Polyphenols and Fruits: Importance of Glycosylation, Chirality, Food Processing and Organic Farming

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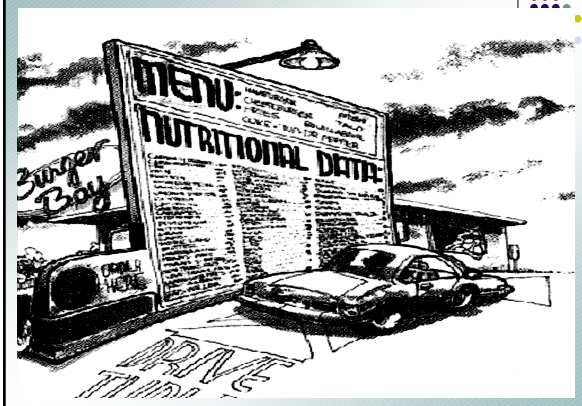
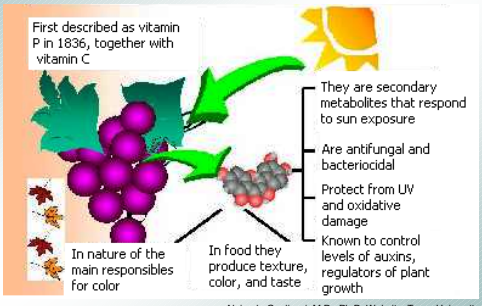


Relevance

- Polyphenols are a large family of compounds.
- Naturally produced plant secondary metabolites.
- Sources: vegetables, fruits, nuts, seeds, grains, beer, wine, tea, honey, herbal remedies, and dietary supplements.

- Dose: tens to hundreds of milligrams/day.
- Research suggests that polyphenols may possess potent anti-oxidant and anti-cancer properties.

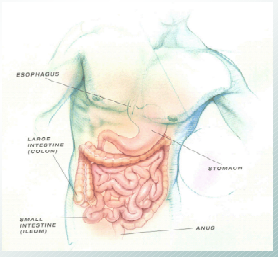
Role in Plants



Alejando Gugliucci, M.D., Ph.D. Website. Touro University-California
http://209.209.34.25/webdocs/BasicScience/gugliucci.htm

After consuming polyphenols?

- Found mainly as glycosides.
- Effect of cooking.
- Stomach acid cleavage.
- Small intestine.
- Phase II metabolism in liver [1].
- Low bioavailability:
  - Greatly metabolized.
  - Rapid elimination.
- Metabolite activity.

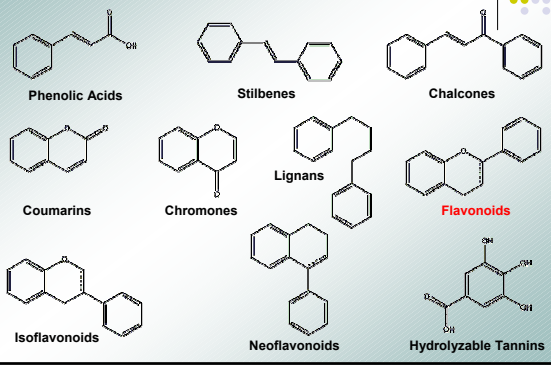


[1] Roupe KA, Remsberg CM, Yáñez JA, Davies NM. Current Clinical Pharmacology, 2006.

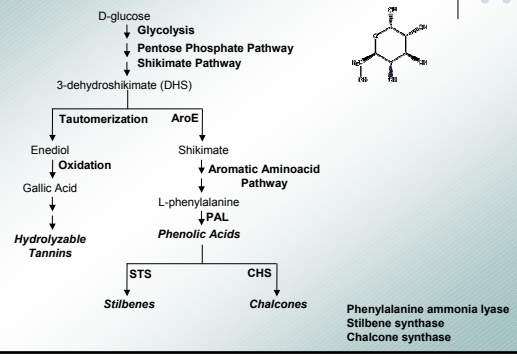
Biological Activities

- Anti-oxidant.
- Anti-platelet.
- Anti-hyperlipidemic.
- Anti-hypertensive.
- Anti-microbial.
- Anti-viral.
- Anti-allergenic.
- Anti-ulcerogenic.
- Anti-neoplastic.
- Anti-inflammatory.
- Anti-atherogenic.
- Anti-hepatotoxic.
- Vasodilator.

