The Organic Center for Education & Promotion

Elevating Antioxidant Levels in Food through Organic Farming and Food Processing

An Organic Center State of Science Review

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January 2005

ACKNOWLEDMENTS

Several members of the Organic Center's Scientific and Technical Advisory Committee (STAC) made important contributions to this State of Science Review. Thanks to Mark Lipson and Steve Diver for raising critical questions regarding the reasons that plants produce polyphenols and antioxidants. Their comments led to a much-improved discussion of this key topic.

Special thanks are due to STAC members, Alison Eldridge, Joe Montecalvo, Craig Weakley, Brian Halweil and Kathleen Merrigan for constructive comments and valuable assistance during the review process.

Karen Lutz Benbrook did an excellent job in preparing both the Executive Summary and full State of Science Review (SSR) for publication. The Executive Summary of this SSR has been published separately and is accessible on the Organic Center website.

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PURPOSE AND OVERVIEW

This State of Science Review (SSR) projects the potential contributions of organic farming methods and food processing techniques on an important dimension of food quality – the polyphenol and antioxidant content of food. This review assesses research comparing antioxidant levels in conventional and organic foods, as well as studies analyzing the impacts of specific production practices that are typically used by organic farmers, but are less often found on conventionally managed farms.

Several sections are highlighted by solid lines and are included as primers for lay readers looking to better understand the basic biology of antioxidants, data sources used in conducting research in this area, and the types of research underway seeking to unravel the linkages between farming, food, and human health. Readers with a background in the relevant sciences may wish to skip these sections.

Four complex questions lie at the heart of this SSR:

1. What roles do polyphenols and antioxidants play in promoting healthy plant growth and helping plants respond to environmental stress and pest attacks?

2. Do these natural plant compounds enhance the nutritional quality of foods and promote animal and human health?

3. Are the levels of polyphenols and/or antioxidants higher in foods produced on organic farms compared to foods grown using conventional farming methods? Are specific polyphenols and antioxidants present at different levels in conventional and organic foods?

4. Do such differences in polyphenol and antioxidant levels enhance the health-promoting benefits of organic foods?

Epidemiological evidence has confirmed that diets rich in fruits and vegetables are associated with reduced frequency and severity of several health problems (Abeywardena et al., 2002; Afaq et al., 2002; Bagchi et al., 2001; Barbaste et al., 2002; Craig 1997; Daniel et al., 1999; Dragsted 2003; Folts 2002; Frei et al., 2003; Galati et al., 2000; Gee et al., 2001; Gerber 2003; Hertog et al., 1993b; Hertog 1996; Lambert et al., 2003; Rao 2003; Reed 2002; Rodrigo et al., 2002; Rotondo et al., 2000; Scalbert et al., 2002; Sun et al., 2002; Watanabe et al., 2002; Willcox et al., 2003; Youdim et al., 2001; Zava et al., 1997b). Scientists have been searching for more than two decades to identify the specific ingredients in fruits and vegetables that account for their many health-promoting benefits. Increasingly, that search points to secondary plant metabolites, many of which are antioxidants, along with levels and mixtures of vitamins, minerals, and fiber content.

Secondary plant metabolites, including enzymes and proteins, are produced by plants to regulate physiology and patterns of growth (Daniel et al., 1999). Some help plants deal with environmental extremes, deter pest attacks, or respond to damage caused when insects or plant pathogens reach damaging levels. Some play a role in repairing injured leaf or fruit tissue through the formation of pigments. Antioxidant-driven plant defenses and wound-healing processes account for the distinctive and sometimes remarkably rich color and flavor of certain fruits and vegetables grown in some regions. After harvest and during storage, fruits and vegetables with higher antioxidant levels are typically able to more effectively slow the onset and progression of post-harvest infections. This property can help extend the shelf life of produce and lessen mycotoxin risks (Daniel et al., 1999).

A small but encouraging set of studies have focused on how farmers can increase average polyphenol and antioxidant levels through management system changes, such as adoption of organic farming methods. A much larger and rapidly growing body of research is exploring the biological mechanisms through which secondary plant metabolites promote healthy crop growth, along with ways to preserve antioxidant levels present in food at harvest. Genetic mapping techniques are being used to isolate the specific genes governing the biochemical synthesize of certain antioxidants (Jones et al., 2003; Le Gall et al., 2003; Niggeweg et al., 2004). Many research teams are trying to identify how antioxidants in food promote animal and human health.

Some antioxidants are remarkably potent in triggering certain biological responses in mammals. In early 2004, one scientific team discovered a secondary plant metabolite in soybeans that is more potent in reducing low-density lipoproteins (LDL, or "bad" cholesterol) than the most potent cholesterol-lowering drugs on the market (Duranti et al., 2004). Other secondary plant metabolites are natural antibiotics that help protect developing crops from certain plant pathogens and extend the shelf-life of harvested fruits (Rauha et al., 2000).

Plant antioxidants are vital constituents in foods, promoting both plant and human well-being. They promote human health by neutralizing cell damage caused by free radicals and dioxygen or peroxide molecules, also called reactive oxygen or reactive nitrogen species. Plant antioxidants should be consumed daily. The level of antioxidants within an individual's body on a specific day reflects that person's diet in the previous few days. Levels in the body tend to spike within a few hours of consuming a meal high in total antioxidants, returning to baseline levels after a few more hours.

Scientists measure the potential of a given food to neutralize free radicals in the body through a number of methods. Most strive to estimate a food's total antioxidant capacity (TAC). This is a measure of the combined ability of all antioxidants in a given food to neutralize free radicals. Many laboratories, including several funded by the U.S. Department of Agriculture (USDA), are working now to more accurately calculate the TAC of common foods and typical diets, a process that will take many years of effort.

Average daily intake and absorption of antioxidants needs to double or triple for the average consumer to benefit fully from the health-protective potential of plant antioxidants

More research is also needed to estimate optimal antioxidant intakes. Existing evidence, however, suggests that most Americans are consuming and absorbing a sub-optimal level of antioxidants, and that average total daily absorption would need to double or triple for people to benefit fully from the health-protective potential of plant antioxidants.

We know of four ways for people to consume and absorb higher levels of antioxidants as part of their daily diets:

- Consume more fruits and vegetables;
- Choose fruits and vegetables that contain relatively higher levels of antioxidants per serving;

- Improve the health of their gastrointestinal tracts in ways that improve absorption of ingested dietary antioxidants;
- Consume foods that are grown, processed and prepared in ways that increase antioxidant levels in foods at harvest, and then preserves those levels as food moves from the farm to consumers.

USDA and the Department of Health and Human Services have just revised their Dietary Guidelines for Americans. Since 1995, the agencies have recommended three to five servings of vegetables and two to four servings of fruits, for a total of five to nine servings of fruits and vegetables every day. Vegetable servings are defined as one cup of raw leafy vegetables; 1/2 cup cooked or chopped vegetables; one ounce of vegetable chips; or 3/4 cup vegetable juice. Fruit servings are defined as one whole fruit, such as a medium apple, banana, or orange, a grapefruit half; 1/2 cup berries, melon or chopped raw fruit; 1/2 cup cooked or canned fruit; 1/4 cup dried fruit; or 3/4 cup fruit juice. For children two to five years old, one serving is two-thirds of the standard size.

The revised dietary guidelines call for two cups of fruit and 2.5 cups of vegetables for a typically 2,000 calorie diet, or about six to nine servings. The National Cancer Institute's "Five-A-Day" promotional campaign supports this revision. Americans are now eating on average about 3.3 servings of vegetables and 1.5 servings of fruit (USDA, 1997).

Eating more servings of fruits and vegetables is among the best ways to increase antioxidant intake; seeking out antioxidant-rich foods is another step in the right direction. The health of a person's gastrointestinal tract can have a big impact on antioxidant bioavailability. Most flavonoids, including many antioxidants, are glycosylated (bound with some form of sugar), which limits their bioavailability (Scalbert et al., 2000a). Most flavonoids become bioavailable after enzymes strip off the sugar in a metabolic process called deglycosylation.

> *Organic farming methods can increase concentrations of antioxidants in vegetables, fruits, grains, and dairy products, and in this way help people elevate their daily antioxidant intake without a proportional rise in calories.*

The fourth general strategy to increase antioxidants in the diet is to seek out foods grown and processed in ways that maximize and retain antioxidant concentrations. Organic farming methods can increase concentrations of antioxidants in vegetables, fruits, grains, and dairy products, and in this way help people elevate their daily antioxidant intake without a proportional rise in calories.

This State of Science Review focuses in particular on the potential of organic farming methods to increase average antioxidant levels in foods. By increasing concentrations of antioxidants in vegetables, fruits, and grains, organic farming methods can help people increase their antioxidant consumption without a proportional increase in caloric intake. Adopting food-processing technologies and cooking methods that naturally help retain a higher percentage of the antioxidants in foods offers perhaps the most significant near-term opportunity to increase average daily antioxidant intakes.

Strawberry harvest in a field of organic berries near Watsonville, CA. Ripeness at harvest is a key variable influencing both vitamin and antioxidant levels.

TERMINOLOGY, METHODS AND SOURCES OF DATA

The tools, concepts, and insights from several fields of science are needed to unravel the role of dietary antioxidants in improving human health. The range of disciplines essential to developing such understanding encompasses most of the biological and agricultural sciences, the medical profession, nutrition science, and food science and technology. An essential place to start is by reviewing the terminology of secondary plant metabolites, polyphenols, and antioxidants.

