Processing Crucifers to Retain Optimal Bioactivity

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# Epidemiological studies on cancer and dietary cruciferous vegetable intake

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Intake</th>
<th>Risk</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>&gt;5 serving/wk</td>
<td>RR 0.49</td>
<td>0.008</td>
</tr>
<tr>
<td>Lung</td>
<td>0.5 serving/d</td>
<td>OR 0.31</td>
<td>CI(0.1 - 0.92)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>&gt;5 serving/wk</td>
<td>RR 0.67</td>
<td>0.03</td>
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<tr>
<td>Prostate</td>
<td>&gt;3 serving/wk</td>
<td>OR 0.59</td>
<td>0.02</td>
</tr>
<tr>
<td>Prostate</td>
<td>5 serving/wk</td>
<td>OR 0.61</td>
<td>0.006</td>
</tr>
<tr>
<td>Breast</td>
<td>Quartile 4</td>
<td>OR 0.50</td>
<td>0.01</td>
</tr>
<tr>
<td>Kidney</td>
<td>Quartile 4</td>
<td>OR 0.53</td>
<td>0.001</td>
</tr>
<tr>
<td>Ovarian</td>
<td>&gt;0.83 serving/d</td>
<td>HR 0.75</td>
<td>0.03</td>
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OR, Odds ratio; HR, Hazard ratio; RR, Relative risk; CI Confidence interval
Crucifers lower risk for cancers more effectively than do vegetables in general

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<thead>
<tr>
<th></th>
<th>RR</th>
<th>Colon</th>
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<tbody>
<tr>
<td>All vegetables</td>
<td>0.75 (p=0.43)</td>
<td>Voorrips et al, 2000 (ratio low: high quintile)</td>
</tr>
<tr>
<td>Cruciferous</td>
<td>0.51 (p=0.004)</td>
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<tr>
<td>All vegetables</td>
<td>0.81 (p=0.15)</td>
<td>Cohen et al, 2000 (ratio &lt;1:&gt;3 servings/wk)</td>
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<tr>
<td>Cruciferous</td>
<td>0.54 (p=0.01)</td>
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</table>
Cruciferous vegetables contain multiple nutrients and phytonutrients

**Nutrients**
- Vitamins A, C, E, folate
- Fiber
- Minerals: Ca, Mn, Mg

**Phytonutrients (Bioactives)**
- Glucosinolates
- Flavonoids
- Selenium

**Nutrients: for growth & development**

**Phytonutrients: slow or reverse chronic disease**
Glucosinolate profile varies across cruciferous vegetables; glucoraphanin is the predominant glucosinolate in broccoli
Glucosinolates are hydrolyzed to bioactive ITC when plants are crushed or chewed.
Effect of Sulforaphane on Incidence of Dimethylbenzanthracene-induced Papillomas in mice

Gills et al, 2006
What is the impact of processing on stability and bioavailability of nutrients and bioactive compounds like sulforaphane?
Broccoli retains substantial amounts of vitamin C during home processing, depending upon the cooking method

- Raw Broccoli: 117.7 mg/100 g FW
- Steam (5’): 117.3
- Microwave (5’): 63.6
- Boiling (5’): 85.6

150 g fresh-cut Marathon broccoli

Vallejo et al, 2002
Sulforaphane has proven unstable in processing of dietary supplements

Residual concentrations of sulforaphane (◊) and phenylethyl ITC (▵) after 20 min thermal treatments. Van Eylen et al, 2007

Sulforaphane is stable below pH 3 Xiao et al, 2007
Novel formulations, including nanoparticles and conjugates are under study
Glucosinolates are relatively stable during cooking, especially steaming

A: Steaming (3.5 min) caused almost no glucosinolate loss from broccoli

B: Microwave (High, 5 min) caused significant breakdown of glucosinolates

C: Conventional boiling (5 min) caused some leaching and some breakdown.

Vallejo et al, 2002
BUT glucosinolates require hydrolysis to produce sulforaphane.

Glucoraphanin $\xrightarrow{\text{Myrosinase}}$ ITC

Sulforaphane $\xrightarrow{\text{Isothiocyanate Anticarcinogen}}$
Myrosinase activity in broccoli florets is only thermally stable below usual cooking temperatures (10 min. heating)

Dosz and Jeffery, 2013
Can dietary glucoraphanin provide sulforaphane without myrosinase?
In rats, purified glucoraphanin shows activity in the colon, but not liver or lung.

Rats were administered 30, 60 or 120 mg/kg glucoraphanin po, purified from broccoli seed, daily for 4 days and killed 24 h after the last dose. 30 mg/kg is approximately the daily amount a rat would ingest, eating a diet containing 10% DW broccoli. Mean +/- S.E. n=6

Lai et al. 2008
INTESTINAL MICROFLORA

$10^{14}$ micro-organisms, >500 different species

Lactobacilli

Streptococci Lactobacilli

Enterobacteria Enterococcus Faecalis Bacteroides Bifidobacteria Peptococcus Peptostreptococcus Ruminococcus Clostridia Lactobacilli and....

