WASHINGTON STATE UNIVERSITY

Horticulture 409 Seminar Class
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Factors Influencing Antioxidant Levels in Plants

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Is the Content of Disease-Reducing Phytochemicals Influenced by Certified Organic Crop Production Practices?

In the Human Quest for Immortality….

- Antioxidants in food are touted as the preventative for practically all that ails us
- Antioxidants putatively with abilities to allay cancer, heart disease, reduce stroke, etc., based on:
  - *in vitro* anti-mutagenicity studies
  - *in-vitro* free radical and singlet oxygen scavenging
  - enzyme inductions
  - high dose rat studies using tumor promoters
  - epidemiology

Why We Need Antioxidants

- Oxidation is the transfer of electrons from one atom to another
  - Essential for metabolism
  - Aerobes: oxygen is ultimate electron acceptor in the ETS (electron transport system) that generates most of our useable energy (ATP)
- Sometimes, electron flow becomes uncoupled, generating free radicals (unpaired single electrons)
  - Oxygen-centered free radicals known as reactive oxygen species (ROS)
    - Superoxide, peroxyl, alkoxyl, hydroxyl, nitric oxide radicals

The Downside of Free Radicals

- Free radicals are very reactive
- Rapidly attack molecules in nearby cells, causing damage that is normally repaired
  - For example, will cause point mutations in DNA, but nearly all mutations are repaired (if healthy)
  - Oxidize lipids, proteins, carbohydrates causing membrane damage and protein modifications
- Oxidative damage considered to play a causative role in aging and several degenerative diseases
  - Heart disease, cataracts, cognitive dysfunction, cancer

Radical Fighters

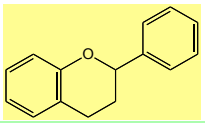
- Endogenous (physiological) and exogenous (dietary) antioxidant systems protect against free radicals
  - Endogenous systems
    - Repair systems
    - Enzymatic defenses
    - Non-enzymatic defenses
  - Exogenous systems
    - Vitamins (for ex., vit C, vit E)
    - Carotenoid pigments (& others)
    - Plant polyphenols

Plant Polyphenols

- Ubiquitous in all plants
  - >8000 phenolic structures known
- Products of secondary metabolism
  - Shikimate pathway
  - Acetate pathway
- Ten main groups; including
  - Phenols
  - Phenolic acids
  - Flavonoids
  - Tannins
  - Lignins

The flavonoids have the highest antioxidant activities among plant polyphenols

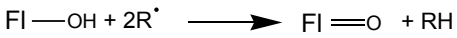
- Chalcones
- Dihydrochalcones
- Aurones
- Flavones
- Flavonols
- Dihydroflavonol
- Flavanones
- Flavanol
- Flavandiol
- Anthocyanidins
- Isoflavonoids
- Biflavonoids
- Proanthocyanidins (condensed tannins)



Basic Flavonoid Structure

How Do They Work?

- Simply speaking, antioxidant systems reduce free radicals by donating a hydrogen atom to the free radical, thus quenching its reactivity



(Fl = Flavonoid)

But not everyone is convinced about health potential…

Los Angeles Times latimes.com

October 27, 2003

Antioxidants: Have they been hyped?

■ Millions of Americans believe in supplements, but science can't back up many of the claims.

By Elena Conis, Special to The Times

Photos

A decade ago, antioxidants — nutrients such as beta carotene and vitamins C and E — were taking the nutrition world by storm. These free-radical fighters, medical experts predicted, would extend human life by protecting us from environmental hazards, cancer and heart disease.

Nevertheless, Epidemiological Studies Suggest…

- Protective effect of consumption of fresh fruits and vegetables against cancer, heart disease, and stroke
- Wine in moderation has been implicated in reducing heart disease risk

And Someone Is Always Willing to Capitalize on Information

http://www.sunsweet.com

SUNSWEET

Prunes Score Highest in Antioxidants

U.S. Department of Agriculture scientists at Tufts University have ranked fruits by their anti-oxidants levels. Many researchers believe anti-oxidants slow the aging process.