Terminology

There are likely far more than 50,000 secondary plant metabolites and some 4,000 flavonoids (Daniel et al., 1999), many of which are antioxidants. Scientists in dozens of disciplines are actively researching the role of secondary plant metabolites in promoting plant and animal health. Many different classification schemes, criteria, and terms have been developed and are encountered in the literature describing the chemical structures, properties, and biological functions of polyphenols and antioxidants.

The voluminous literature on secondary plant metabolites and antioxidants can be confusing. One paper will call a given chemical an antioxidant, another might call it an antioxidant polyphenol, and a third will use the phrase "a flavonoid with antioxidant potential." Others call antioxidants "phytochemicals." The box *Antioxidant Terminology* strives to help non-experts weave their way through the diverse language used to classify and discuss antioxidants and secondary plant metabolites.

Antioxidant Terminology

Secondary plant metabolites are a diverse group of naturally produced chemicals that usually possess no obvious primary function in plant cell growth. They are synthesized by plants in response to external stimuli and often play some regulatory function in a cascade of physiological and metabolic reactions to stress or pest attacks. When a biological function is discovered for a secondary plant metabolite, it is typically relabeled as a vitamin (Brandt et al., 2001).

Polyphenols encompass several classes of weakly acidic chemicals related to, or built upon the phenyl ring. Polyphenols contain one or more phenolic hydroxyl groups directly attached to these carbon-based aromatic phenyl-ring compounds. These are easily oxidized to quinones by reactive oxygen species, a property that helps account for their free radical scavenging capacity.

Polyphenols have diverse functions in plants and are a major class of secondary plant metabolites. The major plant polyphenol by volume is lignin (Daniel et al., 1999). Plant polyphenols may have beneficial and/or detriment effects on mammals. After plants die, phenolic compounds can persist for weeks and affect decomposition. They also have an impact on the shelf life of harvested produce.

Antioxidants are chemicals that oppose or neutralize oxidation in cells. Some of the most important are essential vitamins for which the Food and Drug Administration has established Recommended Dietary Allowances, or RDAs.

Many, but not all, secondary plant metabolites are antioxidants. Normal physiological processes synthesize most of the antioxidants in the human body at any one time, while the balance comes from food. All dietary sources of antioxidants come from secondary plant metabolites. Some are polyphenols; other important ones are not, like alpha- and beta-carotene and lycopene. These important carotenoid antioxidants lack the phenolic ring structures that characterize polyphenols. Antioxidants in milk and dairy products come from the antioxidants and polyphenols in the grass, forage, and grains consumed by cattle.

Antioxidants scavenge free radicals by inhibiting reactions within cells brought about by dioxygen or peroxide molecules, also called reactive oxygen species, or ROS, as well as by reactive nitrogen species. The total supply of antioxidants circulating in the body is the sum of antioxidant enzymes and acids manufactured by the body plus antioxidants consumed in foods.

The body manufactures a number of antioxidant enzymes including gluthathione, a tripeptide consisting of glycine, cysteine, and glutamate; glutathione peroxidase; superoxide dismutase; and catalase. A number of chemicals produced in the brain are also antioxidants. Glutathione peroxidase, together with glutathione, neutralizes hydrogen peroxide, a common free radical, through a reaction that produces water. The body manufactures gluthathione from the amino acid glutamate, and glutathione peroxidase is manufactured from gluthathione. Most antioxidants are beneficial in certain ways within a defined range of doses, yet at high doses, some can be damaging, or lead to no known biological effect. Many, and probably most, antioxidants can also function as pro-oxidants when they exist with an unpaired electron after donating electrons to reactive oxygen species.

Flavonoids, also sometimes called bioflavonoids, are secondary plant metabolites, the majority of which function as antioxidants at the levels commonly found in foods and beverages. Flavonoids encompass a very large and widespread array of water-soluble phenolic derivatives. Some are brightly colored. They are found in the vacuoles of plant cells and are classified by the oxidation state of their pyran ring (part of their chemical structure). There are about a dozen recognized classes of flavonoids. These include flavonols and flavones (typically yellow pigments in plants), flavanones, and isoflavones (a major flavonoid in soybeans). A flavan is the parent ring structure on which the chemical structures of most flavonoids are based.

The body produces several antioxidants that search for free radicals and detoxify them by supplying the missing electron. Antioxidants consumed in food do the same thing, reinforcing this vital health-promoting function.

All secondary plant metabolites and flavonoids are phytochemicals, but the reverse is not necessarily true. Some antioxidants, like resveratrol, are phytoalexins, which are low molecular weight, lipophilic compounds that accumulate at sites of pathogen infection.

Some secondary plant metabolites are anti-nutrients, which are compounds that make proteins or other essential nutrients less bioavailable to humans.

How do all these terms used to describe secondary plant metabolites with antioxidant potential relate to each other?

One set of terms classifies chemicals produced by metabolic processes in plants according to the biological activity or function of the chemical in the plant. Major classes of plant chemicals include enzymes, hormones, and proteins. The term phytochemical encompasses all these classes of chemical compounds naturally produced by plants.

Another set of terms classifies chemicals according to their biological activity or function in mammals. The term vitamin refers to a plant compound that serves an essential function in humans, which may be similar to or very different from the role the chemical plays in plants. The term antioxidant is another example – it describes a class of chemicals that share the capacity to scavenge and neutralize free radicals in mammalian systems. Antioxidants are essential compounds in all organisms: plants, mammals, fungi, bacteria, etc.

Other terms classify secondary plant metabolites according to their chemical structure. These terms include polyphenol and flavonoid. Still other terms classify chemicals based on the type of reaction that produces them or their principle role in, or relation to, common chemical reactions. For example, terms such as polymer, monomer, stereoisomer, glycoside, and aglycone (the non-carbohydrate portion of a molecule that is left after the glycoside is stripped off via hydrolysis) describe chemicals that have been involved in particular, common chemical reactions.

So What's a Free Radical?

Free radicals are oxygen-based or nitrogen-based molecules with unpaired electrons that are generated by a number of metabolic processes within the body. For example, when the body turns foods into energy, free radicals are formed by normal oxidation reactions. Vigorous exercise increases free radical production, as does inflammation, exposure to certain chemicals, cigarette smoke, alcohol, air pollutants and high-fat diets.

As one free radical interacts with another molecule, in effect stealing part of it, a new free radical is created. These reactions often occur in or near cell membranes and can erode the cell's internal integrity. Some free radicals target mitochondria inside cells, affecting their energy-producing capability. Other free radicals attack DNA.

To keep this cycle from spinning out of control and causing dangerous cellular damage, the body produces several natural antioxidants, which are compounds that search for free radicals and detoxify them by supplying the missing electron. Plant-based foods also contain antioxidants, which complement the antioxidants produced by the body. When ingested, some portion of plant antioxidants is absorbed in the gut lining and enters the bloodstream.

Reactive oxygen species encompass both true free radicals and molecules with paired electrons like hydrogen peroxide. Other important reactive oxygen species include singlet oxygen and superoxide anions, hydroxyl, and peroxyl radicals. Free radicals and reactive oxygen species cause cell damage, trigger inflammation, and promote abnormal cell growths, including many kinds of cancer. Antioxidants help prevent tissue damage by combining with free radicals and neutralizing them.

Measuring Antioxidants in Foods

A wide range of factors can influence the mix of polyphenols and antioxidants that a plant manufactures, as well as the levels the plant produces at any given point. These factors include soil type and chemistry, available nitrogen and levels of other plant nutrients, moisture levels, temperature, and pest pressure (Brandt et al., 2002; Daniel et al., 1999; Romero et al., 2004b; Wang et al., 2000; Wang et al., 2002). In general, factors that impose stress on plants tend to trigger a plant's innate defense mechanisms and these mechanisms are driven by and/or entail the synthesis of antioxidants.

Phenolic antioxidants are present in plants at concentrations up to several grams per kilogram (Daniel et al., 1999). In general, levels are higher in the rinds and skins of produce compared to levels in the fruit. A number of chemical assays have been developed to measure different antioxidants. Others are designed to measure total antioxidant capacity, or TAC. In vitro assays are designed to test antioxidant levels in foods, while other assays measure levels in blood (Erlund et al., 2001; Noroozi et al., 2000), lymphocytes (Anderson et al., 2001), or urine (Atkinson et al., 2002; Karr et al., 1997; Milbury et al., 2002; Nielsen et al., 2002).

A number of thorough reviews have compared the strengths and weaknesses of different assays, each of which is uniquely suited to detect and accurately measure certain polyphenols, or certain derivatives of phenolics in certain tissues or media, but no assay is universally accurate (Boxin et al., 2004; Cao et al., 1998; Frankel 1989; Sanchez-Moreno 2002). Many authors report results on polyphenol and antioxidant activity in foods compared to the findings of other teams using different methods. Sometimes correlation coefficients are reported. These reflect the degree of agreement across assays when testing the same food for a given polyphenol or total antioxidant activity. For example, a Norwegian team calculated total antioxidant capacity of a wide range of foods in the typical Norwegian diet using the FRAP (ferric reducing ability of plasma) assay (Halvorsen et al., 2002). They reported a 0.951 correlation coefficient with the results of Wang et al. (1996) on 12 fruits, and correlation coefficients ranging from 0.79 to 0.13 for ORAC (Oxygen-Radical Absorbance Capacity) values based on three different free radicals (ROO’, OH’, and CU^{++}).

Because the biochemistry of antioxidants is so complex and dynamic, it is hard to calculate the portion of total antioxidants in a person that is manufactured by the body (endogenous antioxidants) in contrast to the share from dietary sources (exogenous antioxidants).