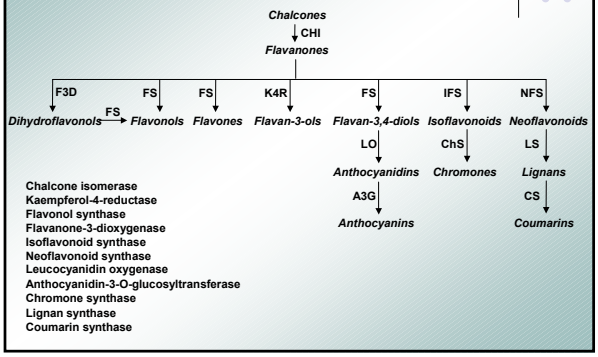
Polyphenol Classification



Metabolism in Plants



Metabolism in Plants



Chalcone isomerase
Kaempferol-4-reductase
Flavonol synthase
Flavanone-3-dioxygenase
Isoflavonoid synthase
Neoflavonoid synthase
Leucocyanidin oxygenase
Anthocyanidin-3-O-glucosyltransferase
Chromone synthase
Lignan synthase
Coumarin synthase

NUTRIENT DATA LABORATORY

Agricultural Research Service

USDA Database for the Flavonoid Content of Selected Foods - 2003

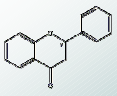
This database was created through a collaborative effort between the USDA and the Epidemiology Group, Jean Mayer USDA Human Nutrition Research Center on Aging, Frances Stern Nutrition Center, Tufts University School of Nutrition Science & Policy, and Tufts New England Medical Center, Boston, MA; the Bell Institute of Health and Nutrition, General Mills, Minneapolis, MN; and Unilever Bestfoods, North America, Englewood Cliffs, NJ

The database contains values for five subclasses of flavonoids:
FLAVONOLS: Quercetin, Kaempferol, Myricetin, Isorhamnetin
FLAVONES: Luteolin, Apigenin
FLAVANONES: Hesperetin, Naringenin, Eriodictyol
FLAVAN-3-OLS: (+)-Catechin, (+)-Gallocatechin, (-)-Epicatechin, (-)-Epigallocatechin, (-)-Epicatechin 3-gallate, (-)-Epigallocatechin 3-gallate, Theaflavin, Theaflavin 3-gallate, Theaflavin 3'-gallate, Theaflavin 3,3' digallate, Thearubigins
ANTHOCYANIDINS: Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin

http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html

Relevance - Flavanones

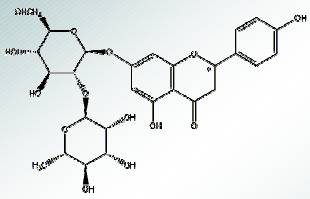
- Found in citrus fruits, tomatoes, peanuts and some herbs (mint, gaviota tarplant, yerba santa, and thyme).
- Called citrus flavonoids.
- Chiral compounds.
- Studied for over 50 years.
- Overlooked chirality.
- No disposition data of the separate enantiomers.
- No biological activity reports of separate enantiomers.
- No validated methods of detection and separation.
- Available only as racemate mixtures (50:50).
- Glycoside and aglycone terminology used interchangeably.



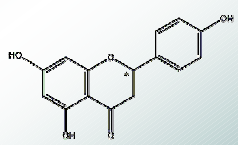
Relevance - Flavonols

- Found in tea, cocoa, vegetables, fruits (strawberry, apples, citrus fruits), wine, and beer.
- Their glycosides (mainly rutin) are found in most of fruits and vegetables.
- Disposition, and some biological activity been determined.
- Studied for over 70 years.
- Concentration reports are very variable.
- Acid hydrolysis is the most common method for aglycones extraction – not specific.

