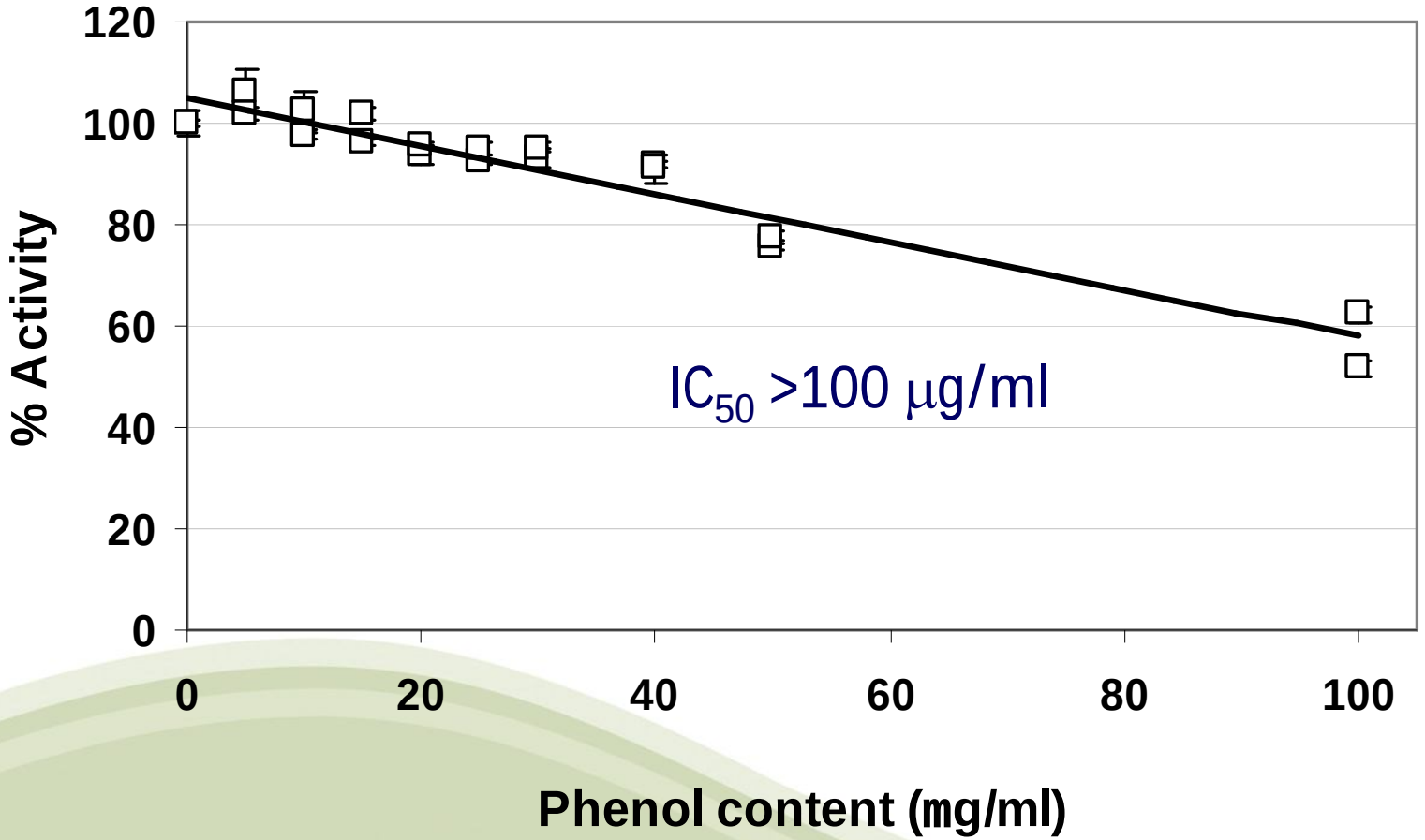