Fruit anti-oxidant score*

Prunes	5770
Raisins	2830
Blueberries	2400
Blackberries	2036
Strawberries	1540
Raspberries	1220
Plums	949
Oranges	750
Pink Grapefruit	483
Cantaloupe	252
Apples	218
Pears	134

*Oxygen Radical Absorbance Capacity is a test tube analysis that measures the total anti-oxidant power of foods and other chemical substances, per 100 grams.

Source: U.S. Department of Agriculture

GRAPHIC BY SAN JOSE MERCURY NEWS

The Latest Spin in the Human Quest for Health….

FoodNavigator.com
Breaking News on Food & Beverage Development

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Does organic food bring added health benefits?

29/07/2004

The benefits of conventionally grown produce over organic foods were the focus of a recent debate between food technologists, with scientists warning it is too early to reach conclusions despite new research that suggests some organic produce could have added health benefits.

Basis for the question…

- Over the last several years, a few published studies have measured the content of various antioxidants and antioxidant activity in fruits and vegetables grown under "conventional" and "organic" practices
- Publicity has accompanied those articles that have shown significantly higher content of antioxidants or antioxidant activity

In particular…

- An ACS press release highlighted an article published in JAFC (probably the best source for studies of antioxidant levels in food)

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

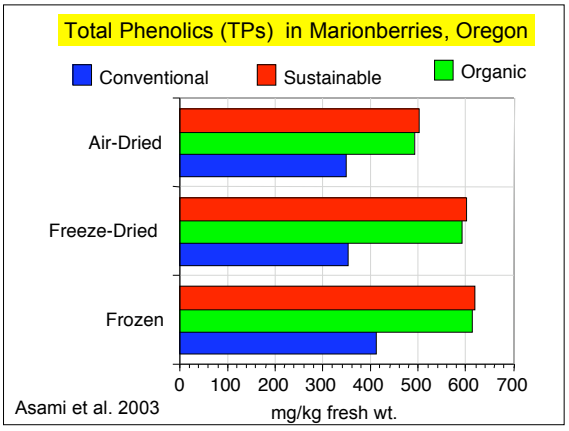
Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices

The Premise…

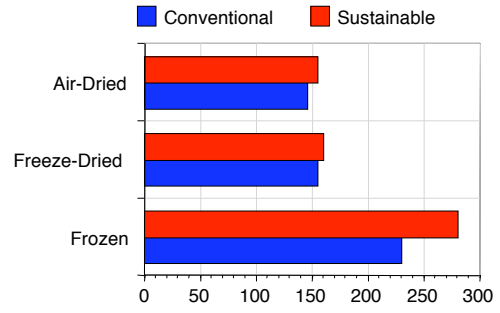
- Asami et al. (2003) state in their introduction:
  - "Differences between the content of phenolic metabolites in organically and conventionally produced fruits and vegetables allows for the possibility that organically grown produce may benefit human health better than corresponding conventionally grown produce."
- By inference, if organically produced food is shown to have higher levels of phenolics than conventionally produced food, it must be healthier--
  - Presumably because a lot of studies suggest polyphenolic antioxidants have beneficial health effects

Experimental Observations…

- Asami et al. 2003 reported that organic and sustainable production practices enhanced the content of ascorbic acid and total phenolics in marionberry (blackberry), strawberry, and corn



Total Phenolics (TPs) in Strawberries, Oregon



Asami et al. 2003

However…

- The authors failed to clearly define differences among their practices
  - For example, there is no scientifically acceptable definition or practices for sustainable agriculture
  - Conventional practices similarly lack definition
    - Indeed, many so-called organic practices are practiced by conventional growers and vice versa (no-tillage; pheromones; resistant plants, etc.)
  - Certified organic practices may use specifically approved pesticides but no mineralized fertilizers