Flavonols



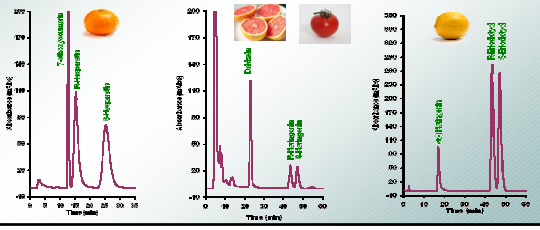
Naringin
(Naringenin-7-O-hesperidose)
(glycoside)
Less Active



Naringenin
(aglycone)
More active

Chromatography

- RH-HPLC:
  - Stationary phase: Chiral columns (Chiralpak AD-RH, Chiralcel OD-RH, Chiralcel OJ-RH).
  - Mobile phase: Mixture of Acetonitrile and Water acidified with H_3PO_4.
  - We directly measure the aglycones, glycosides are measured indirectly after enzymatic hydrolysis.



Enzymatic Hydrolysis

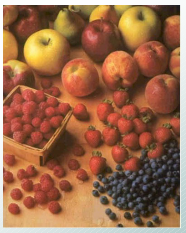
- Acidic hydrolysis mostly utilized.
- Use of HCl or H_2SO_4 to break sugar apart, but can also disrupt polyphenols.
- Enzymatic hydrolysis, adapted from [1], but [2] first ones to used in fruits.

Fruit Material Red-ripe tomato	Concentration Average (mg / 100 g FW)	Concentration (mg / 100 g FW)
Skin	Quercetin = 13.70 Kaempferol = 0.46	Quercetin = 159.7 Kaempferol = 15.9
Flesh	Quercetin = 0.05 Kaempferol = 0.04	Quercetin = 6.74 Kaempferol = 6.17
Seed	Quercetin = 0.11 Kaempferol = 0.04	Quercetin = 6.00 Kaempferol = 7.34

[1] Erlund I, Meririnne E, Alfthan G, Aro A. J. Nutr. 2001;131:235–241.
[2] Yáñez JA, Teng XW, Roupe KA, Davies NM. J Pharm Biomed Anal. 2005;37(3):591-5.
[3] Torres CA, Davies, NM, Yáñez JA, Andrews PK. J Agric Food Chem. 2005; Nov 30;53(24):9536-43

Fruit Disposition

- Hesperetin, Naringenin, Eriodictyol, Ellagic Acid, Phloretin, Quercetin, and Kaempferol:
  - Orange
  - Grapefruit
  - Lemon
  - Lime
  - Tomato
  - Strawberry
  - Cherry
  - Blueberry
  - Pear
  - Apple



Database

2003 USDA Database for the Flavonoid Content of Selected Foods:

Validated Method of Analysis?
Variety?
Maturity, Storage?
False Negatives?
Glycosides or Aglycones?
Enantiomers?
Genetic Modification?
Organic Versus Conventional?

(For mean, = mg/100 g, edible portion)

Description	Subclass	Flavonoid	Mean[1]
Apples, raw, with skin	Flavan-3-ols	(-)-Epicatechin	8.14
		(-)-Epicatechin 3-gallate	0.00
		(-)-Epigallocatechin	0.00
		(-)-Epigallocatechin 3-gallate	0.00
		(+)-Catechin	0.95
		(+)-Gallocatechin	0.00
	Flavones	Apigenin	0.00
		Luteolin	0.00
	Flavonols	Kaempferol	0.00
		Myricetin	0.00
		Quercetin	4.42
Apples, raw, without skin	Flavan-3-ols	(-)-Epicatechin	5.23
		(-)-Epicatechin 3-gallate	0.00
		(-)-Epigallocatechin	0.00
		(-)-Epigallocatechin 3-gallate	0.00
		(+)-Catechin	0.06
		(+)-Gallocatechin	0.00
	Flavonols	Quercetin	1.50