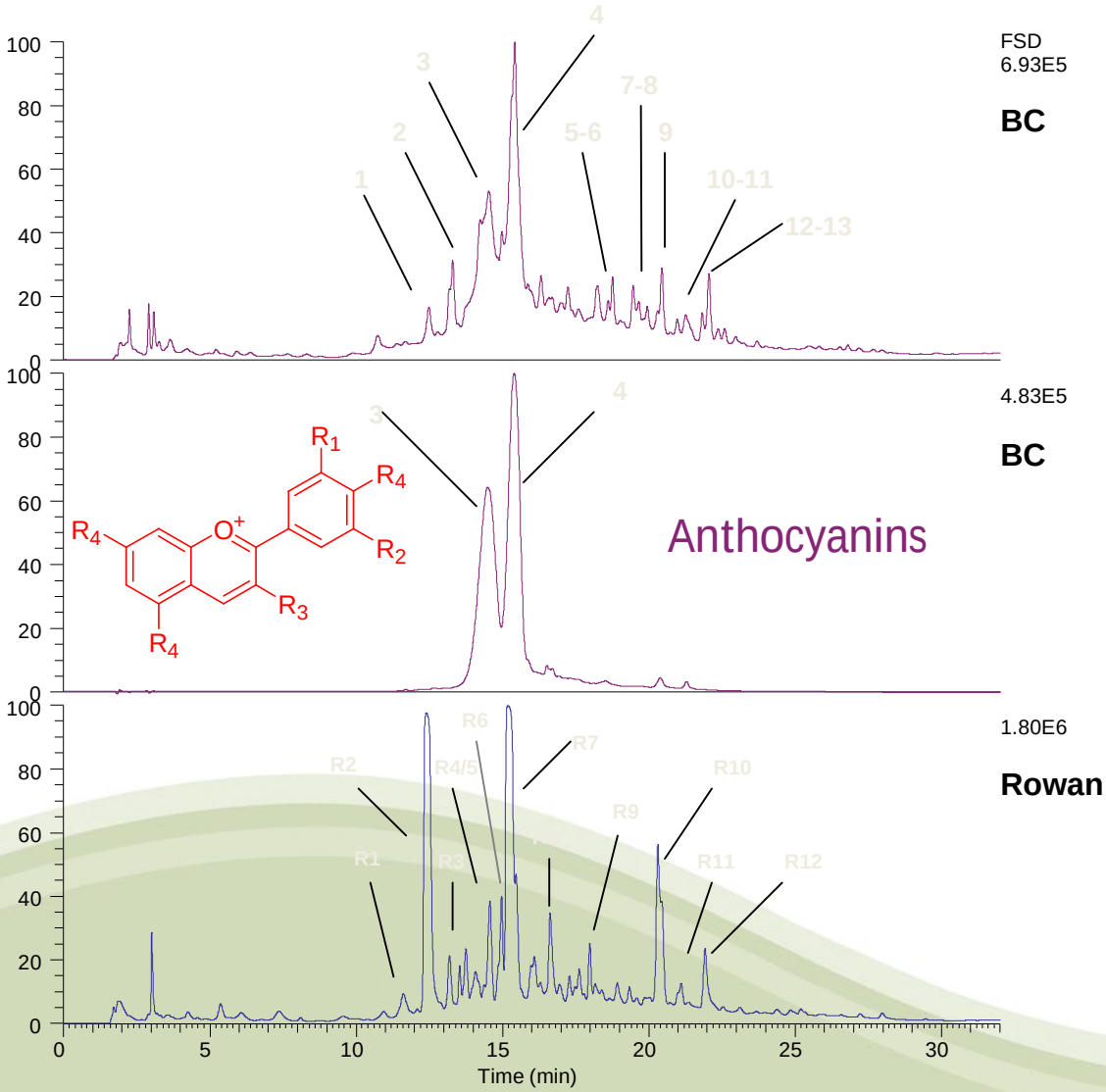


