Antioxidants in Fruits and Vegetables

AO = antioxidant
AOC = antioxidant capacity
Fruit, Vegetables & Health

Fruit & vegetable consumption reduces disease risk

**HOW?**

- Essential vitamins & minerals
- Fiber
- Phytochemicals
  - Carotenoids
  - Polyphenols
  - Tocopherols
  - Ascorbate
  - Isothiocyanates

Antioxidants
Fruit & Vegetable Antioxidants

- Certain cancers
  - Block et al. 1992; Steinmetz & Potter 1996

- Cardiovascular Disease
  - Hertog et al. 1993; Joshipura et al. 1999

- Neurodegenerative disease
  - Kang JH et al. 2005

“...the protection provided against diseases by fruits and vegetables has been attributed to the various antioxidants contained in these foods.” (Ames et al., 1993)
Oxidative Stress in Disease & Aging

- Accumulation of deleterious changes to biological molecules
- Change/damage to DNA, lipids, protein
- Leading to disease & aging
- Due to Oxidative Stress
- Need for biomarkers of oxidative damage linked to disease
The Antioxidant ‘Arms Race’

(Antioxidant capacities per gram of fresh wt.)

Source: 1997 Research at the Jean Mayer USDA Human Nutrition Research Center on Aging, on the antioxidant characteristics of various fruits and vegetables. Journal of Agricultural and Food Chemistry 44:701-705; 3426-3343
Antioxidant Research at AAFC

- Use existing tools to create an inventory of AAFC antioxidant research*
- Survey antioxidant activity *in vitro*
  - Cell-based and non cell-based assays
- Chemical profiling
  - Correlate chemical composition with AOC
- Determine impact of production & processing on AO compounds and AOC
- Animal and clinical studies

* [http://www.fao.org/agrovoc](http://www.fao.org/agrovoc)  FAO’s AgroVoc
F&V AO Research

For each phytochemical AO consider…

• Genetic variation (within & among species)
• Tissue distribution (e.g. seeds, peels)
• Environmental factors (e.g. light, water, disease)
• Effect of maturity (e.g. green vs. ripe red fruit)
• Removal and degradation during processing & storage (e.g. waste products, oxidation)
• Impact of complex food matrix
• Multiple forms (e.g. isomers, esters)
• Antioxidant capacity of in vivo metabolites
Polyphenolic Antioxidants
How well are polyphenol AO absorbed?

adapted from Manach et al. 2005 AJCN & Williamson 2006 Experimental Biology

maximum plasma concentration
## Serum Antioxidants

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid (vitamin C)</td>
<td>Water</td>
<td>50 – 60</td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>Water</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Lipoic acid</td>
<td>Water</td>
<td>0.1 – 0.7</td>
<td></td>
</tr>
<tr>
<td>Uric acid</td>
<td>Water</td>
<td>200 – 400</td>
<td></td>
</tr>
<tr>
<td>Carotenes</td>
<td>Lipid</td>
<td>β-carotene: 0.5 – 1 retinol (vitamin A): 1 – 3</td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (vitamin E)</td>
<td>Lipid</td>
<td>10 – 40</td>
<td></td>
</tr>
<tr>
<td>Ubiquinol (coenzyme Q)</td>
<td>Lipid</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

versus transient serum polyphenol
Cmax of about 0.02 – 2.0 µM
Polyphenol concentrations in vivo are too low to contribute to direct AO protection

- So how do they work?
  - Substantial in vivo and clinical evidence
- Cardioprotection
- Neuroprotection
- Glucoregulation
- Anti-cancer activity
- Direct AO action in the GI tract?
Actions of Polyphenols *in vivo*

- Cell cycling
- Detoxification (Phase II) pathways
- Antioxidant pathways
- Inflammatory Pathways
  Immunomodulation via GALT?
- Mediated through
  - Kinase modulation
  - Cell signal transduction pathways
  - Effects on gene expression
  - Effects on specific enzymes
Antioxidants in Cereal Grains: Highlights of Current Research at AAFC-AAC

Antioxidant Workshop Web Conference
February 3rd, 2010

F. William Collins, ECORC, Ottawa, ON
OVERVIEW

- Summaries of selected AAFC efforts:
  
  E Abdelaal: Anthocyanins in wheat  
  FW Collins: Avenanthramides in oats  
  A Muir: Flaxseed lignans  
  D Oomah: Antioxidants in cereals  

- Consider: Potential roles and opportunities for AAFC?:
  
  Knowledge-based antioxidant research?  
  Building Capacities, (Alliances, Partnerships)  
  Primary Research and/or Product Revelopment?  
  Commodity Frameworks, Connections, Foresighting
Innovation streams

- Inherently Functional Foods (e.g. Fruits & Vegetables)
- Fortified/Man Made Functional Foods (e.g. Baked Goods)
- Nutraceuticals, Natural Health Products (e.g. Extracts, Pills Herbals, etc)

Consider: Where are our efforts focused?

Nutrition Pipeline – Products Evaluation Platform

- Bioactives Research
- In vitro Evaluation
- Cells & Tissue Evaluation
- Animal Models
- Human Studies & Clinical Trials

Priority 1. Enhancing human health and wellness through food, nutrition and innovative products
“Creating opportunities through innovative products”

2 of 3 areas of research associated with antioxidants

1. Grain-based high-lutein functional foods
2. High-antioxidant whole grain foods
3. Anthocyanin-rich products

2. High-antioxidant whole grain foods:
   Characterizing and quantizing targeted compounds

3. Anthocyanin-rich products:
   a) Blue or purple wheat bran – mechanical separation (milling)
   b) Anthocyanin powder (mechanical and chemical separation)
### Primary anthocyanins in blue and purple wheat (μg/g)

<table>
<thead>
<tr>
<th>Anthocyanin</th>
<th>blue wheat</th>
<th>Anthocyanin</th>
<th>purple wheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dp-3-Glu</td>
<td>56.5</td>
<td>Cy-3-Glu</td>
<td>4.0</td>
</tr>
<tr>
<td>Dp-3-Rut</td>
<td>49.6</td>
<td>Pn-3-Glu</td>
<td>2.1</td>
</tr>
<tr>
<td>Cy-3-Glu</td>
<td>20.3</td>
<td>Cy-SucGlu</td>
<td>1.8</td>
</tr>
<tr>
<td>Cy-3-Rut</td>
<td>16.8</td>
<td>Cy-MalGlu</td>
<td>1.2</td>
</tr>
<tr>
<td>Total</td>
<td>153.1</td>
<td>Total</td>
<td>12.8</td>
</tr>
</tbody>
</table>

### 3. ANTHOCYANIN-RICH PRODUCTS:

**b) Blue wheat – mechanical separation (milling) vs extracted**

#### Content of anthocyanins in blue wheat products

<table>
<thead>
<tr>
<th>Product</th>
<th>µg/g</th>
<th>Fold increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole grain</td>
<td>178</td>
<td>-</td>
</tr>
<tr>
<td>Bran fraction</td>
<td>405</td>
<td>2.3</td>
</tr>
<tr>
<td>Pearled grain</td>
<td>41</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol extracted anthocyanin powder</td>
<td>39870</td>
<td>224</td>
</tr>
</tbody>
</table>

### 3. ANTHOCYANIN-RICH PRODUCTS:

a) Blue wheat – mechanical separation (milling), extracted

Anthocyanin composition of blue wheat powdered extract

<table>
<thead>
<tr>
<th>Compound</th>
<th>μg/g</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dp-3-Glu</td>
<td>14,130</td>
<td>44.0</td>
</tr>
<tr>
<td>Cy-3-Glu</td>
<td>9,030</td>
<td>28.1</td>
</tr>
<tr>
<td>Dp-3-Rut</td>
<td>6,840</td>
<td>21.3</td>
</tr>
<tr>
<td>Cy-3-Rut</td>
<td>550</td>
<td>1.7</td>
</tr>
</tbody>
</table>

### 3. ANTHOCYANIN-RICH PRODUCTS:

**Antioxidant properties of anthocyanin products**

<table>
<thead>
<tr>
<th>Product</th>
<th>DPPH(^{-}) (µmol/g) (30 min)</th>
<th>ABTS(^{+}) (µmol/mg) (10 min)</th>
<th>LDL oxidation (CD formation µmol/g)(4 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole grain</td>
<td>36.0</td>
<td>30.7</td>
<td>9.6</td>
</tr>
<tr>
<td>White flour</td>
<td>40.4</td>
<td>45.0</td>
<td>2.7</td>
</tr>
<tr>
<td>Bran fraction</td>
<td>45.8</td>
<td>57.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Anth. powder</td>
<td>2526</td>
<td>2925</td>
<td>0.06</td>
</tr>
<tr>
<td>Dp-3-Glu</td>
<td>831</td>
<td>382</td>
<td>0.04</td>
</tr>
<tr>
<td>Cy-3-Glu</td>
<td>1232</td>
<td>1015</td>
<td>0.31</td>
</tr>
<tr>
<td>Dp-3-Rut</td>
<td>613</td>
<td>430</td>
<td>0.05</td>
</tr>
<tr>
<td>Cy-3-Rut</td>
<td>4346</td>
<td>3261</td>
<td>0.31</td>
</tr>
<tr>
<td>Control (no antioxidants)</td>
<td>-</td>
<td>-</td>
<td>381</td>
</tr>
</tbody>
</table>

**Avenanthramides**: the group of about 35-40 phenolic alkaloids from *Avena* occurring as amide conjugates of substituted *anthranilic* acids

Only found in oats; not present in any other monocot cereal or oilseed.