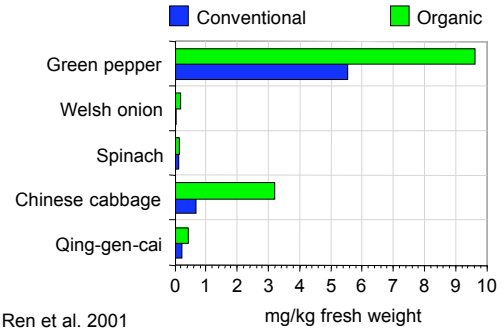
Lack of Information Negates Conclusions

- The sustainable & conventional practices both used herbicides and mineralized fertilizers (corn), no fertilizers (strawberry), or mineralized fertilizers but no herbicides (marionberries)
- Organic practices used organic N (manure), but soil types differed from sustainable or conventional practices
- No information given about irrigation management
- Based on this lack of control for confounding factors and lack of clearly delineated cultivation practices, Felsot and Rosen (2004) concluded that no conclusions could be reached on differences in antioxidant levels

Other Studies

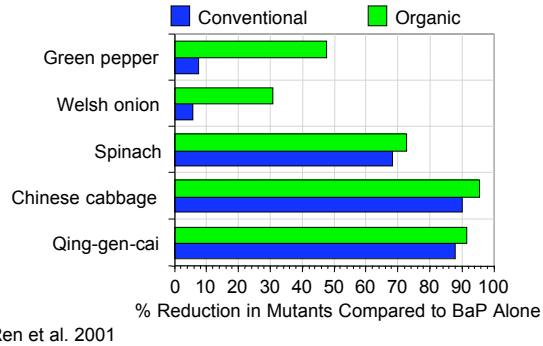
- Ren et al. 2001 (J. Sci. Fd Agric.)
  - Used several measurement methods to study antioxidant levels in several vegetables
  - Organic production practices defined by lack of synthetic pesticide use and use of manure or compost
    - However, chitosan mixed into soil and sprayed several times on leaves for protection against insects and pathogens
    - No information given about conventional practices

Quercitin Concentrations in Vegetables, Japan



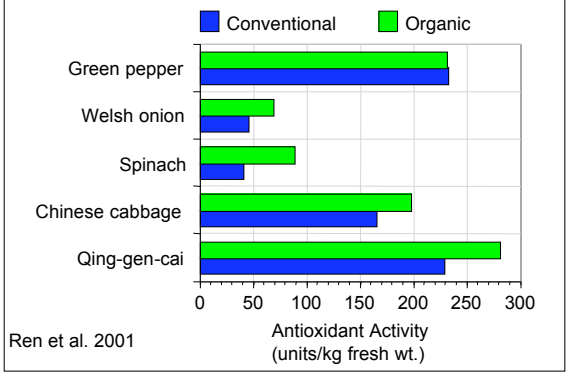
Ren et al. 2001

Antimutagenicity of Vegetable Extracts in Salmonella Mutagenicity Assay with Benzo-α-pyrene (BaP)



Ren et al. 2001

Antioxidant Activity in Vegetables, Japan



Ren et al. 2001

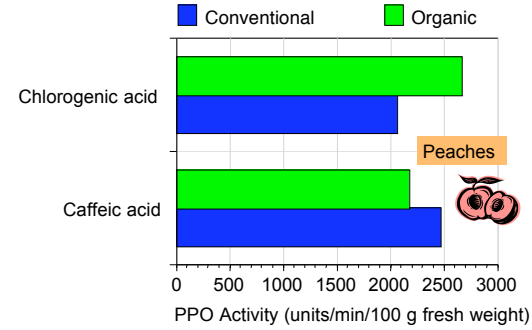
Observations

- Although the greatest difference in phenolic levels and anti-mutagenicity attributed to agronomic practices was observed in green peppers, conventional and organic peppers had similar levels of antioxidant activity

Other Studies

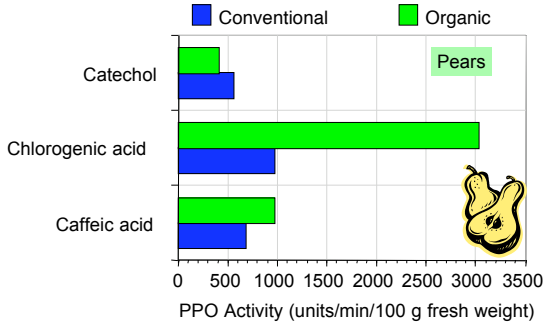
- Carbonaro et al. 2002
  - Peach and pear harvested from the same experimental orchard in Rome, Italy
  - Organic defined as no "chemically synthetic pesticides and largely without the use of soluble mineral fertilizers, within a diverse range of crop rotation and extensive soil tillage"
  - Measured polyphenol oxidase activity (PPO), total polyphenols, ascorbic & citric acid, and vitamin E (tocopherols)

