Biological Activity of Anthocyanins

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Overview

• Natural pigments: generalities

• Technological properties of anthocyanins as food pigments

• Anthocyanins: potential health benefits
Natural pigments

• Naturally-occurring compounds that absorb light at certain wavelengths
• Responsible for imparting colors of foods
• Differ in solubility
  – Water-soluble: anthocyanins
  – Water-insoluble: chlorophyll and carotenoids
Natural pigments

Anthocyanins

Carotenoids

Clorophylls
Food color industry trends

Shaped By Consumer Trends, Food Color Market Will Grow To $2.3 Billion Industry By 2019

Color Additives: Adjusting to Changing Trends with Natural Alternatives


The North American food & beverage colorants market is estimated to grow at CAGR of 4.6% in the period between 2015 and 2020

Natural and organic trends drive European food colourings growth

Food Colorant Trends Show Natural is Critical for Consumers

Color manufacturers are following the "natural" trend by working closely with food, beverage and drug manufacturers to create and stabilize colors derived from natural sources.
Anthocyanins: natural pigments

• Water-soluble pigments responsible for the pink, red, blue, and violet colors of flowers, fruits and vegetables

• Anthocyanins: Anthocyanidins conjugated with sugar

• Color depends on pH

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Anthocyanin structure

Sugar groups:
- Glucose
- Sambubiose
- Rutinose
- Sophorose

<table>
<thead>
<tr>
<th>Aglycone</th>
<th>R1</th>
<th>R2</th>
<th>colour</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
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<tbody>
<tr>
<td>Cyanidin (Cy)</td>
<td>OH</td>
<td>H</td>
<td>Red</td>
<td>535</td>
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<tr>
<td>Peonidin (Pn)</td>
<td>OCH3</td>
<td>H</td>
<td>Bluish-purple</td>
<td>532</td>
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<tr>
<td>Pelargonidin (Pg)</td>
<td>H</td>
<td>H</td>
<td>Orange-red</td>
<td>520</td>
</tr>
<tr>
<td>Malvidin (Mv)</td>
<td>OCH3</td>
<td>OCH3</td>
<td>Purple</td>
<td>542</td>
</tr>
<tr>
<td>Delphinidin (Dp)</td>
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<td>OH</td>
<td>Purple</td>
<td>546</td>
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<tr>
<td>Petunidin (Pt)</td>
<td>OCH3</td>
<td>OH</td>
<td>Purple</td>
<td>543</td>
</tr>
</tbody>
</table>

Cyanidin 3-O-\(\beta\)-D-glucoside
Most abundant anthocyanins in foods and examples of sources

Pelargonidin-3-O-glucoside
Cranberries: 49.16 mg peonidin/100g

Malvidin-3-O-glucoside
Red shiraz: 121.65 mg malvidin/100g

Petunidin-3-O-glucoside
Black beans: 15.41 mg petunidin/100g

Cyanidin-3-O-glucoside
Illawara plum: 555.72 mg cyanidin/100g

Delphinidin-3-O-glucoside
Bilberry: 97.59 mg delphinidin/100g

Peonidin-3-O-glucoside
Radishes: 63.13 mg pelargonidin/100g

Other plant sources of anthocyanins

**Fruits**
- Purple grape
- Red grape

**Vegetables**
- Purple Sweet Potato
- Purple Carrot

**Legumes**
- Black bean
- Purple bean

**Cereals**
- Black rice
- Black peanut
- Blue wheat
- Sorghum
Dietary anthocyanin sources

• Relative abundance of anthocyanin from plants is variable:
  – genetic and agronomic variation
  – light intensity and type
  – temperature
  – harvest time, storage, and processing condition

• Data on food anthocyanins composition and concentration are limited and debated

• Regardless of the variation of anthocyanin concentration in food, it is necessary to establish standardized databases

Azzini et al., 2017. Ox Med Cell Long, Article ID 2740364
Applications of anthocyanins: food colorants
Anthocyanins are of interest to food scientists because:

• they can be used as color additives in food preparations.
• they are not stable during food processing affecting color and biological value.
Anthocyanins as food colorants

