Citrus Phytochemicals
Designer Foods
Functional Foods
Hypernutritious Foods
Nutraceuticals
Nutraceuticals

Nutraceuticals is a generic description of food composites containing natural and biologically active phytochemicals with disease-preventing and life-sustaining functions alone or in combination.
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.
Cancer Producing Compounds

Blocking Agents

Cells Attacked By Cancer Producing Compounds

Suppressing Agents

Cancer

Wattenburg, 1993
Chemopreventive agents

• Blocking agents
  – Flavonoids
  – Indoles
  – Isothiocynates
  – Diallyl sulfides
  – D-limonene
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.
Initiation

Promotion

Phase I enzymes

Oxidative damage
Free radicals

Lycopene, Lutein, Zeaxanthin and Beta carotene

Phase II enzymes

Glutathione S-transferase
Limonoids
Flavonoids

DNA Damage

Procarcinogens
Carcinogens
Electrophiles

Tumor
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>Carotenoids</th>
<th>Oranges</th>
<th>Pink Grf</th>
<th>White Grf</th>
<th>Mandarins</th>
<th>Lemons</th>
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<tr>
<td>Beta Carotene</td>
<td>39</td>
<td>1310</td>
<td>14</td>
<td>38</td>
<td>3</td>
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<td>Alpha Carotene</td>
<td>20</td>
<td>0</td>
<td>1</td>
<td>20</td>
<td>0</td>
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<tr>
<td>Lutein + Zeaxanthin</td>
<td>14</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>12</td>
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<td>Lycopene</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>Beta Cryptoxanthin</td>
<td>149</td>
<td>0</td>
<td>0</td>
<td>106</td>
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<td>Food</td>
<td>Lycopene content mg/100g</td>
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<td>-------------------------------</td>
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<tr>
<td><strong>Tomato (fresh)</strong></td>
<td>0.88-4.20</td>
<td></td>
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<tr>
<td><strong>Grapefruit (raw pink)</strong></td>
<td>3.36</td>
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<td>Tomato (cooked)</td>
<td>3.7</td>
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<td>Tomato (sauce)</td>
<td>6.2</td>
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<td>Tomato Paste</td>
<td>5.40-150.00</td>
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<td>Tomato soup, condensed</td>
<td>7.99</td>
<td></td>
<td></td>
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<tr>
<td>Tomato Powder, drum or spray</td>
<td>112.63-126.49</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>dried</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Tomato Juice</td>
<td>5.00-11.60</td>
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<tr>
<td>Guava (fresh)</td>
<td>5.4</td>
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<tr>
<td>Watermelon</td>
<td>2.3</td>
<td></td>
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<tr>
<td>Papaya (fresh)</td>
<td>2.00-5.30</td>
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<tr>
<td>Ketchup</td>
<td>9.90-13.44</td>
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</tbody>
</table>

Lycopene Variation Among Texas Grapefruits

- Star Ruby
- I-48
- Rio Red
- Ray Ruby
- Henderson
- Ruby Red
- Thomson Pink
- Marsh White
- Duncan White

ppm
Beta carotene

Lycopene

Texas

Florida
LIMONOIDS WITH ANTICANCER ACTIVITY

- Limonin
- Limonin 17-β-D-glucopyranoside
- Limonin carboxymethoxime
- Deoxyxylumonin
INACTIVE LIMONOIDS

- Limonol
- Deoxylimonmonic acid
- Ichangensin
- 17,19-didehydrodilimononoic acid
- Nomilinonic acid 17-ß-D-glucopyranoside
LIMONOIDS WITH PARTIAL ACTIVITY

- Nomilin
- Nomilin 17-β-D-glucopyranoside
- Obacunone
EPIDEMILOGICAL EVIDENCE

1. oral cavity.
2. larynx.
3. esophagus.
4. stomach.
5. pancreas.
6. lung.
7. colon.
8. rectum.
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
**Biological Activity of Citrus Limonoids**

- Anticarcinogenic activities (Lam and Hasegawa, 1989; Lam et al., 1989, 1994; Miller et al., 1989; Gutherie et al., 1997, 1998)
- Act as natural pest control agents (Alford et al., 1986; Klocke and Kubo, 1987)
- Excellent chemotaxonomic markers (Hasegawa and Ifuku, 1994)
Cancer cells used (MTT method)

- The HL-60 (human leukemia cancer)
- SKOV3 (human ovary cancer)
- Hela (human cervical cancer cells)
- BGC-823 (human stomach cancer)
- Bel-7402 (human liver cancer)
- MCF-7 human breast cancer cell
Effect of different limonoids on the percentage of viability MCF-7 cells affected by limonoids.
Foods with cancer preventative properties

- Garlic
- Cabbage
- Licorice
- Soybeans
- Ginger
- Umbelliferae (carrots, celery, parsnips)
- Onions
- Tea
- Turmeric
- Citrus (orange, lemon, grapefruit)
- Whole Wheat
- Flax
- Brown Rice
- Solanaceae (tomato, eggplant, peppers)
- Cruciferous (broccoli, cauliflower, Brussels sprouts)
- Oats
- Mints
- Oregano
- Potato
- Tarragon
- Cucumber
- Thyme
- Barley
- Chives
- Berries

Increasing importance
Health Promoting Compounds

- Flavonoids - Breast cancer and heart diseases
  - Naringin
  - Hesperetin
Antioxidant Activity

• Reactive oxygen Species (ROS) play major role many diseases. To counteract ROS and prevent their possible damage to biological molecules all oxygen-consuming organisms have antioxidant systems.