Subject of at least 8 US and 4 European patents:
- mostly for topical treatment of skin disorders
- based on avenanthramides anti-irritant activity
- 2 on anti-inflammatory activity for therapeutics
## Antioxidant properties of avenanthramides*

<table>
<thead>
<tr>
<th>Compound</th>
<th>DPPH (µmol/g) (30 min)</th>
<th>FRAP assay (EC₁ µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avenanthramide A</td>
<td>19,060 ± 670</td>
<td>343 ± 12.5</td>
</tr>
<tr>
<td>Avenanthramide B</td>
<td>10,030 ± 300</td>
<td>442 ± 2.2</td>
</tr>
<tr>
<td>Avenanthramide C</td>
<td>19,370 ± 950</td>
<td>275 ± 0.1</td>
</tr>
<tr>
<td>Avenanthramide D</td>
<td>0 ± 0</td>
<td>2,901 ± 9.3</td>
</tr>
<tr>
<td>Avenanthramide E</td>
<td>5,750 ± 0</td>
<td>697 ± 0.91</td>
</tr>
<tr>
<td>Avenanthramide F</td>
<td>10,700 ± 670</td>
<td>393 ± 1.7</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>10,980 ± 1,220</td>
<td>2,419 ± 12.4</td>
</tr>
<tr>
<td>Ferulic acid</td>
<td>16,490 ± 1,030</td>
<td>423 ± 1.2</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>31,110 ± 560</td>
<td>362 ± 0.4</td>
</tr>
</tbody>
</table>

Lee-Manion, AM et al, J Agric Food Chem 57 (2009), 10,619
TEAM COLLABORATORS

AAFC
- Process Development,
- Analytical & Structural Chemistry,
- Germplasm Development

F. William Collins
Vern Burrows
Nicole Fillion

USDA TUFTS UNIVERSITY
- Vascular Biology Lab:
  - In vitro studies
  - Mohsen Medani
  - Liping Liu
  - Ligia Zubik
  - Melissa Marko
  - Weimin Guo
  - Mitch Wise (U of W)

Antioxidant Research Lab:
- In vivo human studies
  - Jeffrey Blumberg
  - Paul Milbury
  - Chung-Yen Chen
  - Ting Li
  - Jennifer O’Leary
ANTIATHEROGENIC ACTIVITY IN VITRO

Avenanthramides (C, mixed avenanthramides, ~ 15 ppm) inhibited production of pro-inflammatory cytokines in vascular intima tissues

Avenanthramide C (~ 25 ppm) inhibits vascular smooth muscle cell proliferation in rat aortal tissue

Avenanthramide C (~ 25 - 40 ppm) reverses impaired nitrous oxide (NO) production and inhibits vascular smooth muscle cell proliferation in human aortal endothelial cells

Avenanthramides (C, mixtures, ~ 25 -100 ppm) inhibit activation (phosphorylation) of 2 of the 5 NF-κB subunits

Significantly inhibit proliferation of several colonic cancer cell lines in the magnitude order of HCT116>CaCo-2>LS174>HT29.
CONCLUSIONS FROM BIOACTIVITY STUDIES

- Daily consumption of oat products rich (?) in avenanthramides may play an important role in prevention of chronic inflammatory disease progression
- May play a role in colonic cancer prevention

Purified avenanthramides are bioavailable in humans*
- rapidly taken up from oral administration: $T_{max} \sim 2$hr
- bioavailability estimate: $\sim 0.1\%$ acute dosage

*Chen et al, J Nutr 137: (2007) 1375
AVENANTHRAMIDE PROFILE; WHOLE KERNEL

Apigenin and ferulic acid minor components

Avenan. A, B, & C dominate profile

Over 20 different avenan. detected
### The Problems with Regular Oats

#### Total Avenanthramides in dehulled oats

- **7 Quaker preferred (covered) varieties (Western Canada)**
  - 2002: $2.13 \pm 0.85$ to $23.4 \pm 0.2$ ppm
  - Collins (2004) contract research
- **16 covered varieties (US midwest)**
  - 2005: 2.2 to 11.5 ppm
- **4 hull-less lines (Eastern Canada)**
  - 2006: 1.2 to 33.2 ppm
  - Collins (2007) unpublished
  - 2002-2006: 10.9 to 187 ppm

These current levels are undoubtedly **too low to be effective in any of the bioactivity indicators studied**.
THE PROBLEMS WITH REGULAR OATS

- Large differences in year-to-year levels
- Large genotype X environment differences
- Efforts in Sweden and Finland to increase these levels through processing (steeping, malting, de-branning etc) have been published or are currently patent pending
- Most current technologies rely on malting as a method of increasing avenanthramides
- These disclosures generally show levels can be increased up to about 130% of the starting levels in dry kernels (~2.3-fold)
THE PROBLEMS WITH CURRENT TECHNOLOGIES

1. Still relatively low levels of avenanthramides (typically >250ppm)

2. **Product is germinated oat sprouts roots, shoots and partially-depleted malted kernels.**

3. Most technologies based on “covered” oats (must be de-hulled to produce an edible product)

4. De-hulling and polishing prior to malting can severely compromise kernel integrity = deterioration during malting

5. De-hulling and polishing after malting costly and inefficient limiting commercial potential

Therefore: Using hulless, bald varieties (VAO varieties and lines) may solve some of these problems.
AN INNOVATIVE SOLUTION: “FALSE” MALTING


- Describes processes for producing whole oats and oat-based ingredients with greatly elevated levels of health-promoting avenanthramides; “False Malting”
- Uses “dormant” oats (hulless or de-hulled covered oats) and novel modifications to otherwise standard malting conditions.
- Kernels are pre-treated with dry heat and anaerobic steeping to render them completely dormant but still viable (GA₃-reversible)
- Describes methods for rendering non-dormant oats dormant and thus suitable for the present invention.
AVENANTHRAMIDE PROFILES; WHOLE KERNEL (UN-MALTED vs MALTED)

HPLC profile hulless, hairless, dormoat

Virtually the same profile qualitatively

Total aven. content increased by 2,900 % over control (30 fold)
**Dry fractionation (e.g. Satake milling) to produce bran fractions results in further enrichment of these “unique-to-oats” emerging, health-promoting avenanthramides.**

(e.g. malted VAO 48 Satake milled fractions)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Whole grain before malting (μg/g ± SD)</th>
<th>Whole grain after malting (μg/g ± SD)</th>
<th>10% bran after malting (μg/g ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avenanthramide A</td>
<td>9.1 ± 0.2</td>
<td>186 ± 10.1</td>
<td>336 ± 11.6</td>
</tr>
<tr>
<td>Avenanthramide B</td>
<td>10.8 ± 0.5</td>
<td>325 ± 9.9</td>
<td>565 ± 5.5</td>
</tr>
<tr>
<td>Avenanthramide C</td>
<td>5.3 ± 1.1</td>
<td>303 ± 15.1</td>
<td>426 ± 10.7</td>
</tr>
<tr>
<td>All others</td>
<td>21.4 ± 1.9</td>
<td>1,056 ± 15.4</td>
<td>2,506 ± 19.5</td>
</tr>
<tr>
<td>Total</td>
<td>46.7 ± 2.5</td>
<td>1,879 ± 22</td>
<td>3,830 ± 24</td>
</tr>
</tbody>
</table>
Looking for an ingredient labelling allowance in USA (FDA)

- **Collaborating With Tufts University Under “Growing Forward”**

  a) Short term pharmacokinetic studies in human trials using dietary acceptable vehicle (oat bran test muffin):
  - bioavailability from food source
  - key bio-indicators of antioxidant status
  - potential toxicological/safety compliance
  - ingredient labeling allowance (FDA)

  b) Ongoing long term animal trials with transformed mouse model using supplemented diet feeding trials and antioxidant status indicators
  - Toxicological/safety compliance
  - potential formulation levels for optimum status delivery

- **Ongoing breeding for elite germ-plasm for IP capture**
  a) Evaluating early breeding lines for suitability in process
  b) Working with OIPC using RFP route for potential partners
Flaxseed Lignans

Diglucoside content ~ 10,000-40,000 μg/g
(cf cereals ~0.07-7.64 μg/g)

Bioactivities:
1) Antioxidant: ~ [BHT]
2) Precursor for mammalian lignans; e.g. enterolactone

Relative Bioavailability ?
Metabolic Conversions ?

\[ R = n \text{ fragment, } m = 1: \text{Secoisolariciresinol diglucoside hydroxymethylglutarate complex (native form)} \]

\[ n = 0 + H, m = 1: (+)-\text{Secoisolariciresinol-9,9'-diglucoside} \]

\[ m = 0 + 2H: (+)-\text{Secoisolariciresinol} \]
Biological Activities of Lignans

• Flaxseed lignans have been shown to have a significant beneficial effect in:
  – Cardiovascular Health
  – Diabetes
  – Cancer risk reduction

• Lignans have been shown to have significant antioxidant activity \textit{in vitro} and \textit{in vivo}
  – \textit{In vivo} mode of action is uncertain
    • Phase II enzyme inducer?
  – \textit{In vitro} antioxidant activity in food systems is of the same order of magnitude as BHT
• Cardiovascular Disease
  – Flaxseed and Lignan extracts:
    • Lots of Cell culture and Animal model studies demonstrating positive effects on CVD biomarkers and significant antioxidant activity
  – Flaxseed (10g to 50g/day) - Positive impact on a number of CVD biomarkers in human studies including:
    • LDL-Cholesterol (Bloedon & Szapary 2004, and Patade et al 2008)
    • Platelet aggregation (Bierenbaum et al 1993)
    • Lipoprotein a (Lucas et al 2002, Bloedon et al 2008)
    • Serum apoliporotein B (Lucas et al 2002)
    • Triglycerides (Lucas et al 2002)
HEALTH CLAIMS

• The most readily accessible health claims are Structure-function claims (helps maintain) or Risk reduction claims

• An example might be:
  – Flax lignans help maintain a healthy cholesterol level
  – Flax lignans help reduce the risk of developing prostate cancer
HEALTH CLAIM CHALLENGES

• What claim to pursue?
  – Cardiovascular – Metabolic Syndrome
    • Water soluble product that opens up many consumer product opportunities that are a challenge for fat soluble bioactives such as phytosterols
    • Clinical trail data available and more in the pipeline
    • Limited epidemiological data
      – All in populations with very low levels of dietary intake
    • Crowded market place
  – Cancer Risk Reduction
    • Epidemiological data inconclusive
      – All based on populations with very low levels of dietary intake
    • Clinical trials only possible in a treatment scenario
    • Difficult regulatory environment
HEALTH CLAIM CHALLENGES

• What level of evidence will be required to support the claim?
  – Clinical trials if possible
  – What will be acceptable for long latency health events such as cancer?
    • Can one extrapolate from treatment based clinical trials to prevention claims?

