Phytonutrients:
Polyphenols, Phytosterols and Other Antioxidants

Institute of Himalayan Bioresource Technology, Palampur (H.P.) India
(Council of Scientific & Industrial Research)
Polyphenolics: Flavonoid Group

Common C₆-C₃-C₆ Flavonoid Structures

- Flavonol (F & V, red wine, tea)
- Flavone (celery, parsley)
- Isoflavone (soya)
- Anthocyanin (berries, grapes, plums etc)
- Flavanone (citrus fruit)
- Flavan-3-ol (aka catechins) (F & V, red wine, chocolate, green tea)
**PHYTOSTEROLS**

**Plant Sterols**

- Present in diet (200 - 400 mg / d) in small amounts in vegetable oils, nuts, seeds, legumes.
- Need 1.5-2g for efficacy.
- 2500 tonnes oil needed for 1 tonne plant sterols
PHYTOHORMONES: 
Plant Estrogens

Isoflavones

1. most concentrated in soy beans (genistein, glycine and daidzein)

2. soy bean has 2-4 milligrams isoflavone/gram

3. predominantly genistein found in legumes and pomegranate seeds

17β-estradiol

Glycine

Genistein

Daidzein
CHOLESTEROL FACTS

synthesized from acetyl CoA and eliminated as bile acids
precursor of all other steroids in the body
product of animal metabolism - in foods of animal origin
amphipathic lipid (hydrophobic and hydrophilic portions)
storage form is cholesterol ester found in most tissues.
esSENTIAL structural component of membranes transported in the
circulation in lipoproteins

Cholesterol ester (1st ring only)
R = fatty acid hydrocarbon chain
OTHER ANTIOXIDANTS

1. Phenolic Acids
   Ellagic acid, Chlorogenic acid, P-Coumaric acid
   Cinnamic acid, Ferulic acid, Vanillin, Phytic acid
   Hydroxycinnamic acid

2. Other Non-Flavonoid Phenolics
   Curcumin, Resveratrol, Lignans

3. Glucosinolates
   Phenylethyl Isothyocynate, Benzyl isothyocynate,
   Sulforaphane

4. Indoles
   Indole-3-Carbinol (I3C)
# Examples of Phytonutrients

<table>
<thead>
<tr>
<th>Class/Components</th>
<th>Source</th>
<th>Potential Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FLAVONOIDS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anthocyanidins</td>
<td>berries, cherries, red grapes</td>
<td>bolster cellular antioxidant defenses; may contribute to maintenance of brain function</td>
</tr>
<tr>
<td>Flavanols, Catechins, Epicatechins, Procyanidins</td>
<td>tea, cocoa, chocolate, apple and grapes</td>
<td>may contribute to maintenance of heart health</td>
</tr>
<tr>
<td>Flavanones</td>
<td>citrus fruits</td>
<td>neutralize free radicals which may damage cells; bolster cellular antioxidant defenses</td>
</tr>
<tr>
<td>Flavonols</td>
<td>onion, apple, tea, broccoli</td>
<td>neutralize free radicals which may damage cells; bolster cellular antioxidant defenses</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>cranberries, cocoa, apple, strawberries, grapes, wine,</td>
<td>may contribute to maintenance of urinary tract</td>
</tr>
</tbody>
</table>
Plant Stanols/Sterols

<table>
<thead>
<tr>
<th>Category</th>
<th>Sources</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free Stanols/Sterols</td>
<td>corn, soy, wheat, wood oils, fortified foods and beverages</td>
<td>may reduce risk of CHD</td>
</tr>
<tr>
<td>Stanol/Sterol esters</td>
<td>fortified table spreads, stanol ester dietary supplements</td>
<td>may reduce risk of CHD</td>
</tr>
</tbody>
</table>

Phytoestrogens

 Isoflavones:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sources</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzein</td>
<td>soybeans and soy-based foods</td>
<td>may contribute to maintenance of bone health, healthy brain and immune function; for women, maintenance of menopausal health</td>
</tr>
<tr>
<td>Genistein</td>
<td>soybeans and soy-based foods</td>
<td></td>
</tr>
</tbody>
</table>

IHBT
### Phenolic acids

| Caffeic acid | Ferulic acid | Apple, pear, citrus fruits and some vegetables | May bolster cellular antioxidant defenses; may contribute to maintenance of healthy vision and heart health |

### Isothiocyanates

| Sulforaphane | Cauliflower, broccoli, broccoli sprouts, cabbage, kale, horseradish | May enhance detoxification of undesirable compounds and bolster cellular antioxidant defenses |
## Other Non-Flavonoid Phenolics