Rowanberry proanthocyanidins



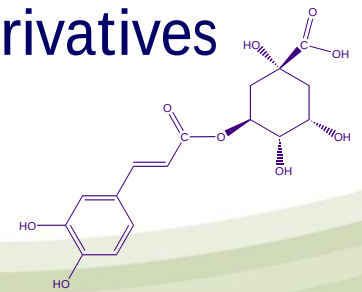
Polyphenol composition



LC-MS analysis shows that the black currant & rowanberry extracts differ greatly in their polyphenol composition

BC is rich in anthocyanins

Rowan in chlorogenic acid derivatives



Co-incubation with acarbose

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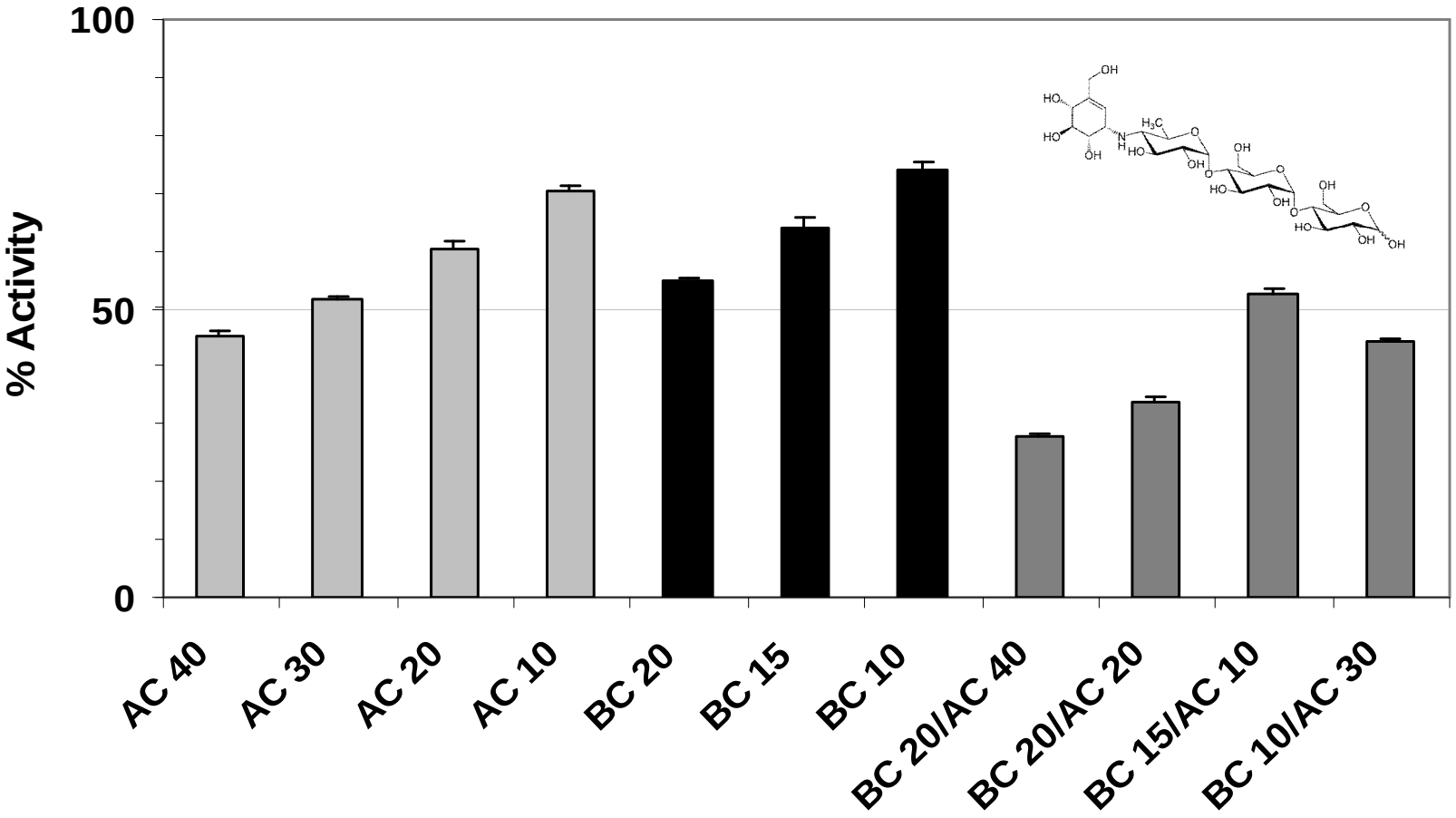

