Anthocyanins: For Color and Health

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Research Areas

Compounds of interest: Flavonoids

- Isoflavones
  - Phytoestrogens
- Anthocyanins
  - Natural colorants
  - Phytonutrients

Areas of work

- Analytical
- Horticultural
- Processing
- Bioavailability
- Health benefits
Introduction

- Epidemiological studies suggest that diets high in fruits and vegetables reduce the risk of a variety of chronic diseases
  - Cancer
    - Strong evidence for GI tract types
  - Cardiovascular disease
  - Obesity
What compounds in fruits and vegetables are responsible for their protective effects?
Anthocyanins
Each Aglycone has a characteristic color and spectra

<table>
<thead>
<tr>
<th>Aglycon</th>
<th>R₁</th>
<th>R₂</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Visible / Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelargonidin</td>
<td>H</td>
<td>H</td>
<td>494 nm</td>
<td>orange</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>OH</td>
<td>H</td>
<td>506 nm</td>
<td>orange-red</td>
</tr>
<tr>
<td>Peonidin</td>
<td>OMe</td>
<td>H</td>
<td>506 nm</td>
<td>orange-red</td>
</tr>
<tr>
<td>Delphinidin</td>
<td>OH</td>
<td>OH</td>
<td>508 nm</td>
<td>bluish-red</td>
</tr>
<tr>
<td>Petunidin</td>
<td>OMe</td>
<td>OH</td>
<td>508 nm</td>
<td>bluish-red</td>
</tr>
<tr>
<td>Malvidin</td>
<td>OMe</td>
<td>OMe</td>
<td>510 nm</td>
<td>bluish-red</td>
</tr>
</tbody>
</table>
In nature, Anthocyanins are always glycosylated

... with one sugar on position 3
In nature, Anthocyanins are always glycosylated and can be acylated... with acids attached to the sugars.

Aliphatic acid (malonic acid)  Cinnamic acid (p-coumaric acid)
Anthocyanins as Natural Food Colorants
Importance of food color

- People judge quality by the appearance of the product
- To keep product standardized
- Many times also correlates with (perceived) flavor or other quality characteristics
What are we looking for?

- "Right" color
  - RED is the color with the highest demand in US
  - Other "attractive" colors are also desirable
- Compatibility with matrix
- Color and pigment stability
- No undesirable aromas / flavors
- **Added value**: potential health benefits
Evaluate plant materials
New and exotic / traditional
Anthocyanin Sources

Berries: Simple pigments

Other Sources: Complex pigments
Berberis boliviana L.

- The Peruvian berry *Berberis boliviana* L. contains unusually high concentration of nonacylated anthocyanins – ~7% by dry weight.

Dried berries could be used as colorants without pigment extraction.

- **15X – 20X the NORMAL Anthocyanin Concentration of Regular Berries!!!**

*Del Carpio Jimenez, Wallace, and Giusti, 2007*
Berberis Anthocyanin Characterization

Peak 1: Delphinidinidin 3 glucoside
Hue Angle of Acylated Anthocyanins in Model Juices

FD&C Red # 40
Radish Extract
Potato Extract
Black Carrot Extract
MegaNatural Red Grape
Red Cabbage Extract

Model juices
pH = 3.5
Radish pigments provided excellent red color and stability to maraschino cherries and model juices and could be used as alternative to the use of FD&C Red No. 40.

Giusti and Wrolstad, 1996
Potato Anthocyanins

Pelargonidin-derivatives

Malvidin-derivatives

Petunidin-derivatives
Pigment Stability

Monomeric ACN (mg/100mL)

Refrigeration (2°C)

Potato

Radish

Potato ACN, $t_{\frac{1}{2}} = 11$ wks

Radish ACN, $t_{\frac{1}{2}} = 24$ wks

Room $T°$ (25°C)
Challenges in Colorant Production

Goal: Produce a Vegetable Juice concentrate

Challenges:
- PPO activity
- Starch Content
- Presence of Glycoalkaloids

Solution:
- Acidity
- PH treatments
- Processing - concentration
- Enzymes?
Evaluating new Radish Cultivars in China!