• What is the dose?
  – Not much dose information
  – Currently epidemiological data not much help
  – No identified habitual flax consuming population to study
CONCLUSIONS

• Diet intake studies indicate:
  – current consumption of flax lignans is not being measured;
  or
  – Flax lignan consumption on average is too low to measure

This represents a tremendous opportunity for the food industry
a) Even modest inclusion rates will have a significant impact on dietary intake
b) Epidemiological and clinical evidence would suggest even a modest increase in long term lignan consumption could have significant beneficial effects on chronic health conditions such as CVD, metabolic syndrome and cancer
“Wheat Quality: Antioxidant activity of wheat millstreams”

- Activities (3 cultivars of CRSW) attributed to phenolics and tocopherols of wheat, with bran and flour exhibiting the highest and lowest activity, respectively

“Antioxidant activity and total phenolics in selected fruits, vegetables and grain products”

- Activities and total phenolics flaxseeds, wheat germ, buckwheat
- Correlation coefficient between total phenolics and antioxidative activities statistically significant.
AAFC in the NFF System: antioxidants

Overview of the Canadian Agriculture and Agri-Food System 2008 (Statistics Canada and AAFC)
(www4.agr.gc.ca/AAFC-AAC/display-affiche.do?id=1228246364385&lang=eng)

Results from the Functional Foods and Natural Health Products Survey – 2007
(Statistics Canada, Working Paper)

Functional Foods and Natural Health Products Database AAFC 2009
(http://www3.agr.gc.ca/apps/ffn/index-eng.cfm)
Antioxidants Workshop
February 3, 2010

Antioxidants in Legumes and Herbs Grown in Canada

Rong Tsao (Rong Cao), Ph.D.

Guelph Food Research Centre, AAFC
93 Stone Road W., Guelph, Ontario, Canada N1G 5C9
Research Activities at AAFC - Legumes/Herbs

- PARC-Summerland, BC
  - Drs. Oomah, Mazza, Ross
- SCPFRC-London, ON
  - Dr. Dhaubhadel
- GFRC-Guelph, ON
  - Drs. Cao (Tsao), Shi
<table>
<thead>
<tr>
<th>Date</th>
<th>Achievement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>Antioxidant capacity and peroxyl radical scavenging activity of bean cultivars correlated highly with phenolic and phytic acid contents</td>
<td>J. Agric. Food Chem. 56: 11312-11319</td>
</tr>
<tr>
<td>2006</td>
<td>39 bean cultivars grown at 4 locations in Manitoba for 3 years were screened for phenolic components and antioxidant activity. Variation in antioxidant activity was mainly due to year and cultivar x year effects. Based on antioxidant activity, bean cultivars may be grouped into four distinct marketing classes in descending order, small, large, navy and white kidney beans.</td>
<td>PulseBeat 2004, 44, 27–28; Final Report to Manitoba Pulse Growers Association (MPGA)</td>
</tr>
<tr>
<td>2006</td>
<td>Lupin genotypes grown in Alberta exhibited weak antioxidant activity that was not related to phenolic contents of seeds</td>
<td>Plant Foods Human Nutr. 61: 91-97</td>
</tr>
<tr>
<td>2005</td>
<td>Two antioxidant systems (lipid peroxidation and DPPH) showed that bean hulls with maximum concentration of phenolics exhibited the strongest antioxidant activity</td>
<td>J. Sci. Food Agric. 85: 935-942</td>
</tr>
<tr>
<td>2005</td>
<td>Extracts of herbal blend from salvia miltiorrhiza, scutellaria barbata, ganoderma lucidum and sea buckthorn berries or leaves showed synergistic antioxidant activity</td>
<td>U. S. Patent 2005/0214394 A1</td>
</tr>
<tr>
<td>2002</td>
<td>Methanolic extracts of pearled bean samples exhibited antioxidant activity by inhibiting DPPH in a dose-dependent manner. Ethyl acetate/acetone and acetone extracted fractions displayed antioxidant, radical scavenging and antimutagenic activities</td>
<td>J. Agric. Food Chem. 50 (24): 6975-6980</td>
</tr>
</tbody>
</table>
Research Activities at London- S. Dhaubhadel

• Regulation of Isoflavonoid Synthesis in Soybean
  – Isoflavonoids accumulation in the seeds
  – Conjugating enzymes: glycosyltransferase; malonyltransferase
  – Factors that regulate isoflavonoid synthesis in soybean seeds and roots
• Anti-oxidative and anti-virus lectin in beans
• Definitions/background information
• Antioxidants-in vitro evidence
• Antioxidants-in vivo evidence
• Antioxidants-mechanisms
• Antioxidants-future perspectives/challenges
What is a legume?

- **A legume** is a plant in the family Fabaceae (or Leguminosae), or a fruit of these specific plants. A 'legume' fruit is a simple dry fruit that develops from a simple carpel and usually dehisces (opens along a seam) on two sides. A common name for this type of fruit is a pod. Well-known legumes include alfalfa, clover, peas, beans, lentils, lupins, mesquite, carob, soy, and peanuts.

http://en.wikipedia.org/wiki/Legume
What are Pulses?

• **Pulses** are the edible seeds of legumes, like lentils, beans, peas and chickpeas. Each of these pulse crops come in a wide range of colours and sizes. The name pulse is derived from the Latin *puls* meaning thick soup or potage.

• Soybean not included

http://www.pulsecanada.com/
Herbs/Native Plants?

- **Culinary herbs and spices** – e.g. oregano, rosemary, thyme, chili pepper
- **Traditional medicinal plants** – e.g. ginseng, clover, kudzu, epimedium, plantain
- **Native Plants** – e.g. Staghorn sumac, inside-out-flower, Osage orange
Approved Health Claims

1. Calcium, Vitamin D, and Osteoporosis
2. Dietary Lipids (Fat) and Cancer
3. Dietary Saturated Fat and Cholesterol and Risk of Coronary Heart Disease
4. Dietary Non-cariogenic Carbohydrate Sweeteners and Dental Caries
5. Fiber-containing Grain Products, Fruits and Vegetables and Cancer
6. Folic Acid and Neural Tube Defects
7. Fruits and Vegetables and Cancer
8. Fruits, Vegetables and Grain Products that contain Fiber, particularly Soluble fiber, and Risk of Coronary Heart Disease
9. Sodium and Hypertension
10. Soluble Fiber from Certain Foods and Risk of Coronary Heart Disease
11. **Soy Protein** and Risk of **Coronary Heart Disease**
12. Stanols/Sterols and Risk of Coronary Heart Disease

http://www.fda.gov/Food/LabelingNutrition/LabelClaims/HealthClaimsMeetingSignificantScientificAgreementSSA/default.htm
Health Claims-Canada

- Sodium and hypertension
- Calcium and osteoporosis
- Saturated and *trans*- fat and cholesterol and coronary heart disease
- Fruits and vegetable and Cancer
- Sugar alcohols and dental caries was apparent.
The Imbalance Causes Various Diseases

Oxidative Stress
(Free Radicals)

Brain:
- Alzheimer’s
- Parkinson’s
- Memory loss
- Depression
- Stroke

Joints:
- Arthritis
- Rheumatism

Lungs:
- Asthma
- Chronic bronchitis

Kidneys:
- Glomerulonephritis
- Chronic renal failure

Multi-Organs:
- Cancer
- Aging
- Diabetes
- Inflammation
- Infection

Heart-Vessels:
- Arteriosclerosis
- Hypertension
- Ischmeia
- Cardiomyopathy
- Heart failure

Eyes:
- Macula degeneration
- Cataract
- Retinal diseases

Fetus:
- Preeclampsia
- IU growth restriction
Phytochemical Antioxidants in Legumes

- Polyphenols
  - Phenolic acids
  - Flavonoids
    - Isoflavones
    - Anthocyanidins
    - Flavanols (Catechins)
    - Flavonols
  - Lignans
- Carotenoids
- Phytosterols
- Saponins
- Folic acid
- Phytic acids

- Other antioxidants
  - Peptides
  - Polysaccharides
Phenolic Antioxidants in Spices & Herbs

- Oregano & Carvacrol
- Clove & Eugenol
- Thyme & Thymol
- Rosemary & Rosemarinic acid
- Chili Peppers & Capsaicinoids
Isoflavones

daidzein

formononetin

orobol

glycitein

genistein

biochanin A

prunetin

pratensein

irilone

pseudobaptigenin

coumestrol

glyceollin

isolupalbigenin
Isoflavones in Red Clover and Kudzu

<table>
<thead>
<tr>
<th>Isoflavones</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochanin A</td>
<td>Me</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Formononetin</td>
<td>Me</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Genistein</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Glycetein</td>
<td>H</td>
<td>H</td>
<td>OMe</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>Daidzein</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>Glu</td>
</tr>
<tr>
<td>Puerarin</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
</tbody>
</table>

![Graph showing chromatograms of Isoflavones in Red Clover and Kudzu.](image)
Condensed flavonoids

Macz-Pop et al., J. Agric. Food Chem. 2006, 54, 536-542
Herbs/Native Canadian Plants
Saponins/Phyto-Myco-sterols

Soyasapogenol A

Ergosterol

Ginsenoside Rb1

Soybean

Mushrooms

north American ginseng
Prenylated Flavonoids and Isoflavonoids

Quercetin

Genistein

Icariin

Vancouveria hexandra

Pomiferin

Maclura pomifera
Phytochemicals as Antioxidants: 

The *in vitro* Evidence
Strong in vitro Antioxidant Capacities

- **Hydrogen Atom Transfer (HAT)**
  - *HAT-based methods* measure the classical ability of an antioxidant to quench free radicals by hydrogen donation
  - E.g., ORAC, PCL, β-CLAMS, Cu-induced LDL peroxidation

- **Single Electron Transfer (SET).**
  - *SET-based methods* detect the ability of a potential antioxidant to transfer one electron to reduce any compound, including metals, carbonyls, and radicals
  - E.g., FRAP, TPC

- **Methods based on both HAT and SET**
  - DPPH (AOAC?)