Co-incubation with acarbose

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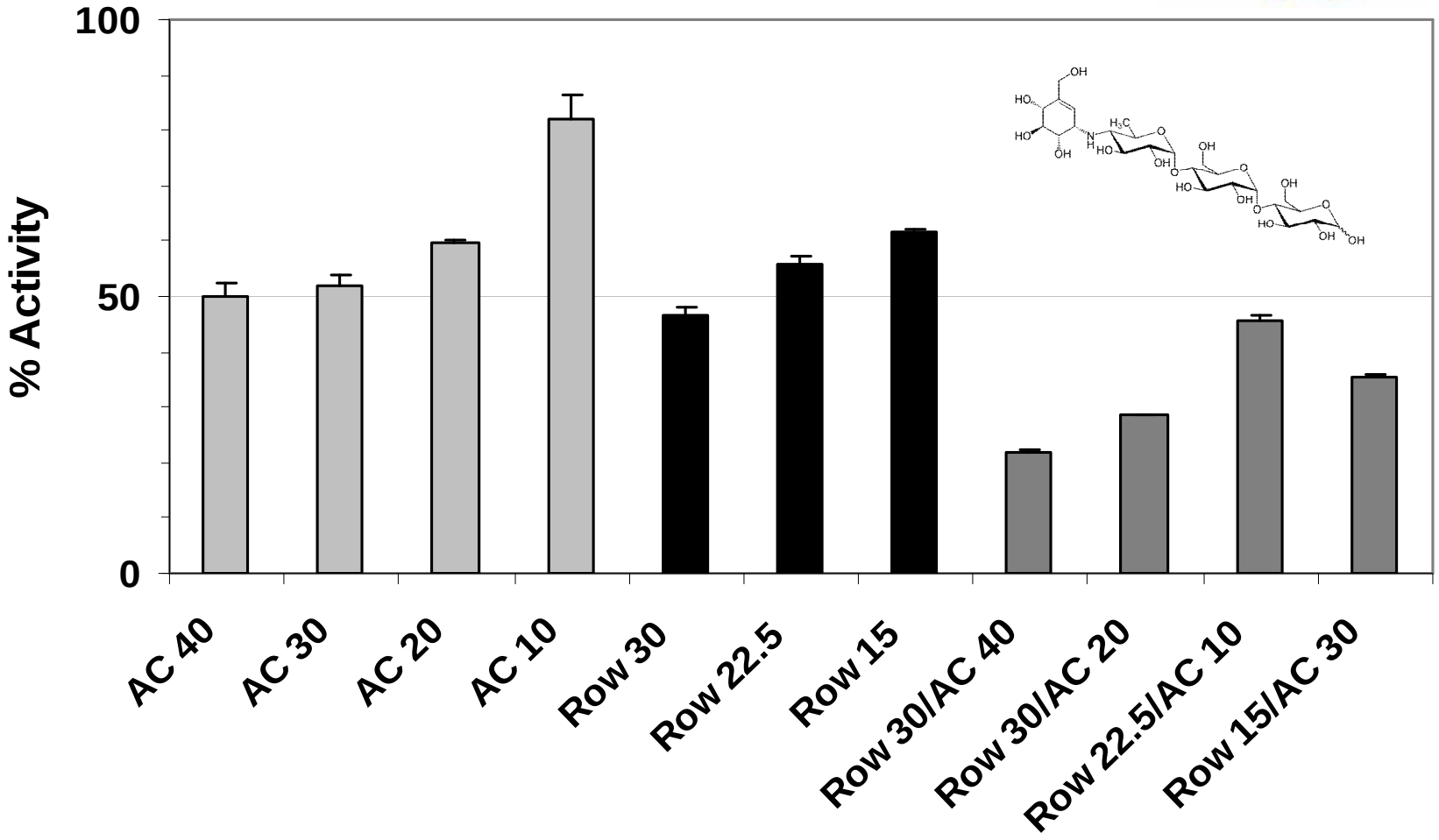