Jiangsu Province, PR China, November 2007
Chemical Structure of a Pg-derivative From Radish

Arrows indicate hydrogens in high proximity

Main challenge: radish colorant free of undesirable aromas / flavors

Solution:
• Cultivar selection
  • Sensory tests
• Processing steps
  • Water as extraction media
  • Physical means for concentration / separation
  • Enzymes?
CONCLUSIONS

- Anthocyanins provided excellent color and stability to a variety of food matrices
- Acylation of anthocyanins is responsible for the added stability of the compounds
- Use of anthocyanins may be challenging. Added value would justify the extra effort!
There is evidence that anthocyanins may contribute to the chemo protective effect of fruits and vegetables.
Supporting evidence

- Purple Sweet Potato (*Ipomoea batatas*)
  - Colorectal carcinogenesis in rats (Hagiwara et al., 2002)
  - Chemoprotective properties using model systems (Konczak-Islam, 2003)
  - Anti-hyperglycemic effect (Matsui et al., 2002)
  - Direct absorption into rats (Suda et al., 2002)
Comparing different anthocyanin pigment profiles

- Bilberry
- Chokeberry
- Grape

Acylated anthocyanins
Our cell line studies showed that...

- All AREs inhibited growth of HT29 colon cancer cells, with little effect on NCM460 non-tumorogenic cells.
- Data suggests that the protective effect of specific anthocyanins or extracts may depend on the chemical structure of anthocyanins present.

Zhao et al., JAFC, 2004
Animal trial: 11 F344 rats / treatment

15 wk ARE treatments: 4g anthocyanin/kg diet

Week 1
AOM injection
(20 mg/kg body wt)

Week 14
Urine, feces

Week 15
Colon, Plasma
Effect of AREs on Aberrant Crypt Foci

Lala et al. 2006. Nutr Cancer 54(1)
Our Animal Studies Show...

- Presence of intact anthocyanins and metabolites in plasma and urine, demonstrating absorption.

- Anthocyanin chemical structure may affect anthocyanins absorption and excretion.

- Anthocyanin levels in feces correlated with inhibition of early cancer lesions, suggesting unabsorbed anthocyanins may be chemoprotective.
Anthocyanin-Rich Extracts (AREs)

**Acylated Anthocyanins**
- Radish (*Raphanus sativus*)
- Purple carrot (*Daucus carota* L.)
- Purple corn (*Zea mays* L.)
- Grape (*Vitis vinifera*)

**Non-acylated Anthocyanins**
- Chokeberry (*Aronia meloncarpa* E.)
- Elderberry (*Sambucus nigra* L.)
- Bilberry (*Vaccinium myrtillus* L.)
## Anthocyanin profiles in seven commercial anthocyanin-rich sources

<table>
<thead>
<tr>
<th>Source</th>
<th>Anthocyanidin</th>
<th>Glycosylation</th>
<th>Acylation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Purple corn</strong> (Zea mays L.)</td>
<td>Cy, Pt, Pn</td>
<td>C3: mono-glycoside</td>
<td>Malonic acid</td>
</tr>
<tr>
<td><strong>Chokeberry</strong> (Aronia melanocarpa E.)</td>
<td>Cy</td>
<td>C3: mono-glycoside</td>
<td>None</td>
</tr>
<tr>
<td><strong>Bilberry</strong> (Vaccinium myrtillus L.)</td>
<td>Dp, Cy, Pt, Pn, Mv</td>
<td>C3: mono-glycoside</td>
<td>None</td>
</tr>
<tr>
<td><strong>Purple carrot</strong> (Daucus carota L.)</td>
<td>Cy</td>
<td>C3: di-, tri-glycoside</td>
<td>One cinnamic acid</td>
</tr>
<tr>
<td><strong>Grape</strong> (Vitis vinifera)</td>
<td>Cy, Dp, Pt, Pn Mv</td>
<td>C3: mono-glucoside</td>
<td>One cinnamic acid</td>
</tr>
<tr>
<td><strong>Elderberry</strong> (Sambucus nigra L.)</td>
<td>Cy</td>
<td>C3: mono-; diglucoside, C3 and C5: di-, tri-glucoside</td>
<td>None</td>
</tr>
<tr>
<td><strong>Radish</strong> (Raphanus sativus L.)</td>
<td>Pg</td>
<td>C3 &amp; C5: tri-glucoside</td>
<td>More than one cinnamic acids</td>
</tr>
</tbody>
</table>
Experimental design