- **Cell-based antioxidant assay**
Antioxidant Activity-Carotenoids

Protection Against Superoxide Radical $O_2^{\cdot-}$

Lutein
Lycopene
β-Carotene

Trolox Equivalent (nmol)

## Anti-clastogenic activity – chromosomal aberration test / CHO

<table>
<thead>
<tr>
<th>Test compound</th>
<th>Concentration (mg/L)</th>
<th>S9</th>
<th>Duration (h)</th>
<th>Rate of teratogenicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>267.0</td>
<td>-</td>
<td>24</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>Lutein + mutagen</td>
<td>133.5</td>
<td>-</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>66.8</td>
<td>-</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>DMSO</td>
<td>50.0</td>
<td>-</td>
<td>24</td>
<td>26*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>6</td>
<td>28*</td>
</tr>
<tr>
<td>MMC</td>
<td>0.5</td>
<td>-</td>
<td>24</td>
<td>66**</td>
</tr>
<tr>
<td>CP</td>
<td>125.0</td>
<td>+</td>
<td>6</td>
<td>44**</td>
</tr>
</tbody>
</table>

*compared with test groups P<0.05; **compared with test groups P<0.05

## Anti-proliferation against Esophageal carcinoma EC9706 cells

### Rate of Proliferation

<table>
<thead>
<tr>
<th>(μg/mL)</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td><strong>PR (%)</strong></td>
<td><strong>A</strong></td>
<td><strong>PR (%)</strong></td>
<td><strong>A</strong></td>
</tr>
<tr>
<td>0</td>
<td>0.482±0.02</td>
<td>100</td>
<td>0.571±0.01</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>0.515±0.01</td>
<td>106.85</td>
<td>0.592±0.02</td>
<td>103.68</td>
</tr>
<tr>
<td>100</td>
<td>0.422±0.01</td>
<td>87.55*</td>
<td>0.398±0.01</td>
<td>69.70*</td>
</tr>
<tr>
<td>150</td>
<td>0.391±0.02</td>
<td>81.12*</td>
<td>0.377±0.03</td>
<td>64.02*</td>
</tr>
</tbody>
</table>

Phytochemicals as Antioxidants: 

The *in vivo* Evidence
Health Benefits - legumes

• **Type 2 Diabetes Mellitus**
  - legumes generally have low-glycemic index values,
  - Diets rich in legumes may decrease the risk of type 2 diabetes by improving blood glucose control, decreasing insulin secretion, and delaying the return of hunger after a meal.

• **Cardiovascular Disease**
  - In 1999, the U.S. FDA approved health claim: “Diets low in saturated fat and cholesterol that include 25 grams of soy protein a day may reduce the risk of heart disease”
  - Other pulses had similar correlation

• **Cancer**
  - Limited evidence on prostate cancer
  - Strong evidence on soy and breast cancer

http://lpi.oregonstate.edu/infocenter/foods/legumes/#intro
Intake Recommendation - legumes

- Substituting beans, peas, and lentils for foods that are high in saturated fat or refined carbohydrates is likely to help lower the risk of type 2 DM and cardiovascular disease.

- Although a number of health-related organizations recommend daily consumption of 5-9 servings (2 1/2-4 1/2 cups) of fruits and vegetables daily (see Fruits and Vegetables), few make specific recommendations for legumes.

- In the USDA Food Guide at the reference 2,000-calorie level, the following weekly amounts are recommended:
  - Dark green vegetables 3 cups/week, Orange vegetables 2 cups/week, Legumes (dry beans) 3 cups/week
  - A serving of legumes is equal to 1/2 cup of cooked beans, peas, lentils, or tofu

Potential molecular sites of metabolic modification

- Methylation
- Oxidation
- Glucuronidation
- Sulphation
- Cleavage
Example – Plasma Levels of Flavonoid Conjugates

Plasma concentrations of antioxidants in young women consuming 16 oz of GF juice for 3 mos

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Concentration (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ascorbate</td>
<td>72±24</td>
</tr>
<tr>
<td>α-tocopherol</td>
<td>19±5</td>
</tr>
<tr>
<td>Coenzyme Q$_{10}$</td>
<td>0.40±0.1</td>
</tr>
<tr>
<td>Naringenin conjugate</td>
<td>0.33±0.5</td>
</tr>
</tbody>
</table>

Pathway of the colonic degradation of rutin - implications for properties of in vivo metabolites

Deglycosylation

Ring fission, water elimination, dehydroxylation

Further degradation

Absorption from the colon

β-Oxidation + glycination

3-hydroxyhippuric acid
How does metabolism affect antioxidant activity tested in vitro?

- Methylation/glycation/conjugation decreases activity
- Ring cleavage products- some may retain radical scavenging ability

<table>
<thead>
<tr>
<th>Isoflavones</th>
<th>TEAC (mM)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>gensitein (5, 7, 4')</td>
<td>2.90</td>
</tr>
<tr>
<td>biochanin A (5, 7, 4' OCH3)</td>
<td>1.16</td>
</tr>
<tr>
<td>daidzein (7,4')</td>
<td>1.25</td>
</tr>
<tr>
<td>formononetin (7, 4'OCH3)</td>
<td>0.11</td>
</tr>
<tr>
<td>genistein 7-glucoside</td>
<td>1.24</td>
</tr>
<tr>
<td>3,4-dihydroxyphenylacetic acid</td>
<td>2.16</td>
</tr>
<tr>
<td>3-methoxy-4-hydroxyphenylacetic acid</td>
<td>1.63</td>
</tr>
<tr>
<td>3-methoxy-4-hydroxybenzoic acid</td>
<td>1.19</td>
</tr>
<tr>
<td>3,4-dihydroxybenzoic acid</td>
<td>1.01</td>
</tr>
</tbody>
</table>

*Pietta, 2000*
Metabolites can be stronger antioxidants

- Daidzein to equol
- SDG to enterolactone, matairesinol and enterodiol
Phytochemicals as Antioxidants:

Mechanisms
In vivo Antioxidant Potential

• Can not actually measure in vivo
• Feed sources of phytochemical antioxidants in the diet in an animal/human model of oxidative stress, and
• measure decrease in oxidative stress, increase in antioxidant status, and/or increase in antioxidant potential *ex vivo*. 
Contrasting *in vitro* and *ex vivo* Effects

- *In vitro* antioxidant activity by FRAP and ORAC very high for Red Delicious, Granny Smith, Fuji apples & proportional to phenolic content. Oligomeric probably a large contributor to this.

- *Ex vivo* oxidation of human plasma – human subjects ate 5 apples each – Table 4 - No Effect

The issue is:

- Do phytochemicals, which are not nutrients, provide antioxidant protection in addition to the endogenous antioxidant defense system?
“Antioxidant activity of phytochemicals in vivo may not depend on their activities as direct scavengers of ROS per se, but rather on the influence of their in vivo forms on the modulation of enzyme/protein functions, intracellular signaling and receptor activities.”

Mechanisms by which antioxidants may be involved

• Quenching free radicals
• Enhancing antioxidant enzyme expression
• Inducing cell-cycle arrest
• Suppression of NF-kB activation
• Inhibiting cycloxygense-2 (COX-2) expression
• More …
### Number of Genes Regulated by Pomiferin

(Cells were treated with 5 μM for 24 h)

<table>
<thead>
<tr>
<th></th>
<th>P&lt;0.05</th>
<th></th>
<th>P&lt;0.01</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Up-Regulated</td>
<td>Down-Regulated</td>
<td>Total</td>
<td>Up-Regulated</td>
</tr>
<tr>
<td>MCF-7</td>
<td>362</td>
<td>153</td>
<td>515</td>
<td>78</td>
</tr>
<tr>
<td>MDA-MB-435</td>
<td>470</td>
<td>221</td>
<td>691</td>
<td>101</td>
</tr>
<tr>
<td>MCF-10A</td>
<td>49</td>
<td>10</td>
<td>59</td>
<td>1</td>
</tr>
</tbody>
</table>
Future Perspectives & Challenges

- Factors affecting the antioxidants
  - Genetics
  - Environmental factors
  - Processing conditions

- Product development

- Health claims?
  - Dosage/prooxidants
  - Bioavailability
  - Antioxidant biomarkers
  - Synergism
  - From *in vitro*, *ex vivo*, to *in vivo*

Moderation is Key
Thank You!
Fruit and vegetables antioxidants: reappraisal of a concept

Yves Desjardins, Ph.D., Agr.
Chair of ISHS Commission Fruits and Vegetable and Health Professor
Institute for Nutraceutical and Functional Food
Goal of the Antioxidant workshop

To provide the FRID group with a better understanding of the level of scientific substantiation needed to support a food health claim related to food antioxidants

- bioactives found in FAV
- FAV relevant to Canada
- comparison of antioxidant power of FAV
1. Ecological function

2. ANTIOXIDANTS

3. Mode of Action?

4. Toward a new concept
Ecological function of phytochemicals of FAV
Sessile organisms if they are to survive, grow and reproduce have to develop strategies to mitigate or circumvent extremes and take maximum advantage when conditions improve.