Mixing of berry extracts

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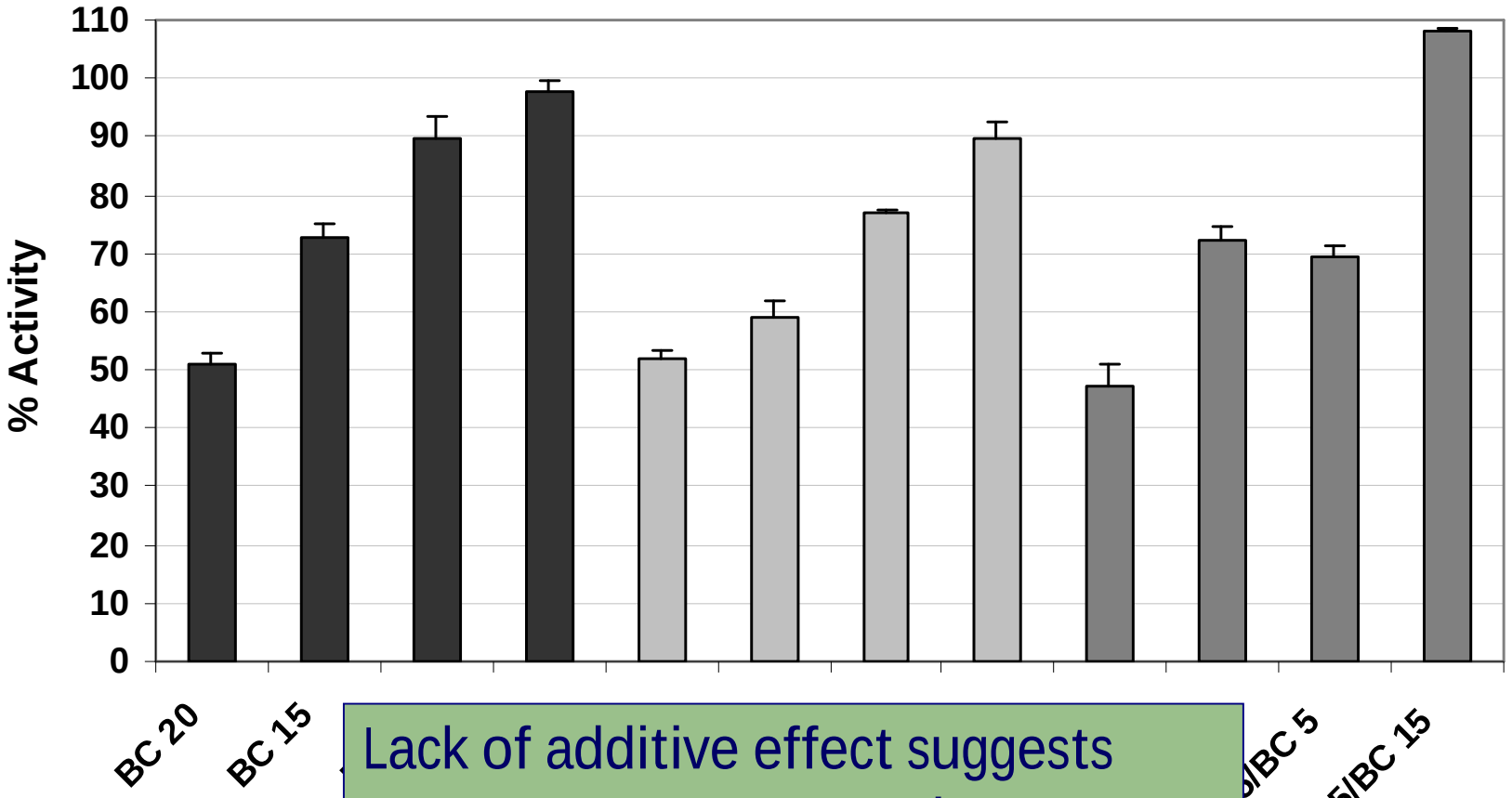

Lack of additive effect suggests components are operating at same site on enzyme?