The measurement of antioxidants is complicated because the biochemistry of antioxidants is so complex. Plants have multiple mechanisms to produce and metabolize antioxidants, just as mammals do. The diversity of polyphenolic secondary plant metabolites reflects the many differences in the carbon skeletal structure of phenolic molecules, as well as differences in their oxidative state (Scalbert and Williamson, 2000). In addition, antioxidants in foods and in people are continuously changing form and even function as a result of glycosylation (reactions with sugar molecules), hydroxylation of aromatic phenolic rings, through polymerization, and as a result of the biosynthesis of various stereoisomers.

Because there are so many sources of antioxidants — both from inside the body and from foods — and because antioxidants are continuously reacting with other molecules and tissues and changing form, it is hard to sort out the portion of total antioxidants in a person’s body that is manufactured by the body (endogenous antioxidants) in contrast to the share from dietary sources (exogenous antioxidants). However, much is known about the relative roles and importance of endogenous and exogenous sources of antioxidants:

- Despite our endogenous antioxidant defense system, two dietary antioxidants (vitamins C and E) are essential for life, and several dietary minerals (iron, copper, selenium, zinc) are required as antioxidant enzyme cofactors.
- The effectiveness of endogenous defenses (especially the antioxidant enzymes) declines with age, increasing the importance of dietary sources of antioxidants in older adults.
- All dietary antioxidants, including the polyphenols, have other mechanisms of action besides and beyond the quenching of free radicals; some of these mechanisms appear important in their health-promoting biological activities.
- Dietary antioxidants cannot substitute for one another, e.g., consuming more vitamin C will not displace the need for vitamin E or replace the action of quercetin.
- The bioavailability of antioxidants is critical for systemic defenses throughout the body, but being bioavailable is not the only way that antioxidants can promote health. Antioxidants in the gut lumen and those that become attached to the gut wall may be important for gastrointestinal defenses and detoxification, and in this way help prevent colorectal cancer and inflammatory bowel diseases.

Characterization and Sources of Data on Antioxidants in Foods

A thorough review article on polyphenols appears in the May 2004 issue of the *American Journal of Clinical Nutrition* (Manach et al., 2004). The authors review both sources and levels in the diet by major class of polyphenols, as well as factors having an impact on bioavailability. The authors point out that, in addition to their beneficial activity as antioxidants, polyphenols also can regulate biological processes in a number of other ways, many of which are just beginning to be studied.

The article describes polyphenols as secondary plant metabolites containing several hydroxyl groups arranged on aromatic rings. The authors note that several thousand polyphenols have been identified and several hundred are known to appear in edible portions of foods. Common classes are *phenolic acids, stilbenes, lignans, and flavonoids,*.

Phenolic acids occur in two classes: derivatives of benzoic acid or cinnamic acid.

Benzoic Acid Derivatives	Cinnamic Acid Derivatives
Protocatechuic acid	Coumaric acid
Gallic acid	Caffeic acid
	Ferulic acid

These phenolic acid derivatives in turn combine with sugars to become glycosylated. Caffeic and quinic acids, for example, combine to form chlorogenic acid, a compound that is found in many fruits and in coffee (Manach et al., 2004). Caffeic acid is generally the most abundant phenolic acid and accounts for between 75 percent and 100 percent of the total hydroxycinnamic acid content of most fruits, with the highest concentrations typically in the outer parts of ripe fruits. Since these chemicals play a role in plant defense mechanisms, it makes sense that plants generally express the highest levels at the point of initial attack by both insects and fungal or bacterial pathogens — that is, the skin of fruits.

Plants are thrifty and generally express the highest levels of polyphenols and antioxidants at the point of initial attack by both insects and fungal or bacterial pathogens — on or in the skins of fruits and vegetables.

Ferulic acid is the most common phenolic acid in cereal grains, which account for most dietary intake of this phenolic acid and as much as 90 percent of total polyphenol content of wheat (Manach et al., 2004). Wheat bran is a particular rich source of ferulic acid (Scalbert and Williamson, 2000), where it is found mostly in the outer parts of the grain. Hence, ferulic acid levels are far lower in baked foods derived from highly processed flour. The health benefits of wheat germ and bran are likely in part due to their relatively high concentrations of ferulic acid.

Linseed is the major source of *lignans* in the diet, although several other cereals, legumes and vegetables contain low levels.

Stilbenes are found in only low quantities in the human diet. Resveratrol is the most widely studied stilbene and may have anticarcinogenic activity (Manach et al., 2004).

Flavonoids share a common chemical structure – two aromatic rings that are bound together by three carbon atoms that form an oxygenated heterocycle. Flavonoids are further divided into six classes:

- Flavonols
- Flavones
- Isoflavones
- Flavonols (catechins and proanthocyanidins).
- Flavanones
- Anthocyanidins

Flavonols are the most common flavonoids in foods, with quercetin and kaempferol being by far the most abundant (Manach et al., 2004). They are generally present at relatively low concentrations on the order of 15 to 30 mg/kg of fresh weight. The richest sources of flavonols are onions, kale, leeks, broccoli, and blueberries. These polyphenols are almost always present in glycosylated forms. Fruits often contain five to 10 different glycosylated forms of a given flavonol.

Flavonol concentrations are highest in or near the peel of fruits since their biosynthesis is stimulated by light. Levels of flavonols in fruits from the same tree or shrub can vary significantly and reflect the intensity of light energy reaching the fruits; levels are often higher on an exposed side of an individual fruit than on its shaded side. In green leafy vegetables, the outer leaves often contain flavonol concentrations more than 10 times the concentrations found in inner leaves. As a result of this pattern of concentration in fruits and vegetables, consuming the outer portions of produce is a key strategy in increasing flavonol intake.

Smaller fruits of the same species, compared to larger fruits, tend to have higher concentrations of flavonols in part because of the relationship between surface area and fresh weight. The larger the fruit, the less skin area there is per gram of fruit weight.

Parsley and celery are the only two known important sources of flavones in fruits and vegetables. Citrus rind contains significant flavones, but is generally not consumed. Some grains contain low levels of flavones.

Isoflavones are found almost exclusively in legumes, with soybeans and processed soybean products being the major dietary source. Isoflavones are phytoestrogens which can mimic the function of hormones in the human body (Manach et al., 2004). The three most common molecules forming isoflavones are genistein, daidzein, and glycitein, each of which can appear in one or more of four forms. Processing has a major impact on the relative concentration of the different forms of isoflavones. Isoflavone levels vary widely, from 580 mg/kg fresh weight in whole beans, to 30 to 175 mg/liter in soymilk.

Flavonols exist in both the monomer form (catechins) and polymer form (proanthocyanidins). The highest concentrations of catechins are present in green tea and chocolate. Apricots are the richest source of catechins among fruits, containing 250 mg/kg of fresh weight. Red wine is another major source. Catechins are not glycosylated and are remarkably stable in most foods (Scalbert et al., 2000b).

Proanthocyanidins are also known as condensed tannins. There are different chemical forms of catechins (dimeric, oligomeric, and polymeric), which react with salivary proteins and produce the astringent taste of many fruits and beverages. As fruit ripens, the degree of astringency declines proportional to changes in proanthocyanidin levels. Because proanthocyanidins appear in so many forms, and their degree and form of polymerization change continuously as fruits ripen, it is hard to measure total levels in many foods.

Flavanones are found mostly in citrus fruits and tomatoes. Mint is also a source. Orange juice is a major dietary source of the flavanone hesperidin. A single glass may contain between 40 and 140 mg of flavanone glycosides, but consuming the same amount of whole fruit will deliver as much as five times more total flavanone because of the relatively higher concentrations in the pulp of the fruit compared to juice.

Anthocyanins are pigments that give fruits and vegetables their color, although some are colorless. Anthocyanins can change color as a function of pH. Food concentrations of cyanidin, the most abundant anthocyanin, is generally proportional to intensity of color and can occur at 24 mg/kg of fresh weight. Levels increase as fruits ripen and are highest in the skins and peels of produce.

In general, most foods contain multiple flavonoids and their characterization is incomplete (Manach et al., 2004). In addition, their chemical form, glycosylation status, and degree of polymerization are often in a state of flux. Hence, measuring levels of specific flavonoids, or total flavonoid, phenolic or antioxidant content, is like taking a snapshot of a rapidly moving target that is also changing form and function.

Measuring levels of specific flavonoids or total antioxidant content in food or the human body is like taking a snapshot of a rapidly moving target that is also changing form and function.

In general, according to Manach et al. (2004), phenolic acid concentrations decline with ripeness, while anthocyanin levels increase. Phenolic acids are directly involved in responses to external stress, like pests, and they contribute to healing by lignification of wounded plant tissues. Manach et al. say:

> *"Although very few studies directly addressed the issue, the polyphenol content of vegetables produced by organic or sustainable agriculture is certainly higher than that of vegetables grown without stress, such as those grown in conventional or hydroponic conditions."*

A Primer on Accessing Information on Antioxidants in Specific Foods

There are several databases that include the levels of selected and total antioxidants in a variety of foods. USDA has been funding work to establish a national baseline of antioxidant levels in common foods, as part of the National Food and Nutrient Analysis Program (NFNAP). A comprehensive study of antioxidants in common foods by a team of USDA scientists has been published (Wu et al., 2004). The data have also become a part of NFNAP and is accessible through the USDA Nutrients Data Laboratory http:www.nal.usda.gov/fnic/foodcomp.

