Citrus Phytochemicals

**Phytochemicals**
- Naturally occurring biologically active components
- Found in plant

**Phytonutrient**
- Plant chemical with known nutrient properties
- Consumed as part of a usual diet

*Biologically active or Protect against Disease*
*Health Canada, 1998*

**Designer Foods**

**Functional Foods**

**Hypernutritious Foods**

**Nutraceuticals**

**Bioactive Compounds**

“Extranutritional” constituents that typically are naturally occurring in small quantities in plant products and lipid rich foods.

*Kitts, 1994 Can J. Physiol Pharmacology 1994*

**Nutraceutical**
- Product isolated or purified from food
- Sold in medicinal forms - pill, powder, capsule

**Functional Food**
- A conventional food
- Consumed as part of a usual diet
- Provide health benefit beyond basic nutrition

*Biologically active or Protect against Disease*
*Health Canada, 1998*

**Chemopreventive agents**

- **Micronutrients**
  - vitamins, beta carotene, molybdenum, calcium
- **Phytochemicals**
- **Synthetics**
  - vitamin derivatives
  - piroxicam
  - tamoxifen
Phytochemicals

- Carotenoids
- Indole
- Saponins

- Coumarins
- Dietary Fiber
- Isoflavones
- Protease inhibitors

Phytochemicals

- Organosulfides
- Isothiocynates
- Indoles
- Dithiolthiones

- Polyphenols
- Flavonoids
- Tannins
- Folic acid

Contd.

Chemopreventive agents

(Based on their mechanisms of action)

- Blocking agents
- Suppressing agents

CANCER CHEMOPREVENTIVE AGENTS

1. BLOCKING AGENTS -- prevent carcinogens from reaching or reacting with the DNA, the genetic information.

2. SUPPRESSIVE AGENTS -- inhibit the expression of cancer in cells that have already been exposed to a carcinogen.

Chemopreventive agents

- Blocking agents
  - Flavonoids
  - Indoles
  - Isothiocynates
  - Diallyl sulfides
  - D-limonene

Cancer Producing Compounds

Blocking Agents

Cells Attacked By Cancer Producing Compounds

 Suppressing Agents

Cancer

Wattenburg, 1993
MECHANISM OF ACTION
(BLOCKING AGENTS)

1. Inhibit the formation of the active carcinogen.
2. Increase the rate at which the active carcinogen is inactivated.
3. Act as scavengers for the active forms of carcinogens.

Chemopreventive agents

• Suppressing agents
  – D-limonene
  – Diallyl sulfides
  – vitamin D
  – vitamin A and retinoids
  – monoterpenes
  – carotenoids
  – polyphenols

Anticarcinogenic mechanisms

• Antioxidant effects
• Increased activity of enzymes that detoxify carcinogens
• Effect on cell differentiation
• Blocked formation of nitrosamines
• Altered estrogen metabolism
• Decreased cell proliferation
• Maintenance of normal DNA repair

THREE-PHASE MECHANISM FOR CHEMICAL CARCINOGENESIS

1. INITIATION - normal cells to latent tumor cells.
2. PROMOTION - latent tumor cells to carcinoma in situ.
3. PROGRESSION - carcinoma in situ to invasive carcinoma.

Health Promoting Compounds

• Carotenoids
  – Lycopene -Prostate Cancer
  – Beta carotene
  – Lutein and zeaxanthin-Blindness
  – Beta cryptoxanthin
**Carotenoid Concentrations**

- Lycopene - Grapefruit
  - 3362 ug/100 g wet wt (Mangels et al., 1993)
  - 350 ug/100 g wet wt (Gross et al., 1987)