- Advantages: lower environmental impact, no toxicity, beneficial effects for human health
- Disadvantages: expensive processing, current sources are inefficient and wasteful, producing byproducts with little or no value

There are several strategies to stabilize anthocyanins:

- Polymeric compounds as copigments
- Polyphenolic compounds with stabilizing effect (for example, Rosemary extract)
- Metallic ions
- -SH group-containing compounds
- Controlled atmospheres
- Spray drying
- Freeze-drying
- Encapsulation
- Genetic modification of crops: gene-encoded acyltransferase or aromatic acyl groups

Zinc and alginate increased the stability of anthocyanins from purple corn in a beverage model.

Beverage model using anthocyanins from purple corn and the combination of zinc and alginate.

Luna-Vital et al. (2017) Protection of color and chemical degradation of anthocyanin from purple corn (Zea mays L.) by zinc ions and alginate through chemical interaction in a beverage model. *Food Research International*, 105, 169-177.
pH and temperature have an important effect in anthocyanins

<table>
<thead>
<tr>
<th></th>
<th>week0</th>
<th>Week 4</th>
<th>Week 8</th>
<th>Week 12</th>
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<tr>
<td>pH 2.0</td>
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<tr>
<td>pH 3.0</td>
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<tr>
<td>pH 5.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 6.0</td>
<td></td>
<td></td>
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<td></td>
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Zinc and alginate helps promoting the stability of anthocyanins from colored corn (PCW)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kool Aid invisible</td>
<td>PCW</td>
<td>0.2 mM ZnCl$_2$</td>
<td>0.4 mM ZnCl$_2$</td>
<td>0.4 % alginate (AA)</td>
<td>0.2 mM ZnCl$_2$ + AA</td>
<td>0.4 mM ZnCl$_2$ + AA</td>
<td></td>
</tr>
</tbody>
</table>

- Zinc and alginate protected corn anthocyanins from degradation in a beverage model
- Zinc and alginate combined improved the stability of color parameters in a beverage
- The protective mechanism relies on the interaction of anthocyanins with zinc and alginate

Luna-Vital et al., 2017. Food Res Int. 105: 169-177
Relevance of anthocyanins on human health
Impact of Obesity

More than one-third (36.5%) of U.S. adults have obesity with 49,190 estimated deaths in 2016.

Obesity-related conditions include heart disease, stroke, type 2 diabetes and certain types of cancer, some of the leading causes of preventable death.

The estimated annual medical cost of obesity in the U.S. was $147 billion in 2008 U.S. dollars; the medical costs for people who are obese were $1,429 higher than those of normal weight.

Diabetes

What is the difference between type 1 and type 2 diabetes?

The difference lies in the causes. Type 1 diabetes results from the destruction of the insulin-producing beta cells in the pancreas. This is usually an autoimmune process.

Type 2 diabetes is a spectrum of abnormalities involving glucose metabolism as well as other metabolic processes. This usually involves insulin resistance, in which higher amounts of insulin are needed to maintain normal glucose levels.

Americans Living with Diabetes: 8% (26 Million)

Americans at risk of developing Type 2 Diabetes: 24% (79 Million)

“Alarm bells are ringing. The CDC estimates that one third of all Americans will develop diabetes and will live 15 years less and lose quality of life. No public health problem compares in scale.”

– Dr. Mehmet Oz

Diabetes in the US

70,000 DEATHS ANNUALLY

174 BILLION Total national cost of diagnosed diabetes

2 out of 3 people with diabetes die from heart disease or stroke.

$1 out of every $5 in total healthcare cost

Cost of caring for someone with diabetes

American Diabetes Association

Centers for Disease Control and Prevention

NHANES National Health and Nutrition Examination Survey
Diabetes-improving properties of phenolic compounds such as anthocyanins in humans

- Improved insulin sensitivity
- Associated with a favorable glycemic response
- Reductions in fasting glucose and insulin levels
- Ameliorated post-prandial increase in plasma glucose
- Lowered insulin demand and index, and lowered glycemia
- Reduced risk for type-2 diabetes

Chen 2011; Wilson 2010; Udani 2011; Granfeldt 2011; Stull 2010; Wedick 2013.
A consensus about the recommended anthocyanin intake is needed

- Nowadays, it does not exist a recommended daily allowance for anthocyanins.