Purple corn
Chokeberry
Bilberry
Purple carrot
Grape
Elderberry
Red radish

**In vitro model:**
HT29 cell line
SRB test
GI50

**SPE**
Semi-purification

**ARE**: anthocyanin-rich extract
**ACN**: anthocyanin fraction
**OPF**: other phenols fraction

**Fractionation (C18 SPE)**
Fractionation of Anthocyanins

- Different solvents used to separate anthocyanin fraction from other phenolics fraction
- Very high efficiency and low cost
- More than 95% pure
Inhibitory effects of fractionations of chokeberry on HT-29 colon cells

Growth Inhibition (% control)

Dose (µg/mL)

ARE: Anthocyanin-rich extract
OPF: Other phenolics fraction
ACN: Anthocyanins fraction
AP: ACN+OPF

ACN: Anthocyanins fraction
OPF: Other phenolics fraction
The GI$_{50}$ of Anthocyanin-rich Extracts from 7 Natural Sources

<table>
<thead>
<tr>
<th>Subset I</th>
<th>Subset II</th>
<th>Subset III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple corn</td>
<td>13.8</td>
<td>68.5</td>
</tr>
<tr>
<td>Chokeberry</td>
<td>31.2</td>
<td>71.2</td>
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<tr>
<td>Bilberry</td>
<td>32.2</td>
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<tr>
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<tr>
<td>Grape</td>
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</tr>
<tr>
<td>Radish</td>
<td></td>
<td>107.7</td>
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<tr>
<td>Elderberry</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

700~2000µg/g in Feces (GI tract)

<1.3µg/mL in plasma

Bioavailability
Summary

- Anthocyanins offer an attractive alternative to the use of artificial colors for products in a wide variety of food products.
- Studies in our laboratory and others are providing convincing evidence that anthocyanin-rich extracts and foods may be protective against chronic disease.
- Anthocyanin-rich commodities may have special appeal due to their potential applications as food colorants and their potential health benefits.
In the news...

Study: Dark-Colored Fruits and Veggies Fight Cancer

Tuesday, August 21, 2007

The darker the berry, the sweeter the juice, so goes the saying. But it turns out the darker berry or plum or grape, for that matter, the stronger the cancer-fighting properties.

Researchers conducting a recent study found that the compounds that give some fruits and vegetables their rich colors are powerful cancer deterrents.

Evidence from laboratory experiments on rats and on human colon cancer cells also suggest that anthocyanins, the compounds that give color to most red, purple and blue fruits and vegetables, also slow the growth of colon cancer cells.

“They contain many compounds, and we’re just starting to figure out what they are and which ones provide the best health benefits,” said Monica Giusti, the lead author of the study and an assistant professor of food science at Ohio State University, in a news release.

The findings, which Giusti presented August 10 at the national meeting of the American Chemical Society in Boston, also bring scientists a step closer to figuring out what fruits and vegetables’ cancer-fighting properties.

Giusti and her colleagues found that in some cases, slight alterations to the structure of anthocyanin molecules make these compounds more potent anti-cancer agents.

In their studies on human colon cancer cells grown in laboratory dishes, the researchers tested the anti-cancer...
Functional Foods Research at OSU...

CAFFRE: Center for Functional Foods Research and Entrepreneurship

- Research from Crop to Clinic to Consumer
- Chemistry of phytonutrients
- Analytical methods development
- Processing effects / chemical alterations of phytonutrients
- Bioavailability, metabolism, and physiological significance of phytochemicals.
THANK YOU !!!

- CREAS - Chile
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- Collaborators:
  - OSU / MD: Berna Magnuson, Joshua Bomser, Steven Schwartz
  - China: P. Jing
- Students and Research Assistants
  - Pu Jing, Jian He, Lucy Zhao, Minnie Malik, Geeta Lala, Qingguo Tian
- Artemis International, Inc / Polyphenolics, Inc. / GlobeNatural International / Agricomseeds / RFI Ingredients
Anthocyanins