Stomach $10^2$ to $10^3$

Duodenum $<10^{4-5}$

Jejunum

Ileum $10^3$ to $10^7$

Colon with appendix $10^9$ to $10^{12}$
The rat cecum, like our proximal colon, holds most microbiota.
When glucoraphanin was introduced into the cecum, sulforaphane appeared in mesenteric plasma from glucoraphanin, but slowly.*
When glucoraphanin was introduced into the cecum, sulforaphane appeared in mesenteric plasma from glucoraphanin, but slowly.
Do humans also hydrolyze dietary glucoraphanin to sulforaphane in the absence of myrosinase?
Human Bioavailability of sulforaphane from glucoraphanin in the absence (powder) or presence (sprout) of myrosinase

Powder: 120 μmol GRP; sprouts 70 μmol GRP + myrosinase
Combination 190 GRP + sprouts myrosinase

Cramer et al, 2011
Human Bioavailability of sulforaphane from glucoraphanin in the absence (powder) or presence (sprout) of myrosinase

24 h Sulforaphane Excretion

0-6 h excretion for sprout and combination was different from powder, p<0.05
Four healthy males ate a single meal of 1) Powder: semi-purified glucoraphanin (120 µmol GRP/ no myrosinase), 2) broccoli sprouts (70 µmol GRP/ active myrosinase) or 3) a combination (190 µmol GRP).

Cramer et al, 2011
Sources of myrosinase

Water cress  Arugula  Kohlrabi  Rutabaga  Wasabi  Drumsticks  Chinese cabbage
Positive impact of thermal processing (3-4 min steaming) on sulforaphane formation in broccoli.
Impact of heating on glucoraphanin hydrolysis to sulforaphane in fresh Marathon broccoli

Marathon broccoli (n=3; 100 g each, a compilation from 4 heads) was heated by a) microwave in a covered dish with 30 mL water; b) boiling in 1L water; c) steaming. Immediately after heating, samples were placed in ice water for 30 sec, strained and homogenized.

Wang et al, 2012
Glucoraphanin has two hydrolysis products, but only Sulforaphane is bioactive.

Matusheski et al, 2006
Bioactivity in Cultured Mouse Liver Cells treated with Sulforaphane or Nitrile

Matusheski and Jeffery, 2001
When glucoraphanin is hydrolyzed, two products are formed: sulforaphane and a nitrile.

Yield varies with genotype:
- Marathon: 12% sulforaphane yield (shown here)
- Brigadier: 47% sulforaphane yield
- Patriot: 22% sulforaphane yield
- Pinnacle: 7% sulforaphane yield

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Wang et al, 2012
Impact of heating methods on glucoraphanin hydrolysis products sulforaphane (blue) and sulforaphane nitrile (gray) in fresh Marathon broccoli

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Wang et al, 2012
Effect of Heating on the nitrile-forming myrosinase cofactor, that directs hydrolysis toward nitrile formation

Recombinant nitrile-forming cofactor (epithospecifier protein) was biosynthesized in *E. coli* transfected with cDNA generated from broccoli mRNA by RT-PCR.

Matusheski et al, 2006
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Matusheski et al, 2006
Frozen broccoli is becoming more popular. What does processing for frozen processing do to sulforaphane bioavailability?
Choice of broccoli blanching temperature

- Blanching 145 sec prolongs shelf life and improves color
- Blanching time is typically based on loss of peroxidase activity
- Lipoxygenase, responsible for off flavor and poor shelf life is less thermally stable
- Lipoxygenase loss complete at a temperature of 76°C for blanching

Dosz and Jeffery, 2013
Impact of blanching for 2.5 min prior to hydrolysis on sulforaphane formation

Dosz and Jeffery, 2013
Choice of broccoli blanching temperature

- Blanching 145 sec prolongs shelf life and improves color
- Blanching time is typically based on loss of peroxidase activity
- Lipoxygenase, responsible for off flavor and poor shelf life is less thermally stable
- Lipoxygenase loss not complete at a temperature of 66°C for blanching

Dosz and Jeffery, 2013
Freeze-dried daikon radish, as a source of myrosinase, produces sulforaphane in frozen broccoli that is then microwave cooked according to instructions.

Dosz and Jeffery, 2013
Sources of myrosinase

- Water cress
- Arugula
- Kohlrabi
- Rutabaga
- Wasabi
- Drumsticks
- Chinese cabbage
TAKE HOME MESSAGE

Fact 1: the health effects of broccoli are strong and deserve to be harnessed

Fact 2: sulforaphane is so unstable, the plant keeps it conjugated (as glucoraphanin) - when free it rapidly degrades

Fact 3: heat-stable glucoraphanin requires an enzyme myrosinase to release sulforaphane

Fact 4: Broccoli processing should protect myrosinase or provide exogenous myrosinase to form sulforaphane
Acknowledgements

Nathan Matusheski  
Grace Wang  
Donato Angelino  
Ren-Hau Lai  
Jenna Cramer  
Joel Gills  
Ed Dosz

Jack Juvik  
John Pezzuto  
Anna Keck

USDA/IFAFS, NRI and NCI funding

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