Anthony Trewavas, 1981
How do plant growth substances work?
Plant evolution strategies

- Continuous growth

- Chemical adaptations
  Secondary Metabolites...
Plant metabolome

Primary metabolism

Secondary metabolism

Phytochemicals

Cellular Metabolism

Growth regulation

Support Lignin

Membrane Fluidity Sterols

Interactions with the environment

Biotic stress

Abiotic stress
Phytochemicals of FAV

4 categories of secondary metabolites

- Polyphenols (6000 + molecules)
  - Flavonoids
  - Hydroxybenzoic acids
  - Phenyl propanoids
  - Lignans
  - Stilbenes

- Isoprenoids (terpenoids)
  - Carotenoids
  - Apocarotenoids
  - Phytosterols
  - Limonoids

- Sulphur compounds
  - Glucosinolates
  - Glutamylcysteine sulphoxides

- Alkaloids
  - 12 groups
  - Capsaicin

Biotic & abiotic stress protection
Roles of secondary metabolites in plants and FAV

**Polyphenols**
- Mechanical support
- Mechanical barrier
- UV light protection
- Anti-fungal (phytoalexins)
- Anti-bacterial
- Anti-viral
- Herbivore deterrent
  (proanthocyanidines)
- Allelopathic plant interactions

**Isoprenoids (terpenoids)**
- Light protection (photosynthesis overflow)
- Insect deterrent
- Anti-fungal (Phytoanticipins)
- Anti-Bacterial
- Allelopathic plant interactions
- Mycorrhizal root formation
- Maintenance of membrane fluidity
- Scavaging free radicals
- Stress hormone (ABA)

**Sulphur compounds**
- Plant/insect interaction
- Feeding deterrent
- Oviposition stimulant
- Anti-fungal (phytoalexins)
- Anti-bacterial

**Alkaloids**
- Herbivore deterrent
- Strong poisons
2

ANTIOXIDANT

DANT
Oxidative theory of disease etiology

- Oxidant
- Antioxidant (Reductant)

Oxidative stress

- Function loss of enzymes
- Increased cell permeability
- Disturbed cell signalling
- DNA lesions
- Apoptosis

Chronic Diseases
- CVD - Atherosclerosis
- Cancer
- Diabetes
- Neurodegenerative Dis.
- Endothelial dysfunction
Cardiovascular diseases

7 Country Study
Flavonoid intake vs long term risk CVD
16 cohorts - Followed over 25 years

Table 4. Correlation Coefficients (r) of Size, Total Anthocyanins (ACY), Total Phenolics (TPH), and Antioxidant Capacity (ORAC and FRAP)\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinium (n = 31)</td>
<td>0.55**</td>
<td>0.93**</td>
<td>0.73**</td>
<td>0.88**</td>
<td>0.79**</td>
<td>0.93**</td>
</tr>
<tr>
<td>\textit{V. corymbosum} (n = 15)</td>
<td>0.84**</td>
<td>0.93**</td>
<td>0.78**</td>
<td>0.96**</td>
<td>0.81**</td>
<td>0.94**</td>
</tr>
<tr>
<td>Vaccinium, 8 species (n = 16)</td>
<td>0.29</td>
<td>0.93**</td>
<td>0.69**</td>
<td>0.85**</td>
<td>0.76**</td>
<td>0.92**</td>
</tr>
<tr>
<td>Rubus (n = 37)</td>
<td>0.45**</td>
<td>0.83**</td>
<td>0.90**</td>
<td>0.85**</td>
<td>0.92**</td>
<td>0.90**</td>
</tr>
<tr>
<td>Rubus hybrids (blackberries) (n = 27)</td>
<td>0.003</td>
<td>0.57**</td>
<td>0.70**</td>
<td>0.38*</td>
<td>0.73**</td>
<td>0.75**</td>
</tr>
<tr>
<td>Ribes (n = 40)</td>
<td>0.46**</td>
<td>0.82**</td>
<td>0.71**</td>
<td>0.74**</td>
<td>0.81**</td>
<td>0.88**</td>
</tr>
<tr>
<td>Ribes nigrum (n = 32)</td>
<td>0.41*</td>
<td>0.63**</td>
<td>0.38*</td>
<td>0.46**</td>
<td>0.44**</td>
<td>0.55**</td>
</tr>
<tr>
<td>all samples (n = 108)</td>
<td>0.43**</td>
<td>0.73**</td>
<td>0.79**</td>
<td>0.80**</td>
<td>0.84**</td>
<td>0.84**</td>
</tr>
</tbody>
</table>

\textsuperscript{a} * = p < 0.05; ** = p < 0.005.
Figure 2. Correlation between total polyphenols (y-axis), $r^2 = 0.98$, and total anthocyanins (y-axis), $r^2 = 0.60$, to TEAC value (x-axis). Average values for rabbiteye blueberries, southern highbush blueberries, and blackberries were used for the plots.
Perspective

Antioxidants in foods and health: problems and fallacies in the field
Frankel, E.N. and J.B. German

"In the last decade, considerable worldwide attention has been given to natural phenolic antioxidants for their potential protective effects against the damage from biological oxidants...

However, very few studies have provided direct evidence that these benefits are actually due to in vivo or cellular antioxidant activity...

Whether or not natural antioxidants can account for the strong epidemiological evidence that consumption of fruits and vegetables is associated with reduced coronary heart diseases, cancer and other age-related diseases is a crucial question that remains unanswered..."
Oxidation network in the body

**Antioxidants**

**Enzymatic**
- SOD
- Catalases
- Glutathione peroxidases
- Peroxiredoxins

**Non-enzymatic**
- Glutathione (mM)
- Uric acid (350 μM)
- Bilirubine
- Transferrin
- Albumin
- Caeruloplasmin

**Pro-oxidants**
- Mitochondria (oxidative phosphorylation)
- NADPH reductase
- Inducible Nitric Oxide Synthase

**Source in the body**

**Dietary antioxidants**
- Ascorbic acid (150 μM)
- Carotenoids
- Polyphenols (low μM)
Antioxidant and oncogene rescue of metabolic defects caused by loss of matrix attachment

Zachary T. Schafer†, Alexandra R. Grassian†*, Loling Song†*, Zhenyang Jiang†, Zachary Gerhart-Hines2,3, Hanna Y. Irie†, Sizhen Gao†, Pere Puigserver†,2 & Joan S. Brugge†

… Trolox treatment induced a substantial increase in the number and average size of colonies, suggesting that antioxidant treatment can enhance the transforming activity of cells that contain oncogenic insults.

… Our work provides a biological rationale for these findings, as antioxidant activity may promote the survival of pre-initiated tumour cells in unnatural matrix environments and thus enhance malignancy. …
Review: antioxidant supplementation does not reduce gastrointestinal cancer


**Commentary**

Despite anecdotal evidence to the contrary, a growing body of scientific evidence suggests that antioxidant supplements may not be effective in disease prevention. The meta-analysis by Bjelakovic et al adds to that body of evidence, showing no protective effect of antioxidant supplements for the prevention of gastrointestinal cancers in high risk patients.

The most serious and unexpected finding was a higher mortality rate in the antioxidant group than the placebo group. The authors estimate that for every million people taking antioxidant supplements, 9000 premature deaths may have occurred. Possible explanations are that many studies used dosages in excess of current recommended daily intake or that some individuals are inherently more sensitive to antioxidants than others. This finding should be interpreted as preliminary, however, as many of the studies recruited high risk populations. The increased mortality rate therefore may not apply to healthy individuals who take antioxidant supplements as part of a healthy lifestyle.

The results of this review are relevant to public health nurses who work in lifestyle prevention, as well as advanced practice nurses who work in primary care or oncology. Given the increasing numbers of people who take supplements for prevention of disease, the results reinforce the importance of health teaching on the safety and efficacy of unregulated nutritional supplements. In light of these results, it may be prudent to advise high risk patients, such as people who smoke or have high alcohol intakes, of increased risks. However, further studies are needed to determine if the increased mortality rate is, in fact, related to the supplements, which supplements, and at what dosage. Thus, at this time it is difficult to infer harm for all people from supplementation. These findings remind all nurses discussing nutrition with their patients that nutritional supplements should be taken with the same care as regulated, prescribed medications.

Claudia Mariano, RN(EC), MSc
East End Community Health Centre
Toronto, Ontario, Canada
4.2.7 Conclusions

The Panel concludes:
Findings from cohort studies conducted since the mid-1990s have made the overall evidence that vegetables, or fruits, protect against cancers, somewhat less impressive. In no case now is evidence of protection judged to be convincing. However, there is evidence that some types of vegetables, and fruits in general, probably protect against a number of cancers. The few judgements on legumes (pulses), nuts, seeds, and (with two exceptions) herbs and spices, reflect the small amount of epidemiological evidence.

Fruits in general probably protect against cancers of the mouth, pharynx, and larynx, and of the oesophagus, lung, and stomach. There is limited evidence suggesting that fruits also protect against cancers of the nasopharynx, pancreas, liver, and colorectum.

