Development of a new plum variety rich in bioactive compounds

M Netzel\textsuperscript{1,2}, K Fanning\textsuperscript{2}, G Netzel\textsuperscript{1}, A Houlihan\textsuperscript{2}, D Zabaras\textsuperscript{1}, D Russell\textsuperscript{2}, R Stanley\textsuperscript{3}

\textsuperscript{1}CSIRO Food and Nutritional Sciences, Coopers Plains\textsuperscript{1} & North Ryde\textsuperscript{3}
\textsuperscript{2}Agri-Science Queensland, Department of Employment, Economic Development and Innovation (DEEDI)
\textsuperscript{3}Queensland Alliance for Agriculture and Food Innovation (QAAFI), University of Queensland
Fruits and Vegetables high in Bioactive Compounds

- Much interest in recent years in developing fruit and vegetable varieties with increased levels of bioactive compounds/phytochemicals (e.g., anthocyanins, carotenoids)

- Reason:
  - To increase potential health benefits
  - As a source for food and nutraceutical ingredients

- **Queen Garnet plum**, a new variety of the Japanese plum *Prunus salicina* Lindl.

- Developed as a **high anthocyanin, high antioxidant** plum; Queensland Government breeding program
Outline

- Queen Garnet plum - “The Plum Story”¹
- Polyphenols/Anthocyanins
- Pilot Study with Human Subjects (Bioavailability & Metabolism)²
- Conclusions and Future Avenues

¹current Queen Garnet plum research is funded by Horticultural Australia Limited (HAL)

²Netzel et al. (2011) J Food Biochem, in press
“The Plum Story”

**Queen Garnet plum**

- Cross made in 1997, seedling planted in 1998
- Selected in 2001
- Grower trials
- Sensory, bio-chemical and processing research
- First commercialized 2007 for fresh market
- Second release 2009
- Awarded to Nutrafruit 2010
“The Plum Story”

Commercialization

- Commercialization won by Nutrafruit Pty Ltd
- Broad acre 1 or 2 variety farming, 80,000 trees by 2012
  - 2 sites - Deniliquin and Inglewood
  - Fruit grown for juice concentrate or anthocyanin products
- Nutrafruit testing new selections to spread season
- Nutrafruit licensing to US, South Africa, Spain and NZ
“The Plum Story”

9000 trees planted at Inglewood, July 2010
“The Plum Story”

Trees growing – November 2010
Queen Garnet plum (QGP) vs. commercial plum varieties (anthocyanins & antioxidant capacity)

- A suitable ‘test-fruit’ for absorption/bioavailability studies (anthocyanins/polyphenols/antioxidants)

*p < 0.05
• Plant polyphenols are significant antioxidant compounds in our diet (intake ~1g/d)
• Green/black tea, coffee, red wine and berries are important dietary sources of polyphenols
• Anthocyanins (major polyphenol subclass) are responsible for red, purple and blue colours in many fruits and vegetables

![cyanidin 3-glucoside](image)

cyanidin 3-glucoside  
→ most abundant anthocyanin in fruits

• Consumption of polyphenol-rich foods is associated with a decreased risk of chronic diseases (eg CVD)
• Significant metabolism of orally ingested polyphenols

• Polyphenol metabolites can provide valuable information re:
  • intake of dietary polyphenols (types & amounts)
  • biological relevance in disease prevention (mode of action)

→ But polyphenol bioavailability & metabolism is still not fully understood
Pilot Study: Queen Garnet plum (QGP) juice as an anthocyanin/polyphenol-rich test beverage to get initial data re the bioavailability and metabolism of QGP anthocyanins/polyphenols in healthy humans.

Focus on urinary excretion of:
- Anthocyanin metabolites with intact flavylium skeleton (e.g., glucuronides)
- Hippuric acid: considered as a polyphenol degradation product / metabolite
Subjects

<table>
<thead>
<tr>
<th>Gender</th>
<th>n = 2</th>
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<tbody>
<tr>
<td>age (years)</td>
<td>range: 26-41</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>24.1 ± 2.1</td>
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Healthy and non-smoker

Administered doses of anthocyanins and total polyphenols

| Anthocyanins (mmol/400ml)
<table>
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<tbody>
<tr>
<td>Cyanidin-3-glucoside</td>
</tr>
<tr>
<td>Cyanidin-3-rutinoside</td>
</tr>
<tr>
<td>Total anthocyanins</td>
</tr>
</tbody>
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| Total polyphenols (mmol GAE/400 ml)
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<tbody>
<tr>
<td>15.6</td>
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</table>

\(^1\) by HPLC and calculated as cyanidin-3-glucoside equivalents; \(^2\) by Folin-Ciocalteu assay and calculated as gallic acid equivalents (GAE).