- Some authors suggest a consumption between $250-400$ mg/d.

- European Prospective Investigation into Cancer and Nutrition (EPIC) estimated a total anthocyanidin mean intake of $64.88 \text{ mg/d}$ and $44.08 \text{ mg/d}$ for men and women, respectively (Turin, Italy).
Absorption and Metabolism of Anthocyanins

- Consumption among the highest of all flavonoids due to their wide distribution in foods.
- Estimated daily intake of anthocyanins in the United States is between **180-215 mg**, may be as low as **12.5 mg** per day.
  - Absorption mainly in the small intestine and stomach.
    - Very efficient epithelial tissue uptake.
    - Gut microbiota cleave glycosidic linkages.
    - Reach peak plasma concentrations quickly.
      - 1-120 nM
- Less than 1% recovery in urine.
- Metabolites and degradation products may play a key role in their biological activity.

Faria 2009; Novotny 2012.
Anthocyanin absorption, distribution, metabolism and excretion based on current information
Anthocyanins are more bioavailable in humans than previously perceived

Pharmacokinetics of anthocyanins and their metabolites in humans

Experimental approach:

• 500 mg of C5-labeled cyanidin-3-glucoside
• 8 healthy male participants
• Collection of samples at 0, 0.5, 1, 2, 4, 6, 24 and 48 h
• Samples were analyzed by HPLC-ESI-MS/MS

Pharmacokinetics of anthocyanins: key results

Seventeen $^{13}$C-labelled compounds were identified

- C$_3$G
- Protocatechuic acid (PCA)
- Phloroglucinaldehyde (PGA)
- 13 metabolites of PCA
- 1 metabolite of PGA
- $C_{\text{max}}$ of the metabolites ranged from 10 to 2000 nM between 2 and 30 h ($t_{\text{max}}$)
- Half-lives of elimination between 0.5 and 96 h

Concentration of C₃G and degradants in plasma, urine and feces

<table>
<thead>
<tr>
<th></th>
<th>C₃G</th>
<th>PCA</th>
<th>PGA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (nM)</td>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (nM)</td>
</tr>
<tr>
<td>Plasma</td>
<td>141</td>
<td>1.8</td>
<td>146</td>
</tr>
<tr>
<td>Urine</td>
<td>334</td>
<td>1-2</td>
<td>337</td>
</tr>
<tr>
<td>Feces</td>
<td>70</td>
<td>6-24</td>
<td>360</td>
</tr>
</tbody>
</table>

C₃G: Cyanidin-3-O-glucoside
PCA: Protocatechuic acid
PGA: Phloroglucinaldehyde
C<sub>max</sub>: maximum concentration
t<sub>max</sub>: time at maximum concentration

Clinical evidence of the beneficial effects of anthocyanins from dietary sources in metabolic disorders
Beneficial effects of anthocyanins: general overview

Azzini et al., 2017. Ox Med Cell Long, Article ID 2740364
Clinical studies retrieved from the [ClinicalTrials.gov](http://ClinicalTrials.gov) database on anthocyanins interventional studies and obesity

**Conditions**
- Overweight
- Type-2 diabetes
- Dyslipidemia
- Insulin resistant
- Childhood obesity

**Sources**
- Blackberry juice (250 mL)
- Blackberry extract (1.4 g)
- Freeze-dried strawberries (25-50 g)
- Blueberry powder (45 g)
- Fresh blackcurrants (80 g)
- Aronia juice (1000 mg/gallic acid eq/100 mL)

**Primary outcomes**
- Plasma concentrations of anthocyanins and metabolites
- Changes in lipids and lipoproteins
- Improvement in insulin sensitivity
- Reduction in glycosylated hemoglobin
- Reduction in blood cholesterol

Number of studies: 10
Time frame: 2010 – 2017
Participants: 18 to 60
Total of participants: 366
Age: 18 to 65 y.o.