The "USDA Database for the Flavonoid Content of Selected Foods – 2003" contains information on five subclasses of flavonoids (accessible at http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.html):

- *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

USDA's flavonoid website contains a March 2003 report in Adobe Acrobat format that reports the levels of the above flavonoids in hundreds of foods (http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.pdf). The same data are also accessible in a Microsoft Access database (http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/flav.mdb), allowing users the ability to create their own tables and input additional values. Table 1 presents information on major antioxidant flavonoids and total antioxidant potential in typical servings of a sample of foods, and draws on this USDA database and Wu et al. (2004)

Table 1. Levels of Common Flavonoids in Selected Foods (mg/100 grams, edible portion) and Total Antioxidant Content of Food per Typical Serving (see notes)

Fresh Food	Quercitin	Kaempferol	Epicatechin	Catechin	Total Antioxidant Capacity per Serving
Apples	4.42	0	8.14	0.95	5,900
Beets	0.13	0	0	0	1,886
Blackberries	1.03	0.08	18.08	0.66	7,701
Blueberries	3.11	0	1.11	0	13,247
Broccoli	3.21	6.16	0	0	982
Celery	0.07	0	0	0	741
Carrots	3.50	0	0	0	344
Cranberries	14.02	0.09	4.2	0	8,983
Cucumbers	0.04	0.06	0	0	60
Green Peppers	0.65	0	0	0	664
Lettuce	2.47	0.07	0	0	1,213
Onions	19.93	0.89	0	0	1,281
Peaches	0	0	0	2.33	1,826
Pears	0.42	0	3.17	0.26	3,172
Plums	1.20	0	2.84	3.35	4,844
Potatoes	0.01	0.05	0	0	4,882
Raspberries	0.83	0	8.26	0.97	6,058
Red Grapes	3.54	0	1.95	0	2,016
Spinach	4.86	0.01	0	0	1,056
Strawberries	0.65	0.79	0	4.47	5,938
Sweet Cherries	1.25	0	9.53	2.17	4,873
Tomatoes	0.57	0.07	0	0	552

Notes: Flavonoid levels are from the "USDA Database for the Flavonoid Content of Selected Foods." "Total Antioxidant Capacity per Serving" is from Table 1 (Wu et al., 2004). The highest value reported for a food was selected in cases where multiple values appear in the table.

An Internet resource, "Dr. Duke's Phytochemical and Ethnobotanical Databases," provides easy access to a list of the chemicals found in nearly all agronomic plants, the biological activities of each chemical, and the amounts of various chemicals typically found in a given plant. It is compiled by James Duke, Ph.D., a botanist and former chief of the National Germplasm Resources Laboratory at USDA. The database is freely accessible at http://www.ars-grin.gov/duke/.

Duke's databases are searchable by chemical name, plant species, and biological activity. A search on "tomato" yields a list of hundreds of chemicals. Clicking on any chemical in the list leads to more detailed information on that chemical's biological activities and concentrations in various other plants. For example, under lycopene, the database lists 16 categories of biological activities associated with lycopene intake. More than two-dozen plants with relatively high levels of lycopene are listed, from highest concentration to least (or unknown concentration).

Variability in Polyphenol and Antioxidant Levels in Food

Steady progress has been made in developing more accurate methods to measure polyphenol and antioxidant content as it fluctuates up and down. Polyphenol/antioxidant content and dynamics are relatively well known in several key crops, including apples, strawberries, spinach, soy products and tomatoes. Several foods that are major sources of a particular polyphenolic compound have also been extensively studied. Examples of well-studied food-polyphenol combinations include resveratrol in red wine grapes, catechins in tea, naringenin in citrus products, lycopene in tomatoes, and isoflavones in soya products.

Still, major analytical challenges remain. The authors of a comprehensive review of polyphenol sources and bioavailability conclude that, "With the current state of knowledge, it is extremely difficult to determine for each family of plant products the key variables that are responsible for the variability in the content of each polyphenol and the relative weight of those variables" (Manach et al 2004).

It is clear that, across the U.S. population, a relatively small number of foods account for a significant portion of the typical person's dietary intake of total polyphenols, flavonoids, and antioxidants. Scientists developing food-antioxidant data for USDA's National Food and Nutrient Analysis Program recently published an article with up-to-date results on more than a hundred foods. They broke the foods tested into four categories based on hydrophilic Oxygen-Radical Absorbance Capacity (H-ORAC) values per serving, using USDA's standard reference guide to define a serving size (Wu et al., 2004). The four groups of foods had H-ORAC scores per serving as follows:

- Very High — between 2,000 and 14,000 H-ORAC units (umol of Trolox Equivalent [TE]/serving);
- High — 1,000 to 1,999 H-ORAC units/serving;
- Moderate — 500 to 999 H-ORAC units/serving; and
- Low — Zero to 499 H-ORAC units/serving.

Common foods in each of these four groups are noted in Table 2 ("Very High" and "High" group foods) and Table 3 ("Moderate" and "Low" group foods), based on the data provided in Wu et al. (2004). The foods within each group are ranked from highest to lowest H-ORAC units within the group. The table also contains the average serving size in grams, calories per serving, and H-ORAC units per calorie. The last column reports the ranking across all foods of H-ORAC units per calorie. This measure of "antioxidant bang per calorie" can be used to help guide the selection of high antioxidant foods for people also working to reduce caloric intake.

Table 2. Common Foods in the "Very High" and "High" Categories or Antioxidant Capacity Ranked by H-ORAC Units per Serving: Average Serving Size, Calories per Serving, and ORAC per Calorie (see Notes)

Antioxidant Category and Fresh Food	Serving Size (grams)	Typical Serving	H-ORAC Units per gram	H-ORAC Units per Serving	Calories per Serving	H-ORAC Units per Calorie	Ranking of Foods by H-ORAC Units per Calorie
Very High							
Blueberry, wild	145	1 cup	92.09	13,353	54	247	1
Artichoke, cooked	84	1 cup hearts	92.77	7,793	42	186	2
Black Plums	88	1 fruit	73.01	4,819	30	161	3
Broccoli Raab, raw	85	1/5 bunch	28.10	2,389	19	126	5
Blackberry	144	1 cup	52.45	7,553	62	122	6
Strawberry	166	1 cup	35.41	5,878	53	111	7
Blueberry, cultivated	145	1 cup	61.84	8,967	83	108	8
Cabbage, Red, cooked	75	1/2 cup	31.46	2,360	22	107	9
Raspberry	123	1 cup	47.65	5,861	64	92	13
Apple (Red Delicious)	138	1 med. fruit	42.34	5,843	72	81	14
Apple (Granny Smith)	138	1 med fruit	38.60	5,327	72	74	15
Sweet Cherry	145	1 cup	33.44	4,849	91	53	19
Bean, Red Kidney	92	1/2 cup	144.04	13,252	310	43	23
Navel Orange	140	1 fruit	17.85	2,499	69	36	24
Prune	85	1/2 cup	83.99	7,139	204	35	28
Bean, Pinto	96	1/2 cup	119.37	11,460	333	34	29
Pear, Red Anjou	166	1 med fruit	17.38	2,885	96	30	31
Grape, Red	160	1 cup	12.60	2,016	110	18	38
Potato, Russett, cooked	299	1 potato	15.27	4,566	290	16	45
Raisin	82	1/2 cup	30.02	2,462	243	10	48
Averages			***53.5***	***6,063***	***116***	***84.5***	
High							
Asparagus, raw	67	1/2 cup	29.15	1,953	13	150	4
Lettuce, Red Leaf	68	4 outer leaves	16.5	1,122	11	102	10
Asparagus, cooked	90	1/2 cup	16.44	1,480	20	74	16
Beet	68	1/2 cup	27.65	1,880	29	65	17
Grapefruit, Red	123	Half	15.13	1,861	37	50	20
Peach	98	1 med fruit	18.13	1,777	38	47	22
Pepper, Yellow	186	1 large pepper	9.56	1,778	50	36	25
Tangerine	84	1 med fruit	16.13	1,355	45	30	30
Onion, Yellow, cooked	105	1/2 cup	12.20	1,281	46	28	33
Apricot	105	3 fruits	13.09	1,374	50	27	34
Grape, Green	160	1 cup	11.18	1,789	110	16	41
Pineapple	155	1 cup diced	7.64	1,184	74	16	43
Potato, White, cooked	173	1 potato	10.41	1,801	114	16	44
Blackeyed Pea	52	1/2 cup	37.07	1,928	175	11	47
Almond	28	1 ounce	42.82	1,216	164	7	53
Low-fat Granola/raisins	60	2/3 cup	21.68	1,301	234	6	57
Toasted Oatmeal Cereal	49	1 cup	20.86	1,022	186	5	58
Averages			***19.2***	***1,535***	***82.1***	***40.4***	

Notes: Serving size and H-ORAC units per gram from (Wu et al., 2004). Calories per serving from the USDA database on the nutrient composition of foods.