Polyphenol Oxidase Activity using Phenolic Acid Substrates in Peaches Grown on a Single Experiment Station in Rome, Italy



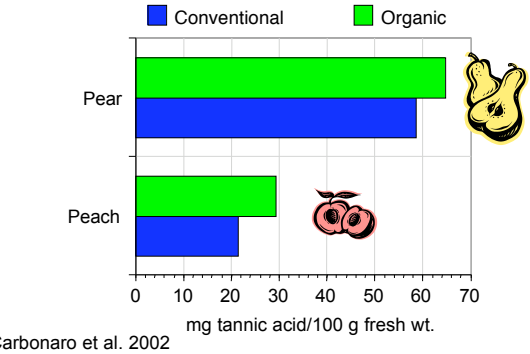
Carbonaro et al. 2002

Polyphenol Oxidase Activity using Phenolic Acid Substrates in Pears Grown on a Single Experiment Station in Rome, Italy

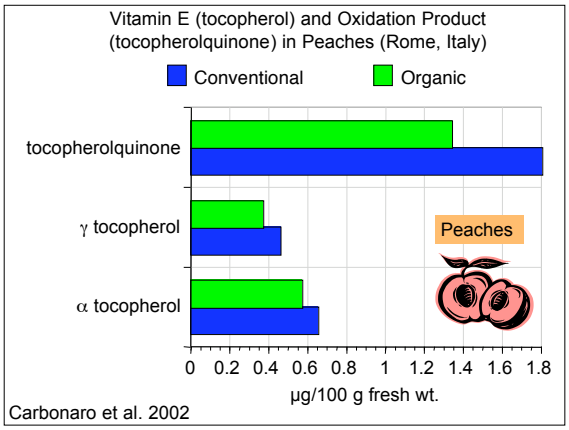


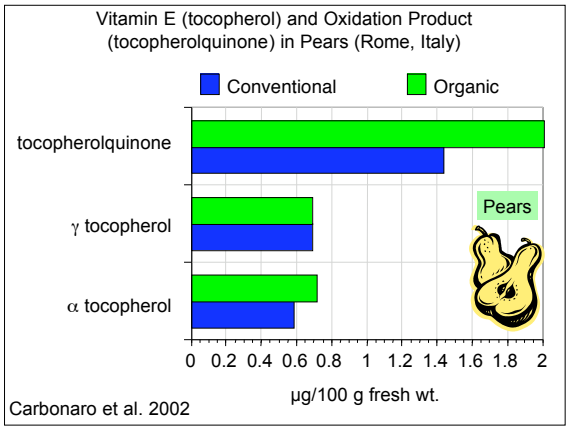
Carbonaro et al. 2002

Total Polyphenols in Pear and Peach Grown on a Single Experiment Station in Rome, Italy



Carbonaro et al. 2002



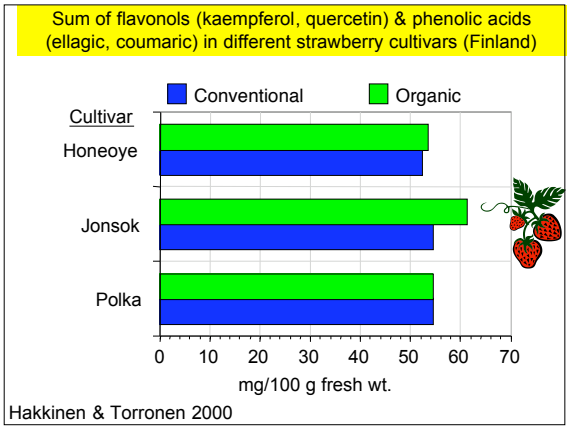


Observations

- Although Carbonaro et al. (2002) observed higher levels of polyphenols in organic pears and peaches, levels of vitamin E were not associated with production practices
  - Also, observed very small differences in vit. C levels
- Although high PPO activity was interpreted as reflecting more antioxidant activity toward specific substrates, PPO is also responsible for browning and off-flavor
  - Thus, it is not clear whether PPO represents a beneficial attribute in fruit (as opposed to leaves)