Azzini et al., 2017. Ox Med Cell Long, Article ID 2740364
Anthocyanin Rich-Black Soybean Testa Improved Visceral Fat and Plasma Lipid Profiles in Overweight/Obese Adults: A Randomized double-blind placebo-controlled trial

- n=80 overweight participants
- 40 received black soybean extract, 40 received placebo
- Dose: 2.5 g/day anthocyanin-rich black soybean testa extracts with high concentrations of anthocyanins (12.58 mg/g)
- Eight weeks
- Age 19 and 65 years
- Body mass index (BMI) >23 (kg/m²)
Significant decreases

- waist circumference (approx. 2 cm),
- triacylglycerols (TG) (≈25%),
- low density lipoprotein cholesterol (LDLc) (≈20%),
- non-high density lipoprotein cholesterol (non-HDLc) (≈15%).

BBT can potentially be developed as a functional food for preventing abdominal obesity with high fiber and low cholesterol diets.
Effect of anthocyanins on obesity and type-2 diabetes

- Inhibition of body weight gain
- Relief of oxidative stress
- Regulation of inflammatory response
- Improvement of insulin resistance
- Alleviation of chronic diabetic complications

Overall effects of anthocyanins in obesity and type-2 diabetes

Inhibition of body weight gain by anthocyanins

- Anthocyanins from black soybean efficiently prevented obesity in rats by inhibiting neuropeptide Y and activating the γ-amino butyric acid (GABA) receptor in the hypothalamus.

- Translational studies using anthocyanins preventing body weight gain in humans is not conclusive yet.

Adult male Sprague-Dawley rats
n= 6
24 mg anthocyanins/kg BW/day
40 days

Satiety
- ↑≈80% GABA-R
- ↓≈50% Neuropeptide-Y
- Both markers measured in the hypothalamus

Metabolic activation
- ↓16% Body weight gain
- ↓≈20% daily average food intake

Badshah et al., 2013. Neuropeptides, 47(5): 347-353
Relief of oxidative stress by anthocyanins

- Obesity and prediabetes increase generation of reactive oxygen species (ROS).

- Anthocyanins may serve as free radical scavengers, however, emerging evidence suggest that they exert modulatory actions on antioxidant signaling molecules.

One-month strawberry-rich anthocyanin supplementation ameliorated oxidative stress markers in humans

- **n=23** healthy participants (men and women, 23 to 31 y.o.)
- **500 g fresh strawberries per day**
- **30 days**

**Plasma**

- **MDA ↓31%**
  - Malondialdehyde
  - Results from the lipid peroxidation of polyunsaturated fatty acids

**Urine**

- **Isoprostanes ↓27%**
  - Prostaglandin-like compounds
  - Formed from the free radical-catalyzed peroxidation of fatty acids

**Urine**

- **8-OHdG ↓31%**
  - 8-Oxo-2’-deoxyguanosine
  - Major product of DNA oxidation
  - Marker of oxidative stress at cellular level


Regulation of inflammatory response by anthocyanins

- Inflammation is a key component of obesity-related metabolic disorders such as type-2 diabetes.
- Several *in vitro* and *in vivo* studies have shown the anti-inflammatory effect of anthocyanins. However, the translation to human studies has not been completely achieved.

*Açaí (Euterpe oleracea Mart.)* beverage consumption improves biomarkers for inflammation but not glucose- or lipid-metabolism in individuals with metabolic syndrome

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ ↓76%</td>
<td>Isoprostanes ↓31%</td>
</tr>
</tbody>
</table>

- Interferon-gamma
  - Cytokine that is critical for innate and adaptive immunity.
  - Is an important activator of macrophages

**Kim et al., 2018. Food Funct, 9:3097-3103**

Improvement of insulin resistance by anthocyanins

- Obesity is strongly associated with insulin resistance, and the improvement of insulin resistance is important in preventing the development of type-2 diabetes.