• Antioxidant enzymes: superoxide dismutase, catalase, and glutathione peroxidase.
Total Antioxidant capacity

• ORAC- Oxygen Radical Absorbance Capacity can be measured by COBAS FARA II analyzer
ORAC of commercial orange juice and vitamin C from grape, grapefruit, tomato, orange, and apple.

Antioxidant Activity of Fruits

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 (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apples</td>
<td>0.71-0.84</td>
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<tr>
<td>Apricots</td>
<td>0.71-1.32</td>
</tr>
<tr>
<td>Bananas</td>
<td>0.59-1.28</td>
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<tr>
<td>Beans</td>
<td>0.27-1.11</td>
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<tr>
<td>Blackberries</td>
<td>0.68-1.19</td>
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<tr>
<td>Carrots</td>
<td>1.17-2.92</td>
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<tr>
<td>Cherries</td>
<td>0.24-0.54</td>
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<tr>
<td>Dewberries</td>
<td>0.51-1.00</td>
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<tr>
<td>Grapes</td>
<td>0.09-0.28</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>3.30-4.50</td>
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<tr>
<td>Lemons</td>
<td>2.8-2.99</td>
</tr>
<tr>
<td>Loganberries</td>
<td>0.59</td>
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<tr>
<td>Oranges</td>
<td>2.34-2.38</td>
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<tr>
<td>Raspberries</td>
<td>0.97</td>
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<tr>
<td>Squash</td>
<td>1.00-2.00</td>
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</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 hypocholesterol effect

Farnandez, et al., 1990
Pectin Hypocholesterolemic Action
Pectin Hemostasis Function

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
Structure of Pectin

- Linear galacturonan
- Rhamnogalacturonan
- Side Chain
Modified Citrus Pectin

- Mol Wt should be less than 10 KD
- MC should be less than 8%
- Galactose and uronic acid may enhance the effect
- Higher Mol Wt and higher MC increase its hypocholesterol effect.

Pienta et al., 1995; Briggs 1997
Fibroblast Growth Factor Signaling System
Factor-Receptor

• Cell needs to communicate to each other.
• Fibroblast growth factor (FGF) is ubiquitous and a mediator of developmental processes in the embryo and homeostasis.

• Inappropriate FGF signal transduction may contribute to defect, tumor growth, cardiovascular disease, diabetes, etc.
• FGF factor, currently 19 gene products
• FGF receptor, (Transmembrane tyrosine kinases) currently 4 gene products
• Heparan sulfate proteoglycans (FGFRHS)
Stimulation

Inhibition

No Stimulation
No Inhibition
Inhibitors (Heparin Mimics)

- Suramin
- Suramin analogs
- pentosan polysulfate
- Carrageenan
- Dextran
- Dextran derivatives
FPLC Elution Profile
Variation of Pectin Content and Composition in Different Citrus Species
Pectin Extraction Rate in Different Citrus Species

Extraction Rate (% of fresh weight)

- Lem
- Tan
- Org
- Gra

F/A: Fresh to Air
L: Liquid
Changes of Pectin Content and Composition Due to Harvest Season
Variation of pectin sugar composition

Percentage (% of total sugar wt.)

Harvest Month

Aug Sep Nov Jan Mar May

rhamnose
arabinose
xylose
mannose
galactose
glucose
In Vitro Effects of Citrus Pectin on the FGF Signaling System
Pectin Inhibit FGF-1 Binding to FGFR1

Specific bound (% of control)

Pectin Concentration (µg/ml)
Pectin Inhibit FGF-1 Binding to FGFR1

Heparin Concentration (µg/ml)

% of Maximum Binding

- Pectin Added
- Heparin Only
Autoradiography of pectin inhibition activity
Inhibition activities of pectin

- Lemon Grapefruit Tangerine Orange

Citrus Species

Inhibition Activity

- Flavedo/Albedo
- Lamella
Inhibition activities of pectin

Inhibition Activity

AUG SEP NOV JAN MAR MAY
Foods with cancer preventative properties

Increasing importance

- Garlic
- Cabbage
- Licorice
- Soybeans
- Ginger
- Umbelliferae (carrots, celery, parsnips)
- Onions
- Tea
- Turmeric
- Citrus (orange, lemon, grapefruit)
- Whole Wheat
- Flax
- Brown Rice
- Solanaceae (tomato, eggplant, peppers)
- Cruciferous (broccoli, cauliflower, Brussels sprouts)
- Oats
- Mints
- Oregano
- Potato
- Cucumber
- Thyme
- Barley
- Chives
- Berries
- Cantaloupe
- Basil
- Tarragon
- Rosemary
### Case Control and Cohort Studies of All Types of Cancer

<table>
<thead>
<tr>
<th>Fruit</th>
<th>No. of studies</th>
<th>Inverse</th>
<th>Positive</th>
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<tr>
<td>Vegetables</td>
<td>55</td>
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<td>9</td>
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<td>Fruits</td>
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<td>Tomatoes</td>
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<tr>
<td>Carrots</td>
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<tr>
<td>Citrus Fruit</td>
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</table>
Phytochemicals in Fruits and Vegetables to Improve Human Health

Project Director: Bhimu Patil

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Start date: Spring 2001 http:// Phytochemicals.tamu.edu