Other Studies

- Hakkinen and Torronen 2000
  - Examined flavonol and phenolic acid contents in strawberries
    - Treatments were cultivars, geographic origin, and cultivation practices (organic vs. conventional)
    - Samples came from 17 different farmers in eastern Finland
    - Three varieties grown on “organic” farms
    - Fruit picked at “optimum stage of ripeness”



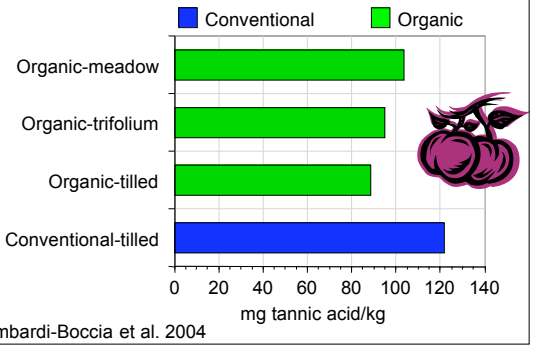
Observations

- In two of three strawberry cultivars studied by Hakkinen & Torronin (2000), antioxidant levels were similar between cultivation practices
- No information given on agronomic practices other than use of the term “organic”

Other Studies

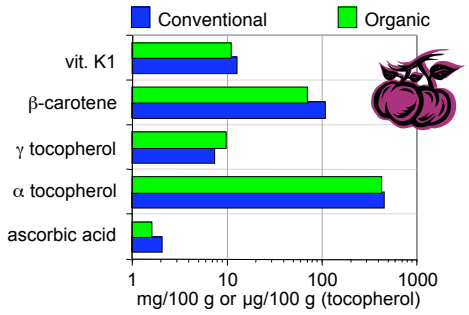
- Lombardi-Boccia et al. 2004
  - Examined phenolics (flavonol, phenolic acids) and antioxidant vitamins in plums grown at a single experiment station in Rome, Italy
  - Compared conventional practices (tilled soil) to three variations of organic practices (tilled soil, trifolium cover crop, undisturbed meadow)

Total phenolics in plums under conventional and three organic practices (Rome, Italy)



Lombardi-Boccia et al. 2004

Vitamin content of plums under conventional and organic (tilled) practices



Lombardi-Boccia et al. 2004

Observations

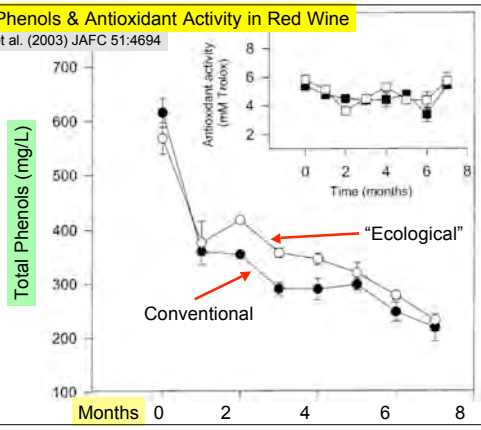
- Lombardi-Boccia et al. (2004) provide some information about cultivation practices
- Phenolic and vitamin levels were higher under conventional practices, but differences (as in other studies) are generally small
- Tillage or lack of tillage seemed to be the biggest factors associated with phenolic levels as evidenced by large differences among the organic production schemes

And Don't Forget the Wine!!