<table>
<thead>
<tr>
<th>Food</th>
<th>Lycopene content mg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato (fresh)</td>
<td>0.08-4.20</td>
</tr>
<tr>
<td>Grapefruit (raw pink)</td>
<td>3.36</td>
</tr>
<tr>
<td>Tomato (cooked)</td>
<td>3.7</td>
</tr>
<tr>
<td>Tomato (juice)</td>
<td>6.2</td>
</tr>
<tr>
<td>Tomato paste</td>
<td>5.40-150.00</td>
</tr>
<tr>
<td>Tomato soup, condensed</td>
<td>7.99</td>
</tr>
<tr>
<td>Tomato powder, drum or spray</td>
<td>11.63-126.49</td>
</tr>
<tr>
<td>Tomato Juice</td>
<td>5.00-11.60</td>
</tr>
<tr>
<td>Guava (fresh)</td>
<td>5.4</td>
</tr>
<tr>
<td>Watermelon</td>
<td>2.3</td>
</tr>
<tr>
<td>Papaya (fresh)</td>
<td>2.00-5.30</td>
</tr>
<tr>
<td>Ketchup</td>
<td>9.90-13.44</td>
</tr>
</tbody>
</table>

Lycopene Variation Among Texas Grapefruits

- Star Ruby
- I-48
- Henderson
- Ho Red
- Ruby
- Ruby
- Red Thames
- Marsh
- Duncan

Beta Carotene
**Limonoids with Anticancer Activity**
- Limonin
- Limonin 17-β-D-glucopyranoside
- Limonin carboxymethoxime
- Deoxylimonin

**Inactive Limonoids**
- Limonol
- Deoxylimonic acid
- Ichangensin
- 17,19-didehydrolimonoic acid
- Nomilinic acid 17-β-D-glucopyranoside

**Limonoids with Partial Activity**
- Nomilin
- Nomilin 17-β-D-glucopyranoside
- Obacunone

**Epidemiological Evidence**
1. Oral cavity.
2. Larynx.
3. Esophagus.
4. Stomach.
5. Pancreas.
7. Colon.

**Limonoid Glucosides**
1. Tasteless.
2. Soluble in water.
3. Human consumption (already present in citrus and citrus products in relatively high concentrations).
4. Can be prepared from by-products of juice processing plants (seeds and citrus molasses).
**Limonoid Concentrations**

- Limonoid glucosides
  - Limonin 17-beta D-glucopyranoside (54-180 ppm)
  - Oranges-320 ppm
  - Grapefruit -195 ppm
  - Lemon-90 ppm
- LG 1000
GST in Intestine for Glucoside Groups

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Water</th>
<th>GluMixA</th>
<th>GluMixB</th>
<th>Naringin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sp Act (μmol/min/mg protein)</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Isolation, Purification and Characterization of Putative Bioactive Compounds

Isolated Citrus Bioactive Compounds

- Limonoids and Flavonoids
- Limonoid Aglycone
- Limonoid Glucosides

Phase I Enzymes CYP450's

Phase II Enzymes GST and QR

Animal Studies

Cell Culture

Colon polyp

Isolation and Characterization Bioactive Compounds
Colon Cancer

Experimental Diets Significantly Reduced Total ACF Number

**Objective**

To investigate the possibilities of various citrus limonoids and flavonoids to reduce plasma cholesterol.

**Total Antioxidant capacity**

- ORAC - Oxygen Radical Absorbance Capacity can be measured by COBAS FARA II analyzer.
Antioxidant Activity of Fruits


Grape
Grapefruit
Tomato
Orange
Apple

ORAC (micromoles Trolox eq./ml)

Strawberry
Plum
Orange
Grapefruit
Grapefruit
Tomato
Grape
Grape

ORAC (micromoles of Trolox equivalents/g of dry matter)

Pectin

• Used traditionally for jelly preparation
• Modified pectin can prevent prostate cancer
• Pectin can reduce levels of serum cholesterol

How much we need?