**Purified Anthocyanin Supplementation Reduces Dyslipidemia, Enhances Antioxidant Capacity, and Prevents Insulin Resistance in Diabetic Patients**

- n=58 participants with type-2 diabetes (men and women, 56 - 67 y.o.)
- 320 mg anthocyanins per day (extracted from blueberries)
- 24 weeks

Blood glucose ↓9%
Insulin sensitivity ↑14% (determined by HOMA-IR)

Insulin levels in blood were not different from the baseline, which suggests that the anthocyanins promoted insulin sensitivity

Clinical evidence of the beneficial effects of anthocyanins from dietary sources on markers of cardiovascular risk
Supplementation of Anthocyanins and Markers of Cardiovascular Disease

➢ 12 studies from 2005 to 2016
➢ n= 27 to 146 each
➢ Total of participants: 1,042
➢ Age: > 18 years
➢ Materials: elderberry extract (500 mg/day), whortleberry extract (1050 mg/day), chokeberry extract (255 mg/day), hibiscus (100 mg/day), purified anthocyanins

Wallace et al., 2016. Nutrients, 8: 32
Effect of anthocyanins in cardiovascular disease markers: lipoproteins

ANC administration

Healthy subjects

7 to 500 mg/day

Slight reduction in total cholesterol (up to -4.5%), no differences in LDL or HDL

Dyslipidemic subjects

Reduction in LDL (up to -32%) and cholesterol (up to -28.9%), increase in HDL (up to 36%)

Wallace et al., 2016. Nutrients, 8: 32
Effect of anthocyanins in cardiovascular disease markers: triglycerides

Healthy and dyslipidemic subjects

Up to 500 mg/day

Overall reduction of triglycerides (up to -37.9%)

Wallace et al., 2016. Nutrients, 8: 32
Effect of anthocyanins in cardiovascular disease markers: blood pressure

**Anthocyanin dose**
- Up to 500 mg/day
  - 19.2 to 500 mg/day

**Condition of the participants**
- Different types of health status
  - Healthy, metabolic syndrome, post-myocardial infarction, prehypertension

**Main outcomes**
- Changes in blood pressure
  - Overall decrease of systolic (up to -8.3%) and diastolic (up to -13.5%) blood pressure

Wallace et al., 2016. Nutrients, 8: 32
Examples of clinical interventions and studies in humans to evaluate the beneficial effects of anthocyanins in cardiovascular disease markers
Improved Lipid Profile in Hyperlipidemic Patients Taking Vaccinium arctostaphylos Fruit Hydroalcoholic Extract: A Randomized Double-Blind Placebo-Controlled Clinical Trial

- \( n = 51 \) with whortleberry, 54 placebo
- Newly diagnosed primary hyperlipidemia
- Dose: 350 mg every 8 h (1.05 g of dry whortleberry daily) for 2 months

Whortleberry (*Vaccinium arctostaphylos*) reduced total cholesterol, triglyceride and LDL, and increased HDL compared with baseline

<table>
<thead>
<tr>
<th></th>
<th>Whortleberry group</th>
<th>Placebo group</th>
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<tbody>
<tr>
<td>Total cholesterol</td>
<td>↓28%</td>
<td>=</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>↓19%</td>
<td>=</td>
</tr>
<tr>
<td>LDL</td>
<td>↓26%</td>
<td>=</td>
</tr>
<tr>
<td>HDL</td>
<td>↑37%</td>
<td>=</td>
</tr>
</tbody>
</table>

Effect of anthocyanins on blood pressure and stress reactivity: a double-blind randomized placebo-controlled crossover study

➢ n=31 healthy men not on antihypertensive medication
➢ Dose: 320 mg anthocyanin twice daily (640 mg anthocyanin /day)
➢ Duration: 4 weeks
➢ Source: bilberries and blackcurrants

Hassellund et al., 2012. J Hum Hypertens, 26: 396-404
Cardiovascular responses to anthocyanins comparing absolute differences across treatment periods

Systolic and diastolic blood pressure was reduced at different times of the study. However, the results are not conclusive, the most appropriate populations, doses, food mixtures and even anthocyanin molecules to maximize health benefits are yet to be found.