There is limited evidence suggesting that carrots protect against cervical cancer; and that pulses (legumes), including soya and soya products, protect against stomach and prostate cancers. There is limited evidence suggesting that chilli is a cause of stomach cancer.
3. Mode of Action ???
Hominids paleolithic hunter-gatherer diet

- 100,000 years ago hominids were essentially carnivores

75% of diet was of animal origin (Cordain 2002)

- Reduction in gut size & metabolic activity
- Increase in brain size necessitates high energy foods
- Inability to chain elongate and desaturate long chain fatty acids (C18)
- Low synthesis of essential taurine
Phytochemicals of plant origin may have provided chemical cues of the environment and food supply

• Caloric restrictions
  • Energy conservation
  • Decrease heat production
  • Slowing growth
  • Delaying reproduction
  • Enhancement of repair mechanisms

  Increased lifespan

  Xenohormetic responses of polyphenols...
Caloric restrictions

- Fasting
- Polyphenols

SIRT-1

Liver
- Liver
- Skeletal Muscle
- White Adipose Tissue
- Pancreatic β Cell

PPARα

PGC-1α

PPARγ

HNF-4α

FOXO1

↑ Fatty Acid Oxidation
↓ Glycolysis

↑ Gluconeogenesis

↑ Mitochondrial Biogenesis
↑ Oxidative Capacity
↑ Fatty Acid Oxidation
↓ Glucose Utilization

↑ Fat Mobilization
↓ Adipogenesis

↑ Insulin Secretion

Adapted from Schwer & Verdin 2008, Cell Met. 104
A Low Dose of Dietary Resveratrol Partially Mimics Caloric Restriction and Retards Aging Parameters in Mice


June 2008 | Volume 3 | Issue 6 | e2264

Polyphenols ??? Xenohormesis
Phytochemical responses are more complex than once thought.

Phytochemicals → Antibiosis / prebiotic effects

Absorption ???
Efflux transporter
ABC transporter

Direct effects in the gut ???

Conjugated phytochemicals → Target tissue and pro-oxidants

Cell Signaling

Liver catabolism

methylation
glucuronidation
sulphatation

P450

ARE/XRE gene expression

Regulatory genes
Antioxidant genes

Cell signaling regulators

Hormesis

Excretion

Hepatoenteric circulation

Disease

Health

Redox status
Towards a new concept...
Responses to phytochemicals are more complex than once thought

Genetic selection / production practices

Plant content
6000+ polyphenols

Ingestion

Absorption

Bioavailable content

Food matrix/other meal components

Processing/culinary practices

Individual characteristics

Poor and incomplete databases of phytochemical content of food

Lack of suitable standards or biomarkers

Interactions with the food matrix e.g. Milk vs catechins

Circulating levels ???

No standard methods of analysis (e.g. PAC)

e.g. Multi Drug Associated Protein ???
Conclusions
SCIENCE AND HORTICULTURE FOR PEOPLE

28th International Horticultural Congress
August 22–27, 2010

Organizers

Gold Sponsor

Bayer CropScience
It is now generally recognized by most international health organization that fruits and vegetables (F&V) consumption can reduce the incidence of many diseases and are thus recommended in health prevention programs. Horticultural science finds itself at the forefront of research on the topic. Indeed much research is needed to understand how bioactive compounds found in F&V are synthesized, what strategies must be implemented to optimize their concentration in horticultural commodities and how to maintain their attributes in the food chain. In this context, it is also crucial for food chemists, nutritionists and clinicians to characterize the bioactive compounds to justify the epidemiological evidences of their protective or curative role on specific diseases. To evaluate the validity and applicability of supportive studies on horticultural commodities.

The ISHS Commission on Human Health Effects of F&V is thus organizing a symposium to actualize knowledge on health effects of F&V and is thus seeking inputs from the scientific community, industry and all professionals involved in this field.

The objective of the symposium “Emerging Health Topics in F&V” is thus to provide a forum for horticultural scientists, nutritionists, dietitians, chemists, biochemists, food scientists, clinicians and physicians to exchange information and bridge the communication gap between the agricultural sciences, nutrition and health sciences. This symposium will be held as part of IHC-2010 and should be an outstanding opportunity for participants to benefit from a very strong scientific program while opening up to the rest of the horticulture sector. It is our great pleasure to invite you to this unique landmark event in the beautiful Lisbon.

Yves Desjardins and Francisco Tomas-Barberan
Convenors IHC 2010 FruH Health
Lisbon IHC-2010 - Symp. 7 Emerging Health topics FAV

Day 1  
Monday August 23rd

8h30  IHC Plenary Lectures  
9h30  2000 attendies  
10h30  Symposium 7  
11h30  2 invited conferences  
12h30  Theme: Cultural approach to improve quality of FAV  
13h30  Lunch Break  
14h30  Poster Session  
15h30  Symposium 7  
16h30  5 conferences  
17h30  Theme: Effect of FAV on Cancer, cardiovascular diseases  
18h30  
19h30  
20h30  

Day 2  
Tuesday August 24th

8h30  IHC Plenary Lectures  
9h30  2000 attendies  
10h30  Symposium 7  
11h30  OECD session  
12h30  3 invited conferences  
13h30  Lunch Break  
14h30  Poster Session  
15h30  Symposium 7  
16h30  OECD session  
17h30  4 invited conferences  
18h30  Wrap-up round table  
19h30  
20h30  

Day 3  
Wednesday August 25th

8h30  IHC Plenary Lectures  
9h30  2000 attendies  
10h30  Symposium 7  
11h30  2 conferences  
12h30  Theme: FAV antioxidants effects on the organism antioxidant network  
13h30  Lunch Break  
14h30  Poster Session  
15h30  Symposium 7  
16h30  OECD session  
17h30  Coordination session of international institutes and networks working on health effects of FAV  
18h30  
19h30  
20h30  
The Analysis of Antioxidants & Antioxidant Potential

Trust Beta
Department of Food Science
University of Manitoba

AAFC Antioxidant Workshop
3 February 2010
Various in vitro methods to measure antioxidant potential

Measuring antioxidant potential in biological samples

Biomarkers for antioxidant status
Antioxidants in Action

RH, H₂O, ^3O₂

↓

Free radicals

↓

DNA, Proteins, Lipids

↓

Tissue damage

(chronic & neurodegenerative diseases, aging)

- Antioxidant potential & health benefits
  - Cancer
  - Cardiovascular diseases
  - Diabetes, etc

In human

T. Beta - University of Manitoba 02-03-10
Measurements of Antioxidant Potential *In Vitro*

- Measurement of oxidation products
- Prevention of oxidation *in vivo* & *in vitro*
  - Scavenging of free radicals
    - (ROO•, •OH, O₂•-, ¹O₂, •NO, •NO₂, OONO-)
  - Inhibition of oxidative enzymes
  - Induction of antioxidant enzymes
  - Chelation of proxidant metal (Fe²⁺ & Cu²⁺) ions
- Cell culture models
Measurements of Antioxidant Potential \textit{In Vitro}

General classification of radical scavenging assays

- **Electron transfer (ET)**
  - \( M(n) + e \) (from \( AH \)) \( \rightarrow \) \( AH^+ + M(n - 1) \)

- **Hydrogen atom transfer (HAT)**
  - \( ROO^+ + AH \) \( \rightarrow \) \( ROOH + A^* \)
  - \( ROO^+ + LH \) \( \rightarrow \) \( ROOH + L^* \)

Huang et al 2005
Shahidi 2007
Assays Based on Electron Transfer

- TEAC (Trolox equivalent antioxidant capacity)
- FRAP (ferric ion reducing antioxidant parameter)
- DPPH (diphenyl-1-picrylhydrazyl)
- Copper(II) reduction capacity
- Total phenols assay by Folin-Ciocalteu reagent (TPC)
Assays Based on HAT

- Oxygen radical absorbance capacity (ORAC)
- Total radical trapping antioxidant parameter (TRAP)
- Crocin bleaching assay
- Inhibited oxygen uptake (IOU)
- Inhibition of linoleic acid oxidation
- Inhibition of LDL oxidation

Huang et al 2005
Shahidi 2007
Other *In Vitro* Chemical Assays

- Singlet oxygen
- Superoxide anion
- HOSC (•OH scavenging capacity)
- Peroxynitrite
- TOSC (total oxidant scavenging capacity)
- Chemiluminescence
Antioxidant Activity of Some Staple Foods

* Analysis by Diphenyl picrylhydrazyl, μmoles Trolox Equivalents/100 grams

WHOLE GRAIN PRODUCTS ARE HIGH IN ANTIOXIDANT ACTIVITY

http://www.wheatfoods.org/pdfs/grains_for_the_health_of_it/

T. Beta - University of Manitoba 02-03-10
### Correlation Analysis (Wild Rice)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPH &amp; TPC</td>
<td>0.92</td>
</tr>
<tr>
<td>DPPH &amp; ORAC</td>
<td>0.80</td>
</tr>
<tr>
<td>ORAC &amp; TPC</td>
<td>0.64</td>
</tr>
</tbody>
</table>

- Qiu 2009

T. Beta - University of Manitoba 02-03-10
Extraction Methods

- Methanol extraction
  - MeOH extract
  - Residue
- Alkaline or Acid hydrolysis
- Ethyl acetate extraction
  - Soluble
  - Insoluble