• To consume about 6g of pectin only about 170 g of grapefruit pulp is sufficient (Baker, 1994)

Pectin Content in Different Fruits (% fresh wt)

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Pectin Content (% fresh wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apples</td>
<td>0.71-0.84</td>
</tr>
<tr>
<td>Apricots</td>
<td>0.71-1.32</td>
</tr>
<tr>
<td>Bananas</td>
<td>0.59-1.28</td>
</tr>
<tr>
<td>Beans</td>
<td>0.27-1.11</td>
</tr>
<tr>
<td>Blackberries</td>
<td>0.68-1.19</td>
</tr>
<tr>
<td>Carrots</td>
<td>1.17-2.92</td>
</tr>
<tr>
<td>Cherries</td>
<td>0.24-0.54</td>
</tr>
<tr>
<td>Dewberries</td>
<td>0.51-1.00</td>
</tr>
<tr>
<td>Grapes</td>
<td>0.09-0.28</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>3.30-4.50</td>
</tr>
<tr>
<td>Limes</td>
<td>2.8-2.99</td>
</tr>
<tr>
<td>Loganberries</td>
<td>0.59</td>
</tr>
<tr>
<td>Oranges</td>
<td>2.34-2.38</td>
</tr>
<tr>
<td>Raspberries</td>
<td>0.97</td>
</tr>
<tr>
<td>Squash</td>
<td>1.00-2.00</td>
</tr>
</tbody>
</table>

Baker, 1997

Dietary Fiber

Dietary fiber denotes all plant cell wall components that cannot be digested by an animal's own enzymes.

• Pectin
• gums
• lignin
• Cellulose
• Hemicellulose
• pentosans
Citrus Pectin Health Benefits

- Modified Citrus Pectin (MCP) prevent cancer metastasis, inhibiting cancer cell proliferation
- Hypoglycemic Effect
- Hypocholesterolemic Effect
- Hemostasis
- Modulate human immune function
- Detoxification

Steps in the process of tumor dissemination

Schematic representation of aggregation of tumor cell to normal cell and pectin function

Raz and LoTan, 1987

Probable mechanism of pectin hypocholesterolemic effect

Farnandez, et al., 1990

Pectin Hypocholesterolemic Action

Pectin can shorten the coagulation time of blood and act as an antagonist of heparin when injected intravenously.

Pectin sulfate can behave as strong anticoagulant.
Parts of the citrus fruit which contain pectin

Components of Pectin
- Molecular Weight
- Polygalacturonic Acid
- Galacturonic Acid Content
- Methoxyl Esters
- Neutral Sugar Content
- Ions
- Proteins

Structure of Pectin

Fibroblast Growth Factor Signaling System
- Cell needs to communicate to each other.

Factor-Receptor
- Side Chain
- Linear galacturonan
- Rhamnogalacturonan
**Inhibitors (Heparin Mimics)**

- Suramin
- Suramin analogs
- Pentosan polysulfate
- Carrageenans
- Dextran
- Dextran derivatives

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**FPLC Elution Profile**

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**Variation of Pectin Content and Composition in Different Citrus Species**

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**Pectin Extraction Rate in Different Citrus Species**
Changes of Pectin Content and Composition Due to Harvest Season

Variation of Pectin Sugar Content

In Vitro Effects of Citrus Pectin on the FGF Signaling System

Pectin Extraction Rate

Variation of pectin sugar composition

Pectin Inhibit FGF-1 Binding to FGFR1
Pectin Inhibit FGF-1 Binding to FGFR1

% of Maximum Binding

Heparin Concentration (µg/ml)

Inhibition activities of pectin

Inhibition Activity

Citrus Species

Lemon Grapefruit Tangerine Orange

Inhibition activities of pectin

Foods with cancer preventative properties

Case Control and Cohort Studies of All Types of Cancer

Fruit     No. of studies
          Inverse  Positive
Vegetables 55       9
Fruits     29       5
Tomatoes   35      10
Carrots    50      7
Citrus Fruit 26      6

Exploring Potential Drug Interaction with Texas Rio Red Grapefruit
Variation of Furocoumarins in Different Varieties

- 9 different varieties are analyzed for the quantitative and qualitative differences in Furocoumarins.
- Bottom line: Optimization of furocoumarin levels.

Varieties Used for this Study...

Rio Red  Thompson Pink
Star Ruby  Duncan
Ray Red  Marsh White
Henderson  Pumello
Ruby Red

Variation of DHB in different Varieties

Variation of Bergamottin in different Varieties

Variation of GF-I-1 in different Varieties.