Hassellund et al., 2012. J Hum Hypertens, 26: 396-404
Anthocyanins: preclinical studies *in vitro* and *in vivo*
Comprehensive *in vitro* and *in vivo* evaluation of anthocyanins and proanthocyanidins from blueberry and blackberry fermented beverages on type-2 diabetes

(Aim 1, Aim 2)

Phenolic Compounds in Berry Wine

(Aim 3)

Glucose Absorption

Oxidative Stress

Inflammation

DPP-IV Activity

(Aim 4)

Insulin Secretion

(Aim 5)

Glucose Levels

Type-2 Diabetes

DPP-IV Activity

Resveratrol: Green
Flavone: Yellow

Diprotin A

-3208.3 kcal/mol

Delphinidin-3-arabinoside

-3227.7 kcal/mol

Malvidin-3-galactoside

-3106.9 kcal/mol
Anthocyanins and proanthocyanidins from blueberry and blackberry alcohol-free fermented beverages

- Beneficial sources of antioxidants
- Inhibitors of carbohydrate-utilizing enzymes
- Potential inhibitors of inflammation

• There is potential for alcohol-free fermented berry beverages to reduce complications associated with chronic inflammatory diseases like type 2 diabetes.
Computational model confirmed binding to DPP-IV:

Delphinidin-3-arabinoside and malvidin-3-galactoside have similarly low interaction energies as diprotin A with DPP-IV enzyme.

Diprotin A -3208.3 kcal/mol  Delphinidin-3-arabinoside -3227.7 kcal/mol  Malvidin-3-galactoside -3106.9 kcal/mol

- The lower the interaction energy, the better the binding.

Johnson et al., 2013. Mol Nutr Food Res. 57(7), 1182-1197
Role of anthocyanins from fermented berry beverages

- Improved glucose tolerance
  - Stimulated insulin secretion & decreased glucose absorption
  - Inhibition of dipeptidyl peptidase IV
  - Increased antioxidant capacity
  - Inhibition of oxidation & ROS scavenging

- Reduced metabolic syndrome complications
- Anti-Inflammatory Properties
- Reduced oxidative stress

- Inhibition of α-amylase and α-glucosidase
- Decreased in vitro markers of inflammation
Colored corn as a source of natural pigments

Peonidin-3-\(O\)-glucoside (P3G)

Pelargonidin-3-\(O\)-glucoside (Pr3G)

Cyanidin-3-\(O\)-glucoside (C3G)

Condensed forms (CF)

Proanthocyanidins from colored corn coproducts and the anti-inflammatory effect from purple and red corn pericarp

Anthocyanins are present mainly in the pericarp and aleurone of colored corn.

<table>
<thead>
<tr>
<th>Peak number</th>
<th>Retention time (min)</th>
<th>Identity</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>11.0</td>
<td>Condensed forms</td>
</tr>
<tr>
<td>2</td>
<td>16.1</td>
<td>Cyanidin-3-glucoside</td>
</tr>
<tr>
<td>3</td>
<td>17.4</td>
<td>Pelargonidin-3-glucoside</td>
</tr>
<tr>
<td>4</td>
<td>18.1</td>
<td>Peonidin-3-glucoside</td>
</tr>
<tr>
<td>5</td>
<td>19.2</td>
<td>Cyanidin-3-O-(6''-malonyl-glucoside)</td>
</tr>
<tr>
<td>6</td>
<td>20.6</td>
<td>Pelargonidin-3-O-(6''-malonyl-glucoside)</td>
</tr>
<tr>
<td>7</td>
<td>21.3</td>
<td>Peonidin--3-O-(6''-malonyl-glucoside)</td>
</tr>
</tbody>
</table>

Anthocyanins are found in different sections depending on the variety.