Table 3. Common Foods in the "Moderate" and "Low" Categories or Antioxidant Capacity Ranked by H-ORAC Units per Serving: Average Serving Size, Calories per Serving, and ORAC per Calorie (see Notes)

Antioxidant Category and Fresh Food	Serving Size (grams)	Typical Serving	H-ORAC Units per gram	H-ORAC Units per Serving	Calories per Serving	H-ORAC Units per Calorie	Ranking of Foods by H-ORAC Units per Calorie
Moderate							
Spinach, raw	40	4 leaves	22.2	888	9	99	11
Lettuce, Green Leaf	40	4 leaves	14.1	564	6	94	12
Broccoli, ccooked	78	1/2 cup	12.3	956	27	35	27
Carrot, raw	61	1 medium	11.6	705	25	28	32
Pepper, Green, raw	119	1 med pepper	5.4	647	24	27	35
Tomato, cooked	120	1/2 cup	4.3	511	22	23	37
Nectarine	136	1 fruit	7.2	979	60	16	40
Banana	118	1 fruit	8.1	959	105	9	51
Corn Flakes	30	1.5 cups	23	691	101	7	54
Oats, Quick (oatmeal)	40	1/2 cup	17.6	705	156	5	60
Oatmeal Raisin Cookie	31	1 Cookie	17.2	532	130	4	61
All Grain Butternut Bread	28	1 slice	19.9	556	Unknown	Unknown	
Averages			***13.6***	***724.5***	***60.5***	***31.6***	
Low							
Cabbage, Common, raw	35	1/2 cup	13.4	469	8	59	18
Lettuce, Romaine	40	4 inner leaves	8.3	331	7	47	21
Celery	60	1/2 cup diced	5.3	320	9	36	26
Cauliflower	50	1/2 cup	6.1	305	12	25	36
Tomato, raw	123	1 med fruit	3.1	385	22	17	39
Pumpkin	116	1 cup cubed	4.1	480	30	16	42
Bean, Snap, canned	68	1/2 cup	2.1	140	10	14	46
Cucumber, no peel	60	1/2 cup	1.1	67	7	10	49
Peach, canned	98	1/2 can	4.2	411	43	10	50
Cantaloupe	160	1 cup cubed	3.0	475	54	9	52
Pea, Green, frozen	80	1/2 cup	5.1	404	62	7	55
Cucumber, with peel	52	1/2 cup sliced	0.9	45	8	6	56
Corn, canned	105	1/2 cup	3.6	379	83	5	59
Watermelon	152	1 cup cubed	1.2	187	46	4	62
Life Cereal	32	3/4 cup	14.2	455	120	4	63
Low-fat Chewy Granola Bar	28	1 bar	14.7	410	109	4	64
Bean, Lima, canned	124	1/2 cup	2.2	267	190	1	65
Averages			***5.4***	***325***	***48.2***	***16***	

Notes: Serving size and H-ORAC units per gram from (Wu et al., 2004). Calories per serving from the USDA database on the nutrient composition of foods.

Note the significant differences between the average H-ORAC units per serving and per calorie across the four food groups:

Food Group	Average H-ORAC Units per Serving	Average H-ORAC Units per Calorie
Very High	6,063	84.5
High	1,535	40.4
Moderate	724	31.6
Low	325	16

Clearly, increasing the average antioxidant capacity of foods in the "Very High" category of foods by 10 percent will make a much larger contribution to rising antioxidant intakes than a 10 percent increase in levels in foods in the "Moderate" or "Low" groups. A 10 percent increase in antioxidant capacity in a serving of food in the "Very High" category would, on average, add about 600 H-ORAC units to a person's daily diet. It would take almost two additional servings of food in the "Low" category to comparably increase daily antioxidant intake.

One additional serving of a food in the "Very High" category would deliver on average 18.6 times more antioxidant intake than an additional serving of food in the "Low" group of foods.

When foods are ranked by H-ORAC units per calorie, rather than in accord with the USDA's classification system used in Tables 2 and 3, the difference between the most antioxidant dense foods and foods low in antioxidant content is even more pronounced. Table 4 presents such a comparison of the top 10 foods ranked by H-ORAC units per calorie, along with the 10 foods at the bottom of this ranking. The antioxidant-dense foods, ranked per calorie, deliver on average 34.5 times more antioxidants per calorie than the average low-density antioxidant foods.

Table 4. Foods High in H-ORAC Units per Calorie Deliver Thirty-Five More ORAC Units per Calorie Than Foods Low in ORAC Units

Antioxidant Category and Fresh Food	H-ORAC Units per Calorie		Antiocidant Category and Fresh Food	H-ORAC Units per Calorie
VERY HIGH			*LOW*	
Blueberry, Wild	247		Cucumber, with peel	6
Artichoke, Cooked	186		Granola, Low-fat	6
Plums, Black	161		Toasted Oatmeal Cereal	5
Asparagus, Raw	150		Oats, Quick (Oatmeal)	5
Broccoli Raab, Raw	126		Corn, Canned	5
Blackberry	122		Oatmeal Raisin Cookie	4
Strawberry	111		Watermelon	4
Blueberry, Cultivated	108		Life Cereal	4
Cabbage, Red, Cooked	107		Low-fat Chewy Granola Bar	4
Lettuce, Red Leaf	102		Lima Bean, Canned	1
Average Top 10 Foods	142		Average Bottom 10 Foods	4

Dietary Intake of Polyphenols and Antioxidants

The average daily dietary intake of flavonoids in the United States has been estimated at about one gram per person (Formica et al., 1995; Kuhnau 1976), with almost half coming from cola, cocoa, beer, and wine. Fruits contributed a little less than one-third of daily consumption (Daniel et al., 1999).

Flavonol consumption has been studied more intensively than other types of flavonoids. A detailed review article published in 2000 in the *Journal of Nutrition* estimated daily flavonoid and phenolic intake at approximately one gram per day, with flavonols (catechins and proanthocyanidins) accounting for the largest share (Scalbert et al., 2000a).

The authors note that fruits and beverages, including beer, wine, fruit juices, tea, and coffee, are the major sources of antioxidants in the diet, with vegetables contributing important amounts of certain specific flavonoids. The plasma concentration of antioxidants rarely exceeds 1 μmols (μmols, one millionth of a mole) after consumption of 10 to100 milligrams (milligram, one thousandth of a gram) of a single antioxidant (Scalbert et al., 2000a). This reflects the relatively tight regulation of plasma antioxidant levels in the human body and helps explain why there is not a corresponding percentage increase in plasma antioxidant concentrations as a function of percentage changes in intake, regardless of the source (Manach et al., 2004).

Foods and beverages contribute roughly equally to total phenolic intake, with phenolic acids accounting for about one-third and flavonoids for two-thirds, although the degree of coffee and/or tea consumption by an individual significantly affects the relative contribution from foods and beverages (Scalbert and Williamson, 2000). Vegetables were estimated to provide 218 milligrams (mg) of total phenols per day in the U.S. diet (Vinson et al., 1998), an estimate regarded as probably high because of the analytical methods used (Scalbert and Williamson, 2000). Fruits are universally regarded as a richer source of phenolics in the diet than vegetables and other foods. Fruits often contain 1 to 2 grams of phenolic compounds per 100 grams of fresh weight (Macheix et al., 1990).

A team of USDA scientists measured concentrations of proanthocyanidins in a wide range of common foods, as part of a coordinated effort to establish a national baseline dataset on polyphenols in foods. They used data from the 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII) to estimate that average daily intake of proanthocyanidins is 57.7 mg per person (two years or older) (Gu et al., 2004). Major sources in the diet are apples (32 percent), chocolate (18 percent), and grapes (18 percent).

A team of Norwegian scientists carried out a detailed study of total antioxidants in foods of plant origin using the FRAP assay. Results are reported as μmols (one-millionth of a mole) per day. Total dietary antioxidant intake in Norway was estimated as about 0.2 μmol per day. The following food groups accounted for decreasing shares of this estimated total intake:

- Fruits, 43.6 percent
- Berries, 27.1 percent
- Cereals, 11.7 percent
- Vegetables, 8.9 percent
- Root crops, 7 percent
- Dried fruits, 1.5 percent
- Pulses (i.e., legumes), 0.2 percent.

Consumption can vary greatly across the population. A study of Dutch dietary intake of quercetin showed more than a 10-fold difference in average daily intake between the 10th and 90th percentile cohorts (Hertog et al., 1993b). Consumption in the United States, Denmark, and Holland has been estimated at 20 to 25 mg per day (Manach et al., 2004). Phenolic acid intake in Germany was found to vary from 6 to 987 mg/day (Radtke et al., 1998). For people eating several servings of fresh fruits and vegetables per day, Manach and co-authors (2004) project total polyphenol intake of at least 1 gram per day.

Accurate estimates are now available for many distinct polyphenols in certain foods and beverages in terms of concentrations per gram of food or liter of beverage, and overall dietary intake. Tea polyphenols have, for example, been studied extensively (Beecher 2003). But given the difficulty of quantifying polyphenol and antioxidant content in foods and the fact that many foods have not been thoroughly tested, it is impossible to project accurately the average total polyphenol and antioxidant intake in the U.S. diet. Bioavailability within the human digestive systems is even more complex to trace and quantify.

The methods used to process fresh produce can have a significant impact on antioxidant levels. By processing food at moderate temperatures and low pressure the loss of antioxidants can be minimized.

Direct Measures of Antioxidants in the Human Body

When animals consume plant foods containing antioxidants, a whole host of factors influence the bioavailability of these antioxidants, and another set determines the biological impact of that portion of antioxidants that is absorbed into the body. Many other factors then influence the degree to which antioxidants deliver health benefits. For example, antioxidants can interact with medicines; the health of a person's gut has an impact on uptake and biological response; and, metabolic or immune-system diseases can either increase or decrease the impact of antioxidants.