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td><img src="image" alt="Curcumin Structure" /></td>
<td>Turmeric may have antiarthritic, antiamyloid, anti-ischemic and anti-inflammatory properties.</td>
</tr>
<tr>
<td>Resveratrol</td>
<td><img src="image" alt="Resveratrol Structure" /></td>
<td>Skin of red grapes and in other fruits, with anti-cancer, anti-inflammatory, blood-sugar-lowering and other beneficial cardiovascular effects.</td>
</tr>
<tr>
<td>Lignans</td>
<td><img src="image" alt="Lignans Structure" /></td>
<td>Flax, rye, some vegetables, may contribute to maintenance of heart health and healthy immune function.</td>
</tr>
</tbody>
</table>

### Indoles (Indole-3-Carbinol)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indole-3-carbinol</td>
<td><img src="image" alt="Indole-3-carbinol Structure" /></td>
<td>Cruciferous vegetables such as broccoli, cabbage, cauliflower, brussels sprouts, collard greens and kale; Anticarcinogenic, antioxidant and anti-atherogenic effects.</td>
</tr>
</tbody>
</table>
Green Tea Flavan-3-ol Structures

(-)-Epicatechin
(+)-Catechin
(+)-Gallocatechin
(-)-Epigallocatechin

(-)-Epicatechin gallate
(-)-Epigallocatechin gallate
(+)-Gallocatechin gallate
Principle phenolics in black tea

Theaflavin

Theaflavin-3'-gallate

Theaflavin-3-gallate

Theaflavin-3,3'-digallate
Flavonol Glycosides

Quercetin-4'-glucoside

200 g fried onion - 257 µmoles

Quercetin-3-rutinoside

300 mL tomato juice - 176 µmoles
Cocoa and chocolate contain monomeric and polymeric flavan-3-ols

(-)-Epicatechin

Proanthocyanidin B₂ dimer

Proanthocyanidin C₁ trimer
Types of Free Radicals

- **Oxygen-centered radicals**
  - Singlet oxygen, superoxide, hydroxyl radicals

- **Sulfur-centered radicals**
  - Thiyl radical (RS•)

- **Carbon-centered radicals**
  - •CCl3, CH2•CHOH

- **Nitrogen-centered radicals**
  - NO•, R2NO•
FREE RADICAL DAMAGE AND DISEASE

Free Radicals → Damage → Disease

- Nucleic acids
- Nucleotides
- Thiols
- Covalent bonding
- Lipids
- Membrane structure

- Heart disease
- Cancers
- Parkinsons
- Arthritis
- Cataracts
- Muscular dystrophy
MODE OF ACTION

REACTIVE OXYGEN SPECIES (ROS)

• Reactive Species

• Includes:
  – hydroxyl radicals (.OH)
  – superoxide anions (O₂⁻)
  – singlet oxygen (1O₂)
  – hydrogen peroxides (H₂O₂)
  – organic peroxides (R-OOH)
  – nitric oxide
• peroxynitrite
# OXIDATIVE STRESS

<table>
<thead>
<tr>
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<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O₂⁻, superoxide anion</strong></td>
<td>One-electron reduction state of O₂, formed in many autoxidation reactions and by the electron transport chain. Rather unreactive but can release Fe²⁺ from iron-sulfur proteins and ferritin. Undergoes dismutation to form H₂O₂ spontaneously or by enzymatic catalysis and is a precursor for metal-catalyzed •OH formation.</td>
</tr>
<tr>
<td>H₂O₂, hydrogen peroxide</td>
<td>Two-electron reduction state, formed by dismutation of •O₂⁻ or by direct reduction of O₂. Lipid soluble and thus able to diffuse across membranes.</td>
</tr>
<tr>
<td>•OH, hydroxyl radical</td>
<td>Three-electron reduction state, formed by Fenton reaction and decomposition of peroxynitrite. Extremely reactive, will attack most cellular components</td>
</tr>
<tr>
<td>ROOH, organic hydroperoxide</td>
<td>Formed by radical reactions with cellular components such as lipids and nucleobases</td>
</tr>
<tr>
<td>RO•, alkoxy and ROO•, peroxy radicals</td>
<td>Oxygen centred organic radicals. Lipid forms participate in lipid peroxidation reactions. Produced in the presence of oxygen by radical addition to double bonds or hydrogen abstraction.</td>
</tr>
<tr>
<td>HOCl, hypochlorous acid</td>
<td>Formed from H₂O₂ by myeloperoxidase. Lipid soluble and highly reactive. Will readily oxidize protein constituents, including thiol groups, amino groups and methionine</td>
</tr>
<tr>
<td>ONOO⁻, peroxynitrite</td>
<td>Formed in a rapid reaction between •O₂⁻ and NO•. Lipid soluble and highly reactive. Will undergo homolytic cleavage to form hydroxyl radical and nitrogen dioxide.</td>
</tr>
</tbody>
</table>
PHENOLICS as ANTIOXIDANT