Fractionation and isolation of compounds from extracts of colored corn

Luna-Vital & de Mejia, 2018. PloS one. 13(7), e0200449
Using pressure-assisted extraction, it is possible to obtain food-grade pigments from colored corn. Colored maize pericarp is extracted under pressure to obtain a purple corn ANC-rich extract (PCW). The anthocyanins are identified by HPLC-MS-MS at 520 nm. The table summarizes the peak names:

<table>
<thead>
<tr>
<th>Peak</th>
<th>Name</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>CF</td>
</tr>
<tr>
<td>2</td>
<td>C3G</td>
</tr>
<tr>
<td>3</td>
<td>Pg3G</td>
</tr>
<tr>
<td>4</td>
<td>P3G</td>
</tr>
<tr>
<td>5</td>
<td>C3G-Mal</td>
</tr>
<tr>
<td>6</td>
<td>Pg3G-Mal</td>
</tr>
<tr>
<td>7</td>
<td>P3G-Mal</td>
</tr>
</tbody>
</table>
Methodology differentiation

3T3-L1 pre-adipocyte cells

Hormone-driven differentiation (IBMX, rosiglitazone, dexamethazone, insulin)
15 days

Differentiation parameters:
- Lipid staining (Oil Red O)
- PPAR-γ levels

Antiobesity parameters:
- Lipase and FAS activity
- Lipolysis
- Molecular docking

Purple corn pericarp water extract
Methodology inflammation

- Fully-differentiated adipocytes
- 8 days
- Purple corn pericarp water extract
- Tumor Necrosis Factor-α
  - cell signaling protein involved in systemic inflammation
- Immunodetection of adipokines and glucose uptake
- Inflammation
- Insulin resistance
Anthocyanins from PCW inhibited adipocytes differentiation in a dose-response manner

Day 0
Control 0.12 mg/mL 0.25 mg/mL 0.5 mg/mL 1 mg/mL

Day 2

Day 4

Day 6

Day 8

Day 10

Oil Red O absorbance at 500 nm

- Control  - 0.5 mg/mL  - 1 mg/mL  - 0.25 mg/mL  - ▲ 0.125 mg/mL

IC\textsubscript{50} = 0.4 mg/mL

Interactions of cyanidin-3-O-glucoside with A) thioesterase domain of fatty acid synthase and B) the three identified pockets of lipoprotein lipase as determined by molecular docking

Effect of anthocyanins from purple corn on proteins related to FFAR1-dependent insulin secretion in iNS-1E cells

Luna-Vital & de Mejia, 2018. PloS one. 13(7), e0200449
Effect of anthocyanins from purple corn on GK-activating potential in HepG2 cells

Hepatocyte

Glucose

Gluco kinase

Glucose-6-phosphate

Glucose metabolism

Gluconeogenesis

Hepatic glucose uptake

Blood glucose

Luna-Vital & de Mejia, 2018. PloS one. 13(7), e0200449
*In vitro* studies using adipocytes, hepatocytes, and pancreatic cells have shown potential benefits improving metabolic disorders.


Conclusions related to diabetes

• Anthocyanins activated FFAR-1 in pancreatic cells.
• D3G was the most effective ANC, followed by C3G; the major ANC in PCW.
• The results of this study suggest that ACN from colored corn are good candidates to be incorporated in the diet during type-2 diabetes treatment.

Luna-Vital & de Mejia, 2018. PloSone. 13(7), e0200449
In an *in vivo* model of obesity, the anthocyanin-rich extract of purple corn prevented body weight gain and reduced fasting blood glucose.

Anthocyanin rich extract reduced liver steatosis in HDF-fed mice

Healthy control

Obese control

200 mg/kg PCW

500 mg/kg PCW

Anthocyanin-rich extract from purple corn reduced the lipid accumulation in adipocytes

Healthy control

Obese control

200 mg/kg PCW

500 mg/kg PCW

Future Perspectives

• Correlation of the anthocyanin chemical composition of foods with the *in vitro* and *in vivo* potential to reduce chronic diseases.

• Effect of processing on the chemistry and composition of anthocyanins in foods and their human health impact.

• Databases of anthocyanin composition and concentration in different food sources.
Take-home message

• Anthocyanins are compounds in foods that can be used by the food industry as natural pigments.

• Anthocyanins have potential to reduce obesity complications, and manage diabetes.

• The development of functional foods with value-added properties is of great interest to the scientific community and to the food industry.
Thank you!

more to love than color: anthocyanins
Thank you