Several studies point out that the polyphenols and antioxidants appearing at the highest concentrations in foods are not necessarily those that are the most bioavailable (Manach et al., 2004). Three factors can work alone or together to undercut bioavailability:

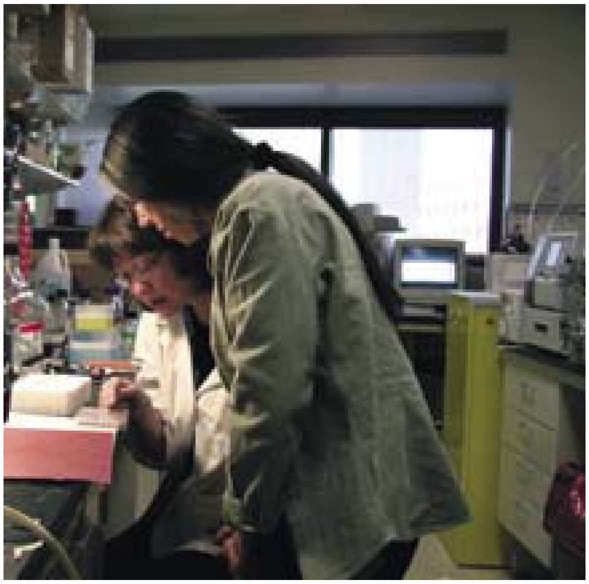
- poor absorption in the gut;
- a tendency to be rapidly metabolized to less available forms; and
- rapid elimination through the gastrointestinal tract.

The aglycone form of polyphenols (the form left after the sugar moiety has been enzymatically removed) can be absorbed in the small intestine, but most polyphenols are present in foods in the form of esters, glycosides, or polymers, which are not readily taken up in the gut. These forms of polyphenols must be hydrolyzed in the gut by intestinal enzymes or microflora before they can be absorbed (Manach et al., 2004). Polyphenols that are not metabolized in the body are eliminated mostly in urine and bile.

Research also suggests that certain polyphenols can increase the body's ability to metabolize and neutralize certain carcinogens, toxic chemicals, and therapeutic drugs (Jaga et al., 2001); (Galati et al., 2000). This can happen because certain polyphenols trigger metabolic processes that are similar to those relied on by the body to metabolize carcinogens, chemicals, and drugs.

Studies of changes in total antioxidant capacity in human blood and urine are two methods scientists use to gain at least a general sense of the portion of antioxidants consumed in a day that are taken up by the body, metabolized, or excreted. But timing matters greatly in measuring antioxidant levels, since the peak response in total antioxidant capacity in studies of human plasma occurs within one or a few hours after ingestion for some antioxidants, but after 10 or more hours in the case of polyphenols that require partial degradation by intestinal flora for absorption (Aziz et al., 1998).

The half-life of some dietary antioxidants is measured in hours, and ranges from one to as many as 24 hours in the case of quercetin, which has a strong tendency to bind to plasma albumin (Scalbert and Williamson, 2000). Another complexity arises from the fact that some portion of ingested and absorbed antioxidants are metabolized in humans to other chemical forms, which often have different antioxidant potential and may be more or less stable (Scalbert and Williamson, 2000). In addition, different methods to measure total antioxidant capacity, whether in foods, blood, or urine, produce sometimes widely divergent results. This is because existing methods employ different free radical generators and target different stages in the normal metabolic reactions that antioxidants go through. Some methods are highly sensitive and accurate when measuring lipid-bound antioxidants, while others are better suited to the measurement of water-soluble compounds. In a recent study by USDA scientists, both lipid and water-soluble antioxidants were measured, and then added together in reporting total antioxidant content (Wu et al., 2004).



Dr. Brigitte Graf, a scientist at the Human Nutrition Resource Center at Tufts University, demonstrates the preparation of a sample for antioxidant testing to Organic Center Board Chair, Theresa Marquez.

Scientists at Tufts University have carried out extensive analyses of flavonoids and total antioxidants both in foods and human blood, urine, and organs (Cao et al., 1998). While multiple assays have been used, Tufts scientists have pioneered applications of the ORAC assay, or Oxygen-Radical Absorbance Capacity. (For further information, use the Tufts University "Nutrition Navigator" at http://navigator.tufts.edu).

Tufts scientists estimate that on an average day most Americans consume less than a third of the dietary antioxidants needed to take full advantage of the health-promoting benefits of antioxidants.

These Tufts scientists have calculated the ORAC units, also called "Anti-Aging Points," for a wide array of foods based on typical serving sizes. They estimate that most Americans consume between 1,200 and 1,600 ORAC units per day, less than a third of the preliminary goal for optimal antioxidant activity of 3,000 to 5,000 ORAC units.

The researchers classified foods into three groups based on antioxidant capacity. High antioxidant foods contain more than 800 ORAC units per typical serving. A 6-ounce glass of Concord grape juice delivers 2,608 ORAC units, while blueberries, blackberries and strawberries all deliver more than 1,000 units. Spinach is the most antioxidant-rich vegetable, containing 1,021 ORAC units per serving.

Tufts scientists have combined assays of antioxidant potential with dietary intervention trials and direct measures of antioxidant activity in human blood and cells. Some exciting results are emerging. One study assessed the ORAC units of the daily diet consumed by 36 adults. Prior to the study, participants consumed 1,670 ORAC units per day. But after consuming 10 servings of fruits and vegetables daily, they increased their average daily ORAC units to 3,300 to 3,500 — much closer to the 5,000-unit upper-end target (Cao and Prior, 1998). In ongoing work, they are assessing the increases in ORAC blood levels following consumption of foods that score high on the ORAC assay.

International Studies on Antioxidant Intake

Through the use of a unique experimental design, a team of Danish scientists attempted to quantify changes in markers of oxidative defense as a result of consumption of realistic quantities of either organic or conventionally grown foods (Grinder-Pedersen et al., 2003). The intervention diets were similar to the diets typically consumed by Danes. The intake and excretion of five flavonoids were measured in this human crossover intervention trial involving 16 subjects. A number of measures of oxidative damage are reported, including the TEAC (Trolox Equivalent Antioxidant Capacity) and FRAP (ferric reducing ability of plasma) assays for total antioxidant capacity.

The organic diet provided substantially more quercetin and kaempferol than the diet consisting of conventional foods. In the case of quercetin, the level in the organic diet was 60 percent higher (4,198 versus 2,632 micrograms per 10 MJ of food). (MJ stands for a million joules and is a measure of food energy. One calorie equals 4.168 joules and hence, a diet delivering 10 MJ per day would contain about 2,400 calories). The kaempferol level in the organic diet was almost double the conventional diet. Excretion of quercetin and kaempferol in urine were significantly higher in the group consuming the organic diet, although there was marked variability across individuals in this trial. The authors suggest that differences in the intestinal flora may account for the high degree of variation in bioavailability observed in this study. Most markers of antioxidant defense did not differ across the two diets, although the organic diet resulted in an increase in protein oxidation and a decrease in total plasma antioxidant capacity compared to baseline.

A dietary intervention trial in France involving healthy adults was designed to measure changes in blood antioxidant levels following consumption of tomato-based products. The typical diet of the study participants was supplemented for three weeks with 96 grams per day of tomato puree, followed by a three-week period during which tomato products were avoided. The tomato-supplemented diet increased plasma lycopene, beta-carotene, and lutein, but did not significantly change total antioxidant capacity, vitamins C and E, and plasma antioxidant trace metals (Tyssandier et al., 2004). But avoidance of tomato products did significantly lower plasma lycopene, vitamin C, beta-carotene, lutein, and total antioxidant capacity.

A novel study focused on the role of sweeteners in the diet in modulating antioxidant capacity. One group of study participants consumed 1.5 grams per day of buckwheat honey, and another group consumed an equal amount of corn syrup (Schramm et al., 2003). The honey delivered a total of 0.79 milligrams of phenolic antioxidants and the corn syrup contained 0.21. The plasma antioxidant status of the group consuming honey increased significantly compared to the group consuming corn syrup. The authors note that the substitution of honey for some of the average 70 kilograms per year of sweeteners in the U.S. diet could appreciably enhance antioxidant status.

Overview of Science on the Human Health Benefits of Antioxidants

Thousands of papers are published each year on the role of antioxidants in promoting health, preventing disease, and meeting nutritional needs. There are several specialized journals focusing largely or solely on the biology of free radicals and antioxidants. These fields of science are central to the growing interest in nutraceuticals, dietary supplements, and use of genomics and biotechnology to enhance plant antioxidant content.

Six criteria have been suggested by Brandt et al. (2004) for identifying health-promoting compounds in fruits and vegetables. These criteria encompass evidence of antioxidant capacity, bioavailability and uptake in humans, and physiological activity and impacts on relevant human tissues. The authors characterize the six criteria as strict and hard to meet, given the sorts of studies that have been carried out to date. The most difficult criterion to meet is the one calling for evidence of concentrations in human tissues from routine dietary consumption that are sufficient to elicit a biological impact, based on animal studies. Only one antioxidant present in carrots — falcarinol — meets all six criteria and appears to play a role in reducing the incidence of certain cancers (Brandt et al., 2004).

While there is strong evidence establishing a link between intake of fruits and vegetables and disease risk, there are no studies that have evaluated consumption of organic fruits and vegetables and disease risk. In addition, there is little evidence for a direct relationship between antioxidant consumption and any particular human health benefit. Several studies designed to search for such a connection have failed to find one. The connections between fruit and vegetable consumption and good health arise from a complex mix of mechanisms and circumstance. In some cases, increased antioxidant intake may simply be a marker for increased fruit and vegetable consumption. In other cases, antioxidants may be affecting health status in ways that are not yet understood. A simple, direct connection between antioxidant intake and health promotion is, in fact, likely to be the exception rather than the rule.