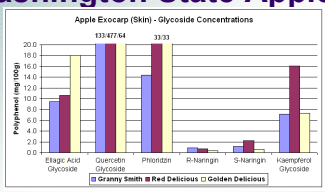
Our Laboratory

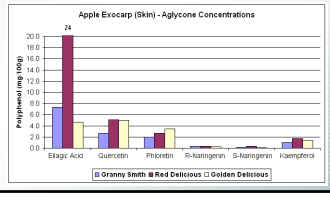
Food	Ellagic Acid Glycoside (mg/100g)	Quercetin Glycoside (mg/100g)	Phloridzin (mg/100g)	R- Naringin (mg/100g)	S- Naringin (mg/100g)	Kaempferol Glycoside (mg/100g)
Granny Smith Skin	9.43	132.59	14.39	0.88	1.14	7.10
Red Delicious Skin	10.62	477.30	33.22	0.67	2.23	16.07
Golden Delicious Skin	18.06	64.31	32.80	0.32	0.60	7.34
Granny Smith Flesh	8.74	6.15	3.98	1.11	1.29	6.64
Red Delicious Flesh	9.80	8.74	2.91	0.53	0.85	6.22
Golden Delicious Flesh	10.79	7.05	4.47	1.65	1.25	7.88

Food	Ellagic Acid (mg/100g)	Quercetin (mg/100g)	Phloretin (mg/100g)	R- Naringenin (mg/100g)	S- Naringenin (mg/100g)	Kaempferol (mg/100g)
Granny Smith Skin	7.33	2.65	1.99	0.32	0.19	1.06
Red Delicious Skin	24.02	5.12	2.68	0.32	0.36	1.70
Golden Delicious Skin	4.65	5.03	3.43	0.25	0.11	1.40
Granny Smith Flesh	7.48	2.20	2.16	0.24	0.10	2.62
Red Delicious Flesh	5.04	2.15	2.26	0.44	0.20	1.95
Golden Delicious Flesh	2.27	2.03	1.98	0.22	0.11	1.05

Variety Dependent
Aglycone and Glycosides
Identified 4 new compounds including naringenin enantiomers
Plus at least 7 other unidentified compounds!

Washington State Apples





Genetic Modification

- Parent genotype: *Ailsa Craig* tomato
- Genotypes:
  - Anthocyanin absent (aa) (LA 3617).
    - Anthocyanin completely absent in all plant parts.
  - Atroviolacea (atv) (LA 3736).
    - Elevated anthocyanin levels in all plant parts.
    - Enhanced photo-responsiveness.
  - High pigment-1 (hp-1) (2838A).
    - Exaggerated photo-responsiveness.
    - Elevated chlorophyll, carotenoids (lycopene and β-carotene), anthocyanins, and ascorbic acid leveles.

Tomato Mutants

Genotypes	Total R-Naringenin (mg / 100 FW) Exocarp	Mesocarp	Seed Cavity	Total S-Naringenin (mg / 100 FW) Exocarp	Mesocarp	Seed Cavity
'Ailsa Craig'	141	24.0	22.0	910	35.4	27.8
aa	191	23.8	22.7	1233	35.4	33.1
atv	182	24.5	23.2	985	37.0	32.8
hp-1	210	24.3	21.9	1160	42.1	28.4

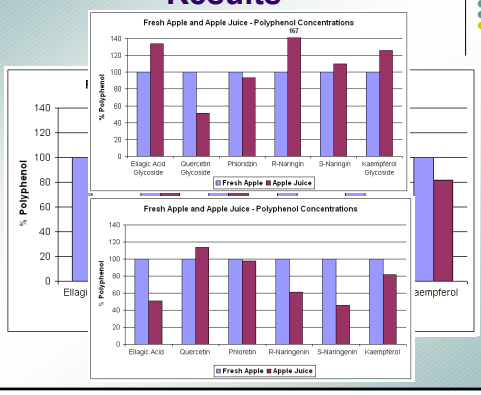
- Parent: *Ailsa Craig*
- Genotypes:
  - Anthocyanin absent (*aa*).
  - Atroviolacea (*atv*).
  - High pigment-1 (*hp-1*).
  - Stereospecific and genotype dependent

Torres CA, Davies, NM, Yáñez JA, Andrews PK. J Agric Food Chem. *2005; 53(24):9536-9543.*

Processed Food Products

- It is believed:
  - Loss of nutritional value.
  - High temperature.
  - Addition of additives.
- Current trend:
  - Fortify foods.
  - Make them more natural-like.
- Future direction:
  - Make processed foods with enhanced health benefits and claims