Summary – α-glucosidase inhibition

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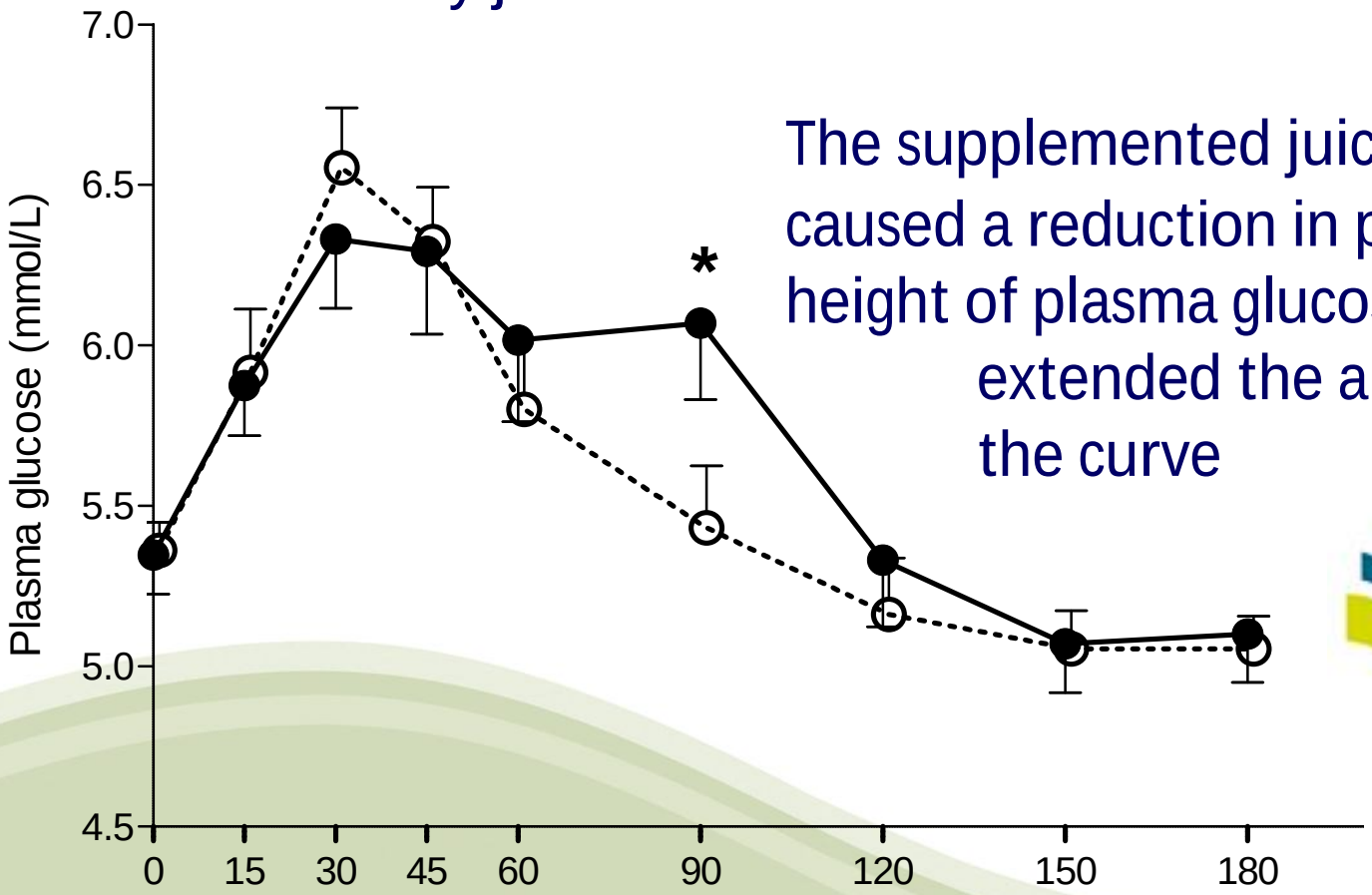
- Berry polyphenols inhibit glucosidase activity *in vitro* at low levels