Still, antioxidant intake almost certainly plays an important role in shaping the relationship between diet and health across a population. Many, if not most, of the health benefits of antioxidants arise from their anti-inflammatory properties within the body (Middleton E Jr 1998; Middleton 1998). Free radical damage is both triggered by inflammation and is itself inflammatory. By lessening free radical damage, antioxidants reduce inflammation. This essential role of antioxidants promotes cardiovascular health (Arai et al., 2000; Hertog et al., 1993a; Huxley et al., 2003; Huxley et al., 2004; Knekt et al., 1996; Knekt et al., 2002; Mennen et al., 2004; Rissanen et al., 2003), inhibits the growth of cancerous tumors and cell masses (Ferguson et al., 2002; Galati et al., 2004; Galati et al., 2000; Knekt et al., 1997; Le Marchand et al., 2000; Morton et al., 1999), slows the aging process in the brain and nervous systems (Bastianetto et al., 2000; Bickford et al., 2000; Joseph et al., 1998a; Joseph et al., 1998b; Martin et al., 2002; Sun et al., 2002), and lessens the risk and severity of neurodegenerative diseases (Mandel et al., 2004), including Alzheimer's (Barkats et al., 2000; Butterfield et al., 2002; Conte et al., 2003; Fontaine et al., 2000; Ono et al., 2003; Yatin et al., 1999), Parkinson's (Grunblatt et al., 2000; Youdim et al., 2001; Youdim et al., 2002), and Huntington's disease. A possible role in lessening the severity of asthma has been observed in a population-based case control study (Shaheen et al., 2001). The flavonoid quercetin has been shown to help relieve chronic pelvic pain syndrome, a common yet hard-to-treat condition (Shoskes et al., 1999).

Complexities in Studying the Human Health Benefits of Antioxdiants

The link between polyphenol intake via diet and health promotion is complicated by many variables (Brandt et al., 2004). The impact of consuming a given level of a particular antioxidant varies widely as a function of variations in overall health status, exposures to chemicals and drugs, abnormal cell growths, wounds, infections and physiological disorders such as diabetes (Beecher 2003). Age, exercise and genetics also play important roles.

In addition, some antioxidants become "pro-oxidants" at higher doses and others can be directly toxic. The emergence of dietary supplements containing antioxidants of plant origin in highly concentrated forms has raised concerns, especially when taken by pregnant women, since flavonoids have been shown to cross the placental barrier and may act as hormone-mimics, or they can block hormone receptors (Galati et al., 2004; Skibola et al., 2000). In the human gut, some well-known antioxidants with potent anti-inflammatory activity have been shown to become pro-carcinogenic in the presence of certain mixes of intestinal microflora and as a function of the degree of progression of cells toward a cancerous state (Chu et al., 2004).

For these reasons it is risky to generalize too broadly about the importance of a given polyphenol or total antioxidant intake. This reality points to the enormous testing, research, and public education challenges that lie ahead.

It is risky to generalize too broadly about the health benefits of a given polyphenol or total antioxidant intake because so many factors influence a person's need for antioxidants and ability to utilize them.

Clearly, one diet will not optimally meet the needs of all Americans. Another key point, well supported by scientific literature, is that the health-promoting benefits of the polyphenols in many foods, if not most, arise from the mixtures of antioxidants in each food, some of which have not been characterized. Dozens of human nutrition intervention trials have tried to isolate the unique effects of individual polyphenols or antioxidants, especially vitamin C and carotenoids/vitamin E. In these trials, people or animals are fed a diet containing high levels of an isolated antioxidant, and the results are compared to a diet delivering the same amount of the isolated antioxidant as part of the mix of antioxidants and polyphenols in whole or largely unprocessed foods.

Repeatedly, whole foods deliver the greater benefit, as confirmed in recent human studies involving free radical DNA damage assays in human urine or lymphocytes and the impact of dietary flavonoids on neurodegeneration (Youdim et al., 2002). The single, dominant antioxidant in Brussels sprouts, onion, and tomatoes had no significant impact on free radical DNA damage, whereas the whole foods did (Halvorsen et al., 2002).

A French research team carried out an ambitious study to assess the impact of lifestyle factors, including diet and antioxidant intake, on human health. They measured oxidative damage in human blood across 88 men and 96 women (Lesgards et al., 2002). The study population was relatively young and healthy. Factors strongly associated with enhanced antioxidant capacity included non-smoking, exercise, and taking vitamin and/or mineral supplements. Conversely, several factors depressed antioxidant capacity — smoking, stress, alcohol, moderate fruit and vegetable intake, and low fish consumption. Several other research teams are exploring the role of polyphenols and antioxidants in explaining the “French Paradox” — the relatively low rate of cardiovascular disease in the French population, despite consumption of a very high-fat diet. Many researchers have concluded that grape polyphenols in wine are at least part of the explanation (Barbaste et al., 2002; Folts 2002; Sun et al., 2002).

Cardiovascular Benefits

Heart disease is the leading cause of death in the United States, accounting for more than 710,000 deaths annually, or about 30 percent of all deaths (Mokdad et al., 2004). A major study of the impacts of diets high in fruits and vegetables on cardiovascular disease and mortality was carried out in Finland. Dietary intakes of fruits and vegetables were assessed and all subjects in the study were divided into five groups as a function of fruit and vegetable intake. Individuals in the highest consumption group had substantially lessened risk of mortality from all causes and cardiovascular disease, compared to the low-consumption group (Knekt et al., 2002). The authors attributed the health-protective impact of the diets high in fruits and vegetables to the antioxidative benefits of the flavonoids.

A similar study involving 1,286 French women showed that diets rich in fruits and vegetables lowered blood pressure (Mennen et al., 2004). In this study, individuals were divided into three groups as a function of fruit and vegetable intake. Women in the high consumption group faced markedly lower cardiovascular risk compared to the low consumption group. Men in this study did not benefit from diets high in fruits and vegetables from the perspective of cardiovascular health, nor did men in a study of U.S. health professionals (Rimm et al., 1996). A Japanese study attributed significant protection from coronary heart disease in women to the relatively high dietary intake of quercetin and isoflavones (Arai et al., 2000).

The oxidation of low-density lipoprotein (LDL) and aggregation of platelets play direct roles in the progression of atherosclerosis (Vivekananthan et al., 2003). A range of observational studies suggests that antioxidant vitamins, especially vitamin E and beta-carotene (precursor of vitamin A) may prevent the initiation and progression of cardiovascular disease. These observations have led to a series of large prospective cohort studies in humans, in which antioxidant vitamin supplements did not consistently result in reductions in the risk and severity of cardiovascular symptoms. Some studies have shown decreases in cardiovascular disease from antioxidant supplementation, while others have not (Cooper et al., 1999a; Cooper et al., 1999b).

Scientists at the Cleveland Clinic Foundation in Ohio published a meta-analysis of randomized trials on antioxidant vitamins and cardiovascular disease and mortality in *The Lancet* (Vivekananthan et al., 2003). Seven trials involving vitamin E and eight on beta-carotene were reviewed. Each trial encompassed more than 1,000 patients. A small but significant benefit was found for supplementation with beta-carotene, but not with vitamin E. Several lines of evidence suggest that the benefits of antioxidant supplementation in preventing heart disease are greatest if the supplementation begins at the early stages of pathogenesis, or even before any symptoms are evident.

The authors note that a number of potentially confounding factors may explain the discrepancy across studies assessing the benefits of antioxidant vitamins and heart disease. In particular, additional antioxidants in foods may play a more important role than vitamin E or beta-carotene. The authors note the possible importance of lycopene and other flavonoids. In addition, Vivekananthan et al. state that:

> *"The natural form of vitamins in food may have biological activity or potency different to those for synthetic vitamin compounds used in supplements. For instance, the natural form of vitamin E, or d-alpha tocopherol (1.5 IU/mg) is more bioavailable than the synthetic form."*

Two scientists in the United Kingdom carried out a comprehensive review of studies on fruit and vegetable intake and cardiovascular disease (Ness et al., 1997). For coronary heart disease, nine of 10 ecological studies, two of three case-control studies, and six of 16 cohort studies "...found a significant protective association with consumption of fruits and vegetables or surrogate nutrient." Three of the five ecological studies reported a protective role for fruits and vegetables in preventing strokes, as did six of eight cohort studies. The authors conclude that the literature supports a strong protective role for fruits and vegetables in the prevention of stroke and a weaker protective effect on coronary heart disease. Again, the association was seen for fruit and vegetable consumption, not estimates of specific antioxidant intake.

Cancer

Cancer is the second leading cause of death in the U.S. and accounts for 553,000 deaths annually (Mokdad et al., 2004). Available treatments include radiation, chemotherapy, and surgical procedures, which are costly, can cause other health problems, and are of varying effectiveness. A wide range of studies has shown that antioxidant plant phenolic compounds are anti-proliferative and can prevent or slow tumor progression (Galati et al., 2004; Galati et al., 2000). Studies of flavonoids extracted from cranberries have demonstrated significant impacts on a number of human cancer cell lines (Ferguson et al., 2002), leading the team to suggest that flavonoid extracts from cranberries might some day find application as a novel anticancer drug. A European team found that the carrot antioxidant falcarinol satisfied six criteria suggested to link consumption of a food antioxidant to a specific health outcome — in this case, a reduction in the risk of cancer (Brandt et al., 2004).

The impact of the flavonoid genistein has been studied in an assay using human breast cancer cells. The dose-response to genistein was similar to estradiol and tamoxifen. The authors concluded that, "...genistein has potent estrogen agonist and cell growth-inhibitory actions over a physiologically achievable concentration" (Zava et al., 1997a). They note that other flavonoids were equally potent as an estrogen agonist or in inhibiting growth, but none were as potent as genistein through both mechanisms.

Recent research has established a specific mechanism leading to the anti-cancer activity of the flavonoid resveratrol. A team of scientists at the University of Virginia has shown that resveratrol can starve cancer by inhibiting the actions of a key protein that helps feed cancer cells (Yeung et al., 2004). Moreover, the chemotherapeutic impact of resveratrol occurred at doses within the range that a person consuming a moderate amount of red wine would ingest.