TPC
DPPH
ORAC, etc

LC-MS-MS

Qiu 2009
<table>
<thead>
<tr>
<th>Class</th>
<th>Wild Rice</th>
<th>Gallic acid equiv</th>
<th>Ferulic acid equiv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>White rice (WR)</td>
<td>46</td>
<td>319</td>
</tr>
<tr>
<td>Mixed</td>
<td>3 blend mix</td>
<td>253</td>
<td>1642</td>
</tr>
<tr>
<td>Processed</td>
<td>Quick cooking</td>
<td>353</td>
<td>2299</td>
</tr>
<tr>
<td>Raw</td>
<td>A black</td>
<td>445</td>
<td>2893</td>
</tr>
<tr>
<td></td>
<td>B black</td>
<td>588</td>
<td>3802</td>
</tr>
<tr>
<td></td>
<td>C scarified</td>
<td>579</td>
<td>3745</td>
</tr>
<tr>
<td></td>
<td>Canadian Lake</td>
<td>419</td>
<td>2727</td>
</tr>
<tr>
<td></td>
<td>Minnesota cultivated</td>
<td>565</td>
<td>3654</td>
</tr>
<tr>
<td></td>
<td>Manomin</td>
<td>585</td>
<td>3784</td>
</tr>
<tr>
<td></td>
<td>Large size</td>
<td>444</td>
<td>2843</td>
</tr>
<tr>
<td></td>
<td>Small size</td>
<td>438</td>
<td>2885</td>
</tr>
</tbody>
</table>

T. Beta - University of Manitoba 02-03-10
Qiu et al 2009
Antioxidant Activity of MeOH Extract

AA of individual wild rice samples

ORAC values >> DPPH scavenging activity values

Qiu et al 2009

T. Beta - University of Manitoba 02-03-10
<table>
<thead>
<tr>
<th>Class</th>
<th>Sample name</th>
<th>Soluble</th>
<th>Insoluble</th>
<th>∑TPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>White rice</td>
<td>12.4</td>
<td>31.5</td>
<td>43.9</td>
</tr>
<tr>
<td>Mixed</td>
<td>3 blend mix</td>
<td>53.0</td>
<td>140.2</td>
<td>193.2</td>
</tr>
<tr>
<td>Processed</td>
<td>Quick cooking</td>
<td>88.6</td>
<td>142.7</td>
<td>231.3</td>
</tr>
<tr>
<td>Raw</td>
<td>A black</td>
<td>137.0</td>
<td>164.0</td>
<td>301.0</td>
</tr>
<tr>
<td></td>
<td>B black</td>
<td>164.6</td>
<td>189.7</td>
<td>354.4</td>
</tr>
<tr>
<td></td>
<td>C scarified</td>
<td>161.4</td>
<td>206.6</td>
<td>368.0</td>
</tr>
<tr>
<td></td>
<td>Canadian lake</td>
<td>105.6</td>
<td>166.0</td>
<td>271.6</td>
</tr>
<tr>
<td></td>
<td>Minnesota</td>
<td>153.8</td>
<td>186.4</td>
<td>340.2</td>
</tr>
<tr>
<td></td>
<td>Manomin</td>
<td>169.1</td>
<td>212.0</td>
<td>381.2</td>
</tr>
<tr>
<td></td>
<td>Large size</td>
<td>124.7</td>
<td>168.8</td>
<td>293.6</td>
</tr>
<tr>
<td></td>
<td>Small size</td>
<td>124.0</td>
<td>172.3</td>
<td>296.4</td>
</tr>
</tbody>
</table>

Qiu et al 2010
Identification of Antioxidants

Eg. flavonoids

- 20% Methanol (100 mL)
- 60% Methanol (150 mL)
- 70% Acetone (100 mL)

- F1: 100 mL 20% MeOH
- F2: 50 ML 60% MeOH
- F3: 50 ML 60% MeOH
- F4: 50 ML 60% MeOH
- F5: 100 mL 70% Ace

T. Beta - University of Manitoba 02-03-10
Schematic representation of LC/MS via route 1

(Anderson and Markham, 2006)
Identification of Differulic Acids in Wild Rice

(Qiu, Liu, Beta 2010)
Identification of Disinapic Acids in Wild Rice

8-8' DiSA
- Aryltetralin form
- Linear form

(Qiu, Liu, Beta 2010)
Antioxidant Potential in Biological Samples

- Include blood plasma, tissues, or urine
- Valid sample processing procedure is critical
- *In vitro* assays need validation to study the impact of antioxidant consumption on reducing oxidative stress (OS) markers
- Valid *in vitro* assays are invaluable tools for clinical studies if combined with data on bioavailability & valid OS biomarker assays

T. Beta - University of Manitoba 02-03-10
Measuring Antioxidant Potential in Biological Samples

- In vitro chemical assays
  - Free radical scavenging assays
  - Metal chelation assays
  - Oxidation products
- Cell culture models and biomarkers including:
  - Inhibition of oxidative enzymes
  - Induction of antioxidant enzymes

Collins 2005
Liu 2007
Cell Culture Models for AO Screening

Cancer
- Antiproliferation
- Cell cycle arrest
- Apoptosis
- Antiangiogenesis
- COX-2 inhibition
- Quinone reductase
- Oxidative DNA damage

Biomarkers
- Inhibition of proliferation
- G1 arrest, G1/S ratio
- Inhibition of apoptosis
- Inhibition of angiogenesis
- COX-2 expression
- Induced QR activity
- 8OH-dG

Liu 2007
Cell Culture Models for AO Screening

CVD
- Inhibition of cholesterol synthesis
- Expression of hepatic LDL receptors
- Bioavailability of AO
  - Flavonoid uptake
  - Carotenoid uptake

Biomarkers
- Cholesterol, SREBP
- LDL receptors, Cellular LDL uptake
- Biomarker
  - Cellular flavonoid uptake
  - Cellular carotenoid uptake

Liu 2007
Cell Culture Models Based on AO Metabolites

T. Beta - University of Manitoba 02-03-10
Flavanols in Green Tea

(Stalmach et al., 2009)

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

(-)-Epicatechin gallate
(+)-Catechin gallate
(-)-Epigallocatechin gallate
(+)-Gallocatechin gallate
Summary of flavonoid metabolites and conjugates formation in human

Dietary Flavonoid e.g. epicatechin procyanidins

Stomach

Oligomeric Flavonoids → Monomeric units

Oligomers cleaved

Small Intestine

O-ring glucuronides

O-methylated glucuronides

O-methylated

aglycone

Colon

Flavonoid → Phenolic acids

Gut microflora

Neurons glia

cells

Blood-brain barrier

O-methylated

Sulphates

Further metabolism

glucuronides

Portal vein

Liver

Kidney

Renal excretion of glucuronides

Urine

T. Beta - University of Manitoba 02-03-10

(Spencer, 2003)
Cell Culture Models for AO screening

Metabolism of AO
- Primary hepatocytes
- Caco-2 colon cancer cells
- HepG2 liver cancer cells

Biomarkers
- Metabolic compound(s)
- Metabolic compound(s)
- Metabolic compound(s)

Liu 2007
Antioxidants in Humans

- Antioxidant capacity of human plasma
  - TRAP, TEAC, ORAC, FRAP (ZERO effect?)
- Antioxidant resistance of lymphocytes & lipids to oxidation
  - Comet assay - DNA damage induced by $\text{H}_2\text{O}_2$
  - Resistance of LDL to oxidation by CuSO$_4$ or macrophages
- Endogenous DNA & lipid oxidation to monitor AO status

Collins 2007
Biomarkers for Antioxidant Status

- Lipid oxidation
  - Plasma malondialdehyde concentrations
  - LDL oxidation
  - $F_2$ - isoprostane
- Protein oxidation
  - Protein carbonyls
- DNA oxidation - ESCODD
  - 8-oxo-7,8-dihydroguanine
- Plasma ascorbic acid

T. Beta - University of Manitoba 02-03-10
Closing Remarks

- Various *in vitro* assays available for food and biological samples
- Validation & harmonization of assays needed for food and biological samples
- Database on content of antioxidants in food sources as basis for potential relationships to disease prevention
- Relevant biomarkers to be selected and validated for function claims related to AOs
Requirements for Food Health Claims Substantiation

Eunice Chao, PhD
Nutrition Evaluation Division, Bureau of Nutritional Sciences
Food Directorate, Health Products and Food Branch
Health Canada

Antioxidant Workshop, February 3, 2010
Outline

- Health claims
  - Definitions
  - 3 categories and regulatory requirements

- *Guidance Document for Preparing a Submission for Food Health Claims*
  - Overview

- Function claims
  - Biomarkers
  - Acceptability of function claims
Health Claims

- Any representation in labelling and advertising that states, suggests or implies that a relationship exists between a food or a component of that food and health (Codex Alimentarius Commission, 2004)

- General or specific, stated or implied

- Must be truthful and not misleading (Section 5 of the Food and Drugs Act)
3 Categories of Health Claims

“Drug” claims (disease risk reduction and therapeutic):
- e.g. “Reduces risk of heart disease”
- e.g. “Lowers serum cholesterol”

Function claims:
- Function associated with health or performance
  - e.g. “Promotes regularity”
- Function of nutrients or energy necessary for normal growth and development
  - e.g. “Calcium aids normal bone and tooth development”

General health claims:
- Claims about healthy eating or dietary guidance
  - e.g. “Include low fat product x as part of healthy eating”
- Do not refer to a health effect
- Include front of package logos, and symbols
### Summary – Food Health Claim Requirements

<table>
<thead>
<tr>
<th>Type of Health Claim</th>
<th>Must be truthful and not misleading</th>
<th>Brings food under drug regulations (unless specifically permitted in food regulations)</th>
<th>Pre-market assessment required</th>
<th>Regulatory Amendment required</th>
<th>Guidance provided (CFIA Guide to Labelling etc.)</th>
<th>Conditions for type of food that can carry claims set out in FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Drug” claims (disease risk reduction or therapeutic claims)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Function claims (associated with health or performance)</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td>yes (guidance)</td>
<td></td>
</tr>
<tr>
<td>Function claims (as applied to nutrients and energy necessary for normal growth and development)</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td>yes (regulated)</td>
<td></td>
</tr>
<tr>
<td>General health claims (including logos, symbols related to healthy eating)</td>
<td>yes</td>
<td></td>
<td></td>
<td></td>
<td>yes (on specific topics)</td>
<td></td>
</tr>
</tbody>
</table>
Guidance Document for Preparing a Submission for Food Health Claims


Applicable to all types of health claims (except general health claims that do not refer to health effects; abbreviated review process available for function claims for nutrients with recommended intakes)

Update of the principles for claim validity in Interim Guidance Document

- Systematic approach
- Transparency
- Comprehensiveness
- Human evidence
- High level of certainty
- Demonstration of causality
- Biological relevance of the claimed effect
- Feasibility of consumption of effective dose
- Health claim wording
- Focus on one food/health relationship
Main Requirements

- Details pertaining to proposed health claim
- Status of health claim in other jurisdictions
- **Characterization of food/food constituent**: the composition and manufacturing of the food meets quality standards
- Characterization of health effect
- Evaluation of claim validity
- References
Characterization of Food/Food Constituent

Questions/issues to consider:

- **Food category** *(e.g. fruits)*:
  - What foods typically fall under the category?
  - Any foods to be excluded from the health claim?