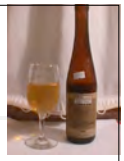
- Zafrilla et al. (2003) compared phenolic content and antioxidant activity in conventional and "ecological" wine initially and during storage
  - Conventional grapes (red & white) treated with pesticides (all synthetic fungicides
  - Organic grapes treated with "natural" pesticides (sulfur & pheromones)

Total Phenols & Antioxidant Activity in Red Wine

Zafrilla et al. (2003) JAFC 51:4694



Observations



- Zafrilla et al. (2003) differentiated agronomic practices by pesticide use but no other practices were discussed
- No statistically significant differences in total phenol content nor antioxidant activity was seen initially nor over the course of wine aging

Linking Old Observations with a New Hypothesis

- A common hypothesis among researchers studying antioxidants as affected by cultural practices:
  - Putative lack of pesticide use on organic crops leaves them vulnerable to insect & pathogen damage
  - Damage induces "hypersensitive" response that results in oxidative burst and eventual biosynthesis of antioxidants
  - Thus, organic crops have more antioxidants than pesticide protected crops

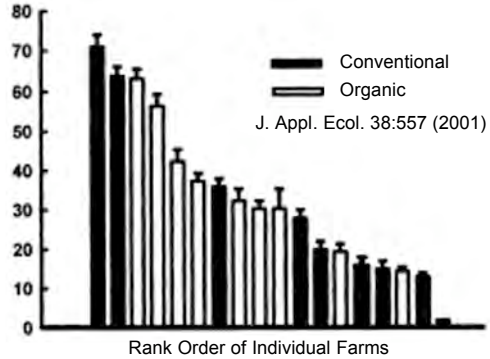
However…

- The "lack of pest protection" hypothesis rests on the myth that organic crops are not protected
  - Recall that a number of pesticides are registered for certified organic production
  - There is no evidence that organic crops are more vulnerable to pests
    - For ex., Carbonaro et al. (2002) said they saw no evidence of damage on their peaches and pears
  - Insect and pathogen infestations are usually spotty and often don't affect a whole field
    - Thus it is possible that many plants within a large field don't suffer significant pest damage

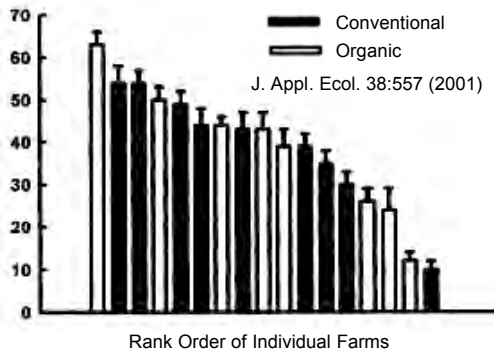
Recent evidence contradicts the expectation of more pests on organic crops

- Letourneau & Goldstein (2001) studied pest damage and arthropod community structure on 18 commercial farms (mix of conventional and certified organic)
- Concluded:
  - Insect pest damage levels varied across the spectrum of farm mgt. practices but were not associated with whether the farming operation was organic or conventional
  - Organic & conventional farms did not differ significantly for any type of damage to tomato foliage
  - Fallow mgt., surrounding habitat and transplant date explained the major variability among farms
  - Insecticide intensity was a weaker factor

Flea Beetle Damage (% damage) on Tomato Leaves
(copied from Letourneau and Goldstein 2001)



Puncture Damage (% damage) on Tomato Fruit
(copied from Letourneau and Goldstein 2001)



Moving On…

- Whether pest damage increases antioxidant levels is a worthy question to pursue, but…
- Better control of experimental design is needed (control soil type, irrigation, harvest)
- Researchers comparing cultural methods need to explicitly state the details of the agronomic practices
- Fruit and vegetables need to be examined for signs of pest damage
- Sampling replication should be at the level of the field, not in the lab

Be Aware

- In addition to large variations among cultivars, other factors can affect antioxidant levels
  - Fertilizer: nitrogen rate and availability
  - Irrigation regime; infiltration capacity
  - Ripeness
  - Holding times before analysis

In My Opinion

- Studies thus far have not proven that antioxidant levels are higher under any one agronomic practice
- Levels vary according to many factors
  - Probably most consistent is the cultivar effect and ripeness
- What we may be seeing in the studies comparing "organic" and "conventional" is just random error
  - We tend to forget that all statistical tests have Type I and Type II errors
  - We need to consider biological plausibility

For More Information

- http://feql.wsu.edu
  - Food & Environmental Quality Lab
- http://wsprs.wsu.edu/
  - WA State Pest Management Resource Ctr.
- http://aenews.wsu.edu
  - Agrichemical & Environmental News
- afelsot@tricity.wsu.edu

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