- 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



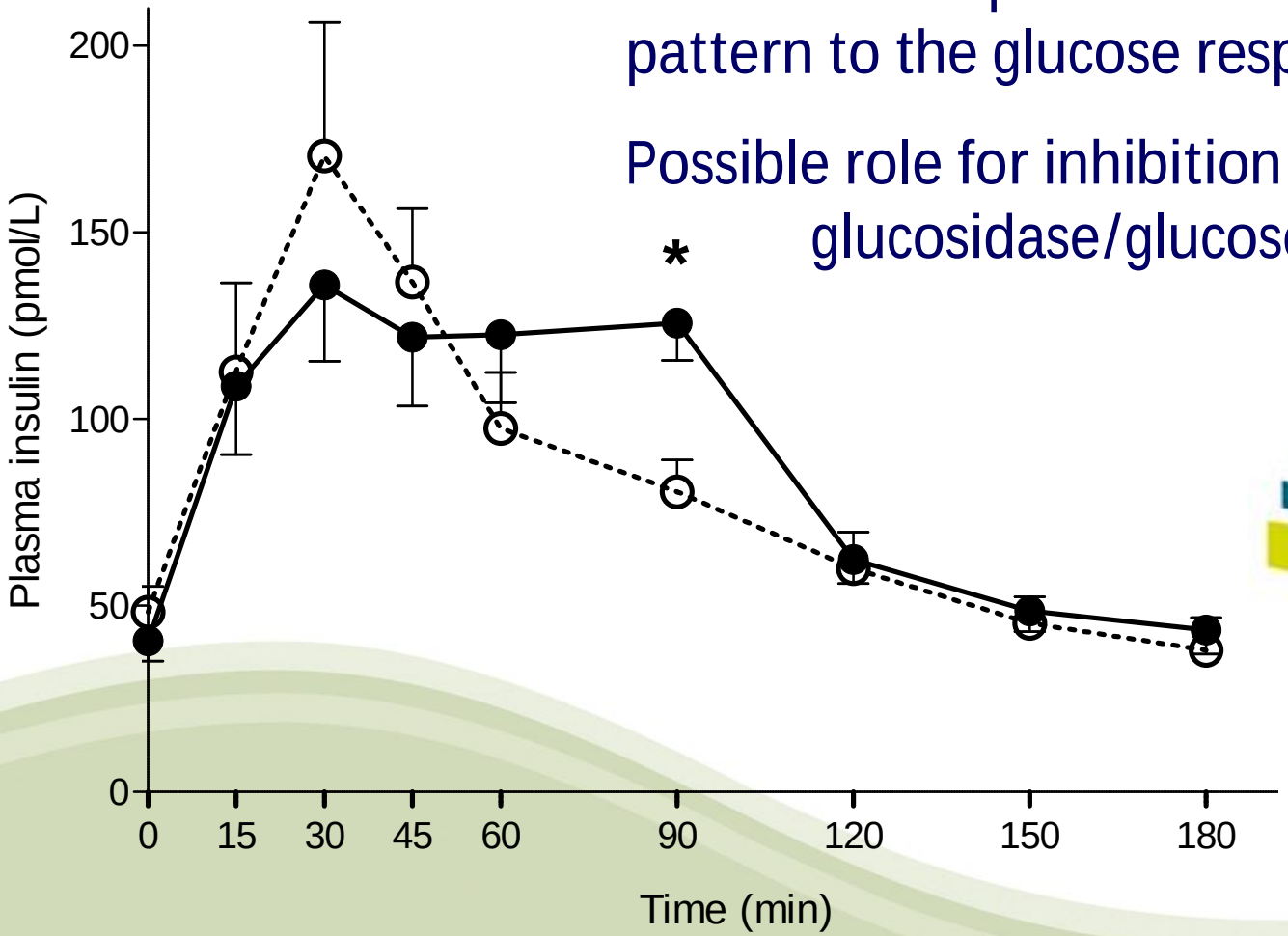
The supplemented juice (●) caused a reduction in peak height of plasma glucose and extended the area under the curve

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?



Summary

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- 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

Acknowledgements

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Thank you for your attention

The James Hutton Institute

JHI at Invergowrie on the north bank of the River Tay

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