- **Whole (unprocessed) food** *(e.g. apple)*:
  - Nutritional composition; minimum effective intake of the food

- **Foods containing an inherent bioactive substance** *(e.g. galacturonic acid in apples)*:
  - Nutritional composition; minimum effective intake of the bioactive substance and the amounts and types of food to be consumed
  - Specifications, manufacturing process and quality system
  - Analytical tests and results
  - Stability
Questions/issues to consider:

- **Food containing an added bioactive substance** (e.g. yogurt with *L. casei* 431)
  - **End product**: similar to question/issues applicable to foods containing an inherent bioactive substance
  - Novel if genetically modified → Novel food notification (Division 28, Part B of *Food and Drug Regulations*)

- **Bioactive substance (added to the food)**:
  - Specifications, manufacturing process and quality system
  - Analytical tests and results
  - Stability
  - Novelty: novel food notification may apply
    - No history of safe use in foods
    - Possibly novel delivery technology (e.g. microencapsulation)
Characterization of Health Effects

Questions/issues to consider:

- Why is the claimed effect important?
- Is the health effect important to Canadians?
- What endpoints or biomarkers are used?
- Why is a particular endpoint or biomarker chosen to measure the health effect?
Evaluation of Claim Validity

13 steps in the retrieval and evaluation of the totality of relevant evidence on the food/health relationship to allow for an assessment of:

- Causality
- Generalizability to target population
- Biological relevance
- Feasibility
Overview of steps

Step 1. Describe the search strategy for literature retrieval
Step 2. Implement the search strategy for literature retrieval
Step 3. Develop inclusion and exclusion criteria to filter the literature
Step 4. Filter the literature
Step 5. Generate reference lists of included and excluded studies
Step 6. Tabulate studies
Step 7. Evaluate study quality
Step 8. Tabulate study findings per health outcome
Step 9. Assess causality
   Step 9a. Rate consistency
   Step 9b. Rate the strength of the association
   Step 9c. Discuss the relationship between food exposure and the health effect (dose-response)
Step 10. Discuss generalizability of the data to the target population
Step 11. Discuss the physiological meaningfulness of the effect of the food exposure
Step 12. Discuss the feasibility of consuming an effective amount of the food
Step 13. Make conclusions
Step 13 - Make conclusions

Based on evidence from previous steps (and evidence from other meta analyses and authoritative statements, if desired) make concluding remarks

- Propose claim wording that accurately reflects the substantiated health outcome

- Propose and justify conditions for a food to qualify for the claim
  - E.g. Minimum amount of the food/food constituent eligible to carry the claim
  - E.g. Maximum amount to be consumed, if applicable
  - E.g. Minimum/maximum levels of key nutrients in the food (e.g. low in saturated fat)

- Propose labelling statements to manage potential risks, if necessary (e.g. statement indicating maximum limit not to be exceeded)
Function Claims

- Claims about the specific beneficial effects that the consumption of a food or a constituent of a food (i.e. nutrient or other component) has on normal functions or biological activities of the body

- Related to positive contribution to health, or physical or mental performance

- Based on the role that the food or the food constituent plays when consumed as part of normal dietary patterns
### Function Claims

#### Characterization of health effects

- “Biomarker”: a biological marker that can be objectively measured and assessed as an indicator of normal biologic processes

- Has the health relevance of the “biomarker” been demonstrated in humans?

- Other endpoints: related to physical or mental performance (e.g. muscle fatigue, cognitive function)
Acceptability of Function Claims

- **Wording**: clearly states a **specific physiological effect** relevant to health or performance and meaningful to consumers

**Accepted**
- Helps build antibodies (protein)
- A factor in normal early fetal development (folate)
- Protects blood lipids from oxidation (green tea)
- Promotes regularity (coarse wheat bran)

**Could be considered if substantiated**
- Reduces the absorption of cholesterol from the gut
- Fluid and electrolyte replacement
- Helps to delay muscle fatigue
- A factor in normal cognitive function
Acceptability of Function Claims (cont’d)

- Potentially misleading

  - Broad (unspecific) claims with poorly defined health effects
    - e.g. *Supports immune health / Supports a healthy immune system*
    - Subject to multiple interpretations
    - Potentially misleading unless each interpretation is scientifically supported

  - Wording does not reflect the substantiated health outcome
    - e.g. *Contributes to healthy cholesterol*
    - Studies involve individuals with high blood cholesterol - same studies that support a “drug” claim “*lowers serum cholesterol*”
Summary

- **Regulatory requirements vary** with the type of health claims.

- **Evidence requirements comparable** for all claims about health benefits or effect:
  - *Guidance Document for Preparing a Submission for Food Health Claims*
  - **Exception**: abbreviated documentation process for nutrients meeting specified criteria

- **Function claim**
  - Clear, meaningful statement about a specific physiological effect relevant to health or performance
  - Context and entirety of message important in distinguishing from “drug” claims
Regulating Novel Foods in Canada

Luc Bourbonnière,
Section Head, Novel Foods Section, Eval-BMH
Food Directorate
Health Canada
Canadian Food & Drugs Act

Novel Foods Regulation

- Canada Gazette Part II, October 27, 1999
- Notification prior to sale or advertising
a) History of Safe Use

A substance may be considered to have a history of safe use as a food if it has been an ongoing part of the diet for a number of generations in a large, genetically diverse human population where it has been used in ways and at levels that are similar to those expected or intended in Canada.
Novel Food Definition (1999)

b) Novel Process that Causes a Major Change

“Major Change” means, in respect of a food, a change in the food that, based on the manufacturer’s experience or generally accepted nutritional or food science theory, would place the modified food outside the accepted limits of natural variations for that food with regard to:

- the composition, structure or nutritional quality of the food or its generally recognized physiological effects
- the manner in which the food is metabolized in the body, or
- the microbiological safety, the chemical safety or the safe use of the food
c) Genetic Modification

A food that is derived from a plant, animal or microorganism that has been genetically modified* such that

- the plant, animal or microorganism exhibits characteristics that were not previously observed in that plant, animal or microorganism,
- the plant, animal or microorganism no longer exhibits characteristics that were previously observed in that plant, animal or microorganism,
- one or more characteristics of the plant, animal or microorganism no longer fall within the anticipated range for that plant, animal or microorganism.

* “genetically modify” means to change the heritable traits of a plant, animal or microorganism by means of intentional manipulation
Novel whole foods and food ingredients may appear in the marketplace through:

- the importation of new products
- the introduction of a new species as a food source
- the use of new processing techniques
- changes in the genetic make-up of the microorganisms, plants and animals from which foods are derived
The Safety Assessment of Novel Foods

The safety assessment of novel foods follows a stepwise process of addressing relevant factors that can include:

- History of use
- Dietary exposure
- Details of novel process
- History of organism(s)
- Characterization of derived plant/microorganism
- Genetic modification considerations
- Nutritional considerations
- Toxicology considerations
- Allergenicity considerations
- Chemical considerations
- Microbiological considerations
Examples of Novel Foods

No history of safe use as a food

- Trehalose
- Vegetable diacylglycerol (DAG) oil

Is manufactured using a process not previously applied to that food

- High hydrostatic pressure treated RTE meats
- UV treated apple juice/cider

A food derived from a genetically-modified organism

- Imidazolinone tolerant wheat
- Insect resistant corn (Cry3Bb1)
So how does this apply to antioxidants and other bioactives?

Someone wants to add an ingredient to food for the purpose of making a antioxidant claim

Example 1: Adding fresh blueberries, dried blueberries, or minimally processed blueberries are not novel
  ▪ No need for an assessment under Division 28

Example 2: purified blueberry extract with concentrated antioxidants
  ▪ No longer blueberries
  ▪ Does the extract have a history of safe use as a FOOD INGREDIENT?
    • If not, it’s a novel food

Example 3: you traditionally bred a blueberry line so that it has a 10X increase in antioxidant levels
  ▪ Does the antioxidant levels fall within anticipated range for blueberries grown in Canada for food purpose.
    • If not, it’s a novel food
Health Canada’s Website:

http://www.hc-sc.gc.ca/fn-an/gmf-agm/index_e.html


Summaries of Novel Food Safety Assessments

Novel Food Regulations

Revised Guidelines for the Safety Assessment of Novel Foods

Thank you!