Phenolic antioxidant mechanism

OH

[Chemical structure showing the antioxidant mechanism]

semi-quinone

quinone
Two-Stage Oxidation of Quercetin

Orthoquinone

Hydrogen-bond stabilized semiquinone

Extended paraquinone

(J. Agric. Food Chem. 2003;51:1684-90)
Structure – activity relationships

Increasing Antioxidant Potential

Galangin → Quercetin → Myricetin

Kaempferol → Apigenin → Taxifolin
## ANTIOXIDANT MEASURE

<table>
<thead>
<tr>
<th>Item</th>
<th>Antioxidant Capacity (µ mol Trolox/g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Tea</td>
<td>927</td>
</tr>
<tr>
<td>Green Tea</td>
<td>814 30</td>
</tr>
<tr>
<td>Spinach</td>
<td>129 6</td>
</tr>
<tr>
<td>Beet</td>
<td>81 25</td>
</tr>
<tr>
<td>Leaf Letuce</td>
<td>49 7</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>46 11</td>
</tr>
<tr>
<td>Garlic</td>
<td>46</td>
</tr>
<tr>
<td>Onion</td>
<td>40 2</td>
</tr>
<tr>
<td>Cabbage</td>
<td>32 2</td>
</tr>
<tr>
<td>Carrot</td>
<td>26 8</td>
</tr>
<tr>
<td>Corn</td>
<td>22 4</td>
</tr>
<tr>
<td>Potato</td>
<td>15 5</td>
</tr>
<tr>
<td>Cucumber</td>
<td>15 2</td>
</tr>
<tr>
<td>Sweet potato</td>
<td>14 2</td>
</tr>
</tbody>
</table>

Consumed Dietary Phenolics Metabolism in Humans

Dietary phenolics

Small intestine → LIVER

Colon

Faeces

Ileostomy bag

Tissues

Kidney → General circulation

Urine
Colonic degradation of anthocyanins

(Aura et al., Eur J Nutr 44, 133-142, 2005)
Fate of Rutin In The Large Intestine

Quercetin → ring fission → 3,4-Dihydroxyphenylacetic acid

3,4-Dihydroxyphenylacetic acid → 3-Hydroxyphenylacetic acid
3,4-Dihydroxyphenylacetic acid → 3-Methoxy-4-hydroxyphenylacetic acid
1. The maximum plasma concentrations attained after a polyphenol-rich meal, which are in the range of 0.1–10 μmol/L.

2. Catabolism of polyphenols in humans usually occurs only as a result of microbial activity in the (large) intestine.

3. Human tissues are exposed to polyphenols via the blood, which is the only route through which dietary polyphenols can reach tissues and their cells, except for the cells lining the intestinal tract.

4. Phenolic acids account for about one third of the total intake and flavonoids account for the remaining two thirds. The most abundant flavonoids in the diet are flavanols (catechins plus proanthocyanidins), anthocyanins and their oxidation products. The main polyphenol dietary sources are fruit and beverages (fruit juice, wine, tea, coffee, chocolate and beer) and, to a lesser extent vegetables, dry legumes and cereals.

5. Endogenous plasma antioxidants, especially ascorbate, are required for disposal of the resultant phenoxy radicals.

6. Over 95% of the intake passes to the colon and is fermented by the gut microflora. A fraction of the resulting microbial metabolites is absorbed and appears in the plasma primarily as mammalian conjugates.

7. More than 2 d are needed for the phytoestrogen metabolites to reach the baseline concentrations in plasma and urine after the consumption of soy milk and flaxseed, respectively.
• Ellagitannins (antioxidants) are not absorbed
• Metabolized in vivo to bioavailable urolithins
• Urolithins are weakly antioxidant
• Large inter-individual variability
TISSUE DISTRIBUTION

Non-detected-in


Plasma concentrations

- Below 1μM
Thank You .....!

Institute of Himalayan Bioresource Technology, Palampur (H.P.) India
(Council of Scientific & Industrial Research)

Post Box No. – 06
Palampur-176061 (H.P.) India
Website: http://www.ihbt.res.in
E-mail: director@ihbt.res.in
EPABX: 91-233338-39,230742-43,230431
Fax: 91-1894-230433 / 230428