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

Ashley Boath, Dominic Grussu, Derek Stewart & Gordon J. McDougall

The James Hutton Institute

gordon.mcdougall@hutton.ac.uk

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Berry research at the James Hutton

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

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

The 3 main causes of premature death in Scotland led to the “5 a day” programme - Government-led Mass Intervention to alter our diet and improve health

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**
How can polyphenols affect human health?

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

- 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 Activity

Control  BC  ROW  BB  LB  AB  CB  SB  RB

Inhibitory at 50 µg/ml

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Lipase inhibition

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

Inhibition of starch digestion

Amylase chops into fragments
\( \alpha \)-glucosidase nibbles off glucose
α-amylase inhibition

Strawberry and raspberry most effective

Phenols (g)

% Inhibition

Previous work suggested that the inhibitory components in raspberry were ellagitannins.

Tannins bind to amylase and prevent starch digestion?


These assays were conducted at a concentration of 100 μg/mL.Berry polyphenols inhibit to different extents.
Yellow vs. Red Raspberries

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.
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

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

IC$_{50}$ values for whole Rowan extract $< 1 \mu$g/ml GAE and Rowan PAC fraction 8 = 1 \mu g/ml GAE

Does this confirm that PACs wholly explain amylase inhibition?
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

% Control Amylase activity

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<tr>
<th></th>
<th>Control</th>
<th>Rowan 1</th>
<th>Rowan + BSA 1</th>
<th>Control + BSA 1</th>
<th>Rowan 2</th>
<th>Rowan + BSA 2</th>
<th>Control + BSA 2</th>
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</thead>
<tbody>
<tr>
<td>% Control Amylase activity</td>
<td>100</td>
<td>40</td>
<td>60</td>
<td>100</td>
<td>50</td>
<td>70</td>
<td>110</td>
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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

- 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

Inhibition by black currant

IC$_{50}$ = 20 μg/ml

Inhibition by rowanberry

IC$_{50}$ = 30 µg/ml
Rowanberry proanthocyanidins

IC_{50} > 100 \mu g/ml
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

Rowanberry/Acarbose (g/ml)
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?
Summary

- Berry polyphenols inhibit enzymes involved in starch and lipid digestion \textit{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
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JHI at Invergowrie on the north bank of the River Tay

Questions?

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