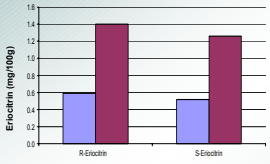
Results

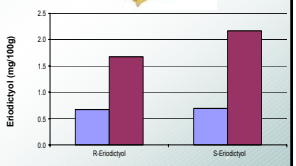


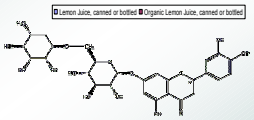
Organic vs. Conventional

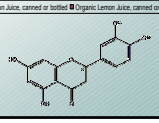
- Health claim:
  - Organic foods are better for you.
  - More phytochemicals.
  - No chemical use.
- Polyphenol-secretion inducers:
  - Sun exposure.
  - Fungal or bacterial attack.
  - UV exposure and oxidative damage.
  - Maturity stage.

Organic versus Conventional: Lemon Aid

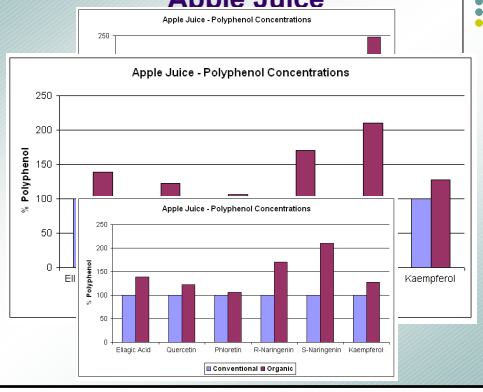




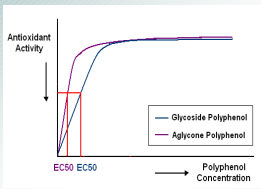


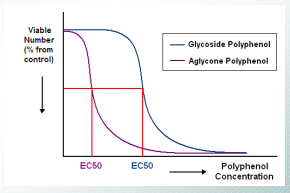


Organic Versus Conventional Apple Juice

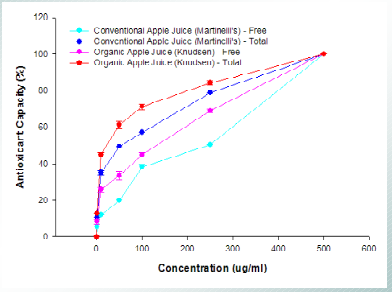


Pharmacological Activity

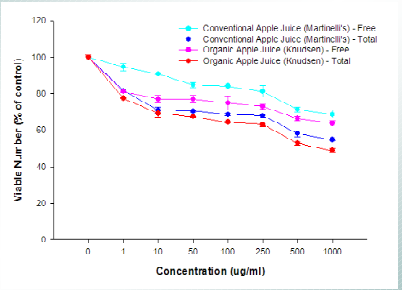




Organic Versus Conventional Apple Juice:Anti-Oxidant Activity



Organic Versus Conventional Apple Juice: Breast Cancer Activity

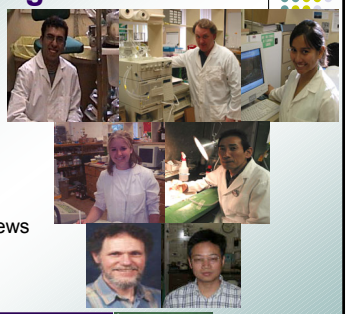


Conclusions

- Development and validated quantitation methods for polyphenols are required.
- Disposition and activity differences between glycosides, aglycone and enantiomers are important.
- USDA database needs refining.
- Detected polyphenols not reported in USDA database.
- Genetic modification may affects polyphenol concentrations.
- Food processing affects polyphenol concentrations.
- Organic farming may increase concentrations of some polyphenols.
- Alterations in polyphenols have implications for nutritional and health benefits of produce and food products.

Acknowledgments

- Davies Lab:
  - Jaime A. Yáñez
  - Dr. Neal M. Davies
  - Nicole Miranda
  - Connie Remsberg
  - Dr. Yusuke Ohgami
- Collaborators – WSU
  - Dr. Preston K. Andrews
  - Dr. Canming Xiao



The Organic Center

Washington Tree Fruit Research Commission

WARF