Ingestion of QGP juice (400 ml) or water (400 ml; control) + standardised meal 1 week wash-out phase

T0 Baseline collection

Urine collection
Pilot Study – Urine Analysis

Preparation of urine samples by solid phase extraction (SPE)

SPE

Analysis of:
- anthocyanins
- anthocyanin metabolites
- hippuric acid by HPLC-PDA-ESI/MS

Additional analysis of:
- Antioxidant capacity (ORAC, FRAP, Total phenolics)
- Malondialdehyde (biomarker for oxidative stress; HPLC)
- Ascorbic acid & Uric acid (important antioxidants in biological fluids; HPLC)
## Pilot Study – Results Anthocyanins/Metabolites

**A1: QGP juice (HPLC-PDA @ 520 nm)**

- **Peaks**
  - 1. cya monoglucuronide: m/z 463/287
  - 2. cya-3-gluc: m/z 449/287
  - 3. cya-3-rut: m/z 595/287
  - 4. peo monoglucuronide: m/z 477/301
  - 5. peo-3-gluc: m/z 463/301
  - 6. peo-3-rut: m/z 609/301
  - 7. cya monosulfate: m/z 367/287

*not yet identified metabolites

**A2: urine after the ingestion of 400 mL QGP juice (HPLC-PDA @ 520 nm)**

Urinary excretion profile of QGP anthocyanins and metabolites

Total urinary excretion:
12.7 (9.5-15.9) µmol/24 h (~0.5% of the administered dose) → 84% as metabolites

Data: means and range (n=2)

1Sum of cya-3-gluc and cya-3-rut
2Sum of cya monoglucuronide, cya monosulfate, peo monoglucuronide, peo-3-glucoside, and peo-3-rutinoside
Urinary hippuric acid excretion/24 h by two healthy male subjects

- Intake of 15.6 mmol total polyphenols resulted in a 3.5 mmol increase in hippuric acid.
- Biotransformation rate of ~22% (ratio: total polyphenols intake vs. increase in hippuric acid excretion/24 h).
- Similar results with green and black tea\(^1\)

Data: means and range (n=2)

\(^1\)Mulder et al. 2005
Pilot Study – Results Additional Analysis

**Urinary excretion/24 h by two healthy male subjects:**
- Antioxidants assayed by FRAP, ORAC, and Total phenolics
- Malondialdehyde
- Ascorbic acid & Uric acid

**Control (400 mL water) vs. Treatment (400 mL QGP juice)**

Data: means and range (n=2); control-data as 100%
Conclusions

• QGP demonstrated an **outstanding anthocyanin** and **antioxidant content** (~7 times higher than the commercial varieties tested)

• **High biotransformation** of ingested QGP anthocyanins/polyphenols (mainly glucuronidated and methylated metabolites as well as ~3-fold increase of hippuric acid)

• **Significance of metabolites** for human health needs to be clarified!

• **Decreased MDA** excretion may indicate that some QGP phenolics and/or metabolites have antioxidant function *in vivo*  

→ Needs to be confirmed in a full clinical trial
Future Avenues

- **Identification** and **quantification** of **metabolites**, particularly colon metabolites, should be the focus in further studies (blood, urine, faecal excretion).
- **Interaction of metabolites** with chronic disease processes (‘mode of action’) should be investigated by using appropriate cell based assays and animal models.
- The ‘right’ **analytical tools** and **methods** are crucial (e.g. LC-MS, NMR, labelled compounds).
Acknowledgements

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Food and Nutritional Sciences
Michael Netzel

Phone: + 61 (0)7 3214 2172
Email: michael.netzel@csiro.au
Web: www.foodscience.csiro.au

Thank you!