Galati and O'Brien (2004) point out that flavonoids can interfere with several steps in the development of cancer. They can protect DNA from oxidative damage that leads to abnormal cell proliferation. They can inhibit cancer promoters and activate carcinogen-detoxification systems (Galati et al., 2004). In addition, the pro-oxidant activity of some flavonoids can lead to toxic impacts and/or serve as another anticancer mechanism. A potent antioxidant in canola oil has recently been discovered and tested for antimutagenic, antibacterial, and anti-proliferative impacts (Kuwahara et al., 2004). The discovery of this antioxidant in canola oil may have implications for the oil-refining process, since current technology removes a high portion of many polyphenolic compounds in oils.

Diabetes

Diabetes is one of the most costly diseases in the United States. Across the population, but particularly among teenagers, the incidence of diabetes is rising, earning the label "epidemic" in some quarters. Diabetes and its complications is the sixth leading cause of death in the U.S., accounting for about 70,000 deaths annually (Mokdad et al., 2004). Recent research suggests that some polyphenols in plants can increase the sensitivity of the body to insulin, at least in some patients, thereby delaying the onset of type II diabetes or slowing the progression of the disease.

A cross-sectional study in the United Kingdom showed that frequent consumption of fruits and salads reduced the risk of type II diabetes (Williams et al., 1999). Substantial evidence exists linking consumption of whole grains to reduced risk for development of type II diabetes.

FACTORS AFFECTING SECONDARY PLANT METABOLITE LEVELS IN FOODS

Many factors influence the levels of secondary plant metabolites, polyphenols, and antioxidants in food. One set of factors arises on the farm and includes plant genetics, farming practices, soil fertility, the weather, pest pressure and pest management systems, and harvest time and ripeness. Another set of factors come into play as food leaves the farm and make its way to consumers, and how food is stored in the home and cooked can alter levels, sometimes dramatically. Determining how to cost-effectively preserve the polyphenol and antioxidant content of fruits, vegetables, and grains at the time of harvest, as it moves toward consumption, is a major challenge and ongoing research need.

Vitamin, mineral, and antioxidant levels in food often decline as crop yields increase, a phenomenon called the "dilution effect".

Reviews Addressing the Impacts of Organic Farming Systems

Two food scientists working at the University of Otago in New Zealand published a comprehensive review in 2002 entitled "A Comparison of the Nutritional Value, Sensory Qualities, and Food Safety of Organically and Conventionally Produced Food" in *Critical Reviews in Food Science and Nutrition* (Bourn and Prescott, 2002). The review covers the impact of farming methods and fertilization practices on the nutritional content of foods, especially vitamin, nitrate, mineral and protein contents. Impacts on taste and sensory parameters are also assessed. While dozens of studies are covered in this review, only a few assessed changes in antioxidant or polyphenol levels — beyond impacts on vitamins C and E.

On the topic of vitamin C levels, the review by Bourne and Prescott (2002) notes several studies of matched pairs of crops grown under conventional and organic systems. While several studies have shown higher levels in organic foods, others have shown the opposite. The authors note that::

> *"Because vitamin C content is readily affected by maturity at harvest, storage conditions (e.g. temperature), surface bruises, and the presence of oxygen, irrespective of farming system, it is not surprising that there is considerable variation in results both within and among studies."*

The authors note that research focusing on polyphenols in organic foods has just begun, but that a substantial literature exists on polyphenols in conventional foods. In addition, they highlight the fact that many studies have shown an inverse relationship between nitrogen applications and phenolic compound concentrations. In fields where high levels of nitrogen are readily available to plants, they can and typically do grow rapidly and attain a relatively large size, but concentrations of polyphenols and some vitamins are typically lower. This phenomenon has been referred to in the past as the "dilution effect" (Davis et al., 2004; Farrell 2003). This finding has been replicated and explained genetically in an elegant study carried out by USDA scientists involving tomatoes grown under conventional high-nitrogen or sustainable, lower-nitrogen systems (Kumar et al., 2004)

A paper presented at the 1998 International Federation of Organic Agriculture Movements (IFOAM) scientific conference compared phenolic compound levels in conventional and organic apple orchards in which the same cultivar was grown and found higher levels in the organic blocks

(Weibel et al., 2000). Scientists working at the Danish Institute of Agricultural Sciences published a comprehensive review of the impact of organic farming methods on the nutritional value of plant foods in the *Journal of the Science of Food and Agriculture* (Brandt et al., 2001). The authors conclude that the only consistent advantages of organic plant-derived foods are higher levels of vitamin C and lower levels of nitrate.

The Weibel et al. review explains that there are five major classes of chemical constituents in plants that affect health: vitamins, minerals, proteins, carbohydrates and secondary plant metabolites. In reference to the first four classes of chemical constituents, the team states that:

> *"...no human or animal studies have shown effects on health of using plant foods with relatively high or low contents of any of the four types of nutrients, unless the other parts of the diet were distinctly deficient in one of these nutrients."*

The authors argue that, in evaluating the "nutritional value" of foods in developed countries, vitamins, minerals, protein and carbohydrates are not important targets since any deficiencies can be so easily addressed. They go on to say:

> *"For secondary plant metabolites the situation is very different. None of them are known to be absolutely necessary for a long and healthy life... This is true by definition, since once a specific biological function for a secondary plant metabolite is discovered, it is typically redefined as a vitamin. (Brandt et al., 2001)."*

Some secondary plant metabolites are known as "anti-nutrients" because they block the uptake nutrients in the human digestive system. Consumption of food high in anti-nutrients can help counteract the adverse effects of overeating.

While none are essential to good health, the authors note that many studies have shown that consumption of one or a combination of secondary plant metabolites can prevent or lessen the severity of disease or slow the aging process. They state that:

> *"In fact, it is well established that the greater the daily intake of vegetables and fruit, the smaller the risk of the major deadly diseases in Western society, including cancer, cardiovascular disease and diabetes."*

Because of the vast number of secondary plant metabolites, and the almost limitless array of mixtures present in foods, the authors recommend that future research focus on groups or mixtures of compounds. The authors also make a fascinating point regarding the impact of secondary plant metabolites on food intake in populations blessed with ample access to food. Some secondary plant metabolites are anti-nutrients that block the uptake of proteins and/or other essential nutrients. Most populations that have access to essentially unlimited supplies of food tend to overeat, increasing the risks of a variety of diseases. Consumption of fruits and vegetables high in anti-nutrient secondary plant metabolites will tend to counteract the adverse effects of overeating by reducing the bioavailability of excess nutrients.

The Brandt et al. (2001) review also surveys literature on the impacts of farming methods on secondary plant metabolites, noting that soil fertility levels tend to consistently have an impact on secondary plant metabolite levels. The authors note that organic crops are typically grown in fields with lower levels of readily available nitrogen, and as a result, most organic foods tend to have lower nitrate and protein levels (grains are a likely exception). They also explain that plants grown in organic systems "have more intrinsic resistance [to pests] than conventional ones, since they can cope so relatively well without the protection of pesticides" (Brandt et al., 2001). On the key question of the levels of secondary plant metabolites in organic versus conventional foods grown under similar conditions, they highlight the lack of well-designed studies and the often-conflicting results:

> *"...we will dare to estimate levels of plant defense-related secondary metabolites in organic vegetables to be 10-50% higher than in conventional ones. The differences are probably smaller in fruits."*

They also point out that as conventional producers move toward farming systems that include many of the core practices on organic farms, a trend that is underway in the U.S. on a portion of the land base, the differences in secondary plant metabolite levels between organic and conventional foods will likely narrow.

Interest in and research on secondary plant metabolites and antioxidants in organic versus conventional foods has only been underway a few years. There is no mention of secondary plant metabolites in a major review article published by German scientists in 1997 entitled "A Comparison of Organically and Conventionally Grown Foods – Results of a Review of the Relevant Literature" (Woese et al., 1997).

Impacts of Organic Farming Methods on Antioxidant Secondary Plant Metabolites

In a well-designed study published in the *Journal of Agricultural and Food Chemistry*, a team of Italian scientists analyzed the impact of organic and conventional production practices on polyphenol levels in plums, a fruit widely grown in the Mediterranean region that is known to be high in antioxidant content. Concentrations of three vitamins were measured — ascorbic acid, vitamin E, and beta-carotene (Lombardi-Boccia et al., 2004). The organic and conventional blocks were the same variety and were grown on the same experimental farm, eliminating many possible sources of variation. Organic blocks were at least 600 meters from conventional blocks and were surrounded by a thick hedge.

The organic blocks were managed in three ways:

- tilled soil, just like the production practice used on the conventional blocks;
- use of a trifolium cover crop sowed the previous fall and mowed throughout the subsequent production season; and
- soil covered with natural, mixed meadow, also mowed during the season.

The plum study covered three growing seasons. The organic plums contained higher concentrations of several minerals including zinc and magnesium. The total sugar content did not vary significantly. The organic plums grown with the meadow cover crop had the highest concentrations of tocopherols. Organic plums under tilled soil contained higher levels of beta-carotene than the conventional plums, although the conventional plums contained more total polyphenols. The levels of polyphenols in the organic plums managed with a trifolium cover crop were higher in six of nine phenolics measured, the same in one case, and were lower in two cases.

Scientists working at the University of California-Davis carried out a study of total polyphenol and secondary plant metabolite levels in two berry crops and field corn under conventional, "sustainable, low input," and certified organic farming methods (Asami et al., 2003). The team assayed the total phenolic content of marionberries and corn under all three production systems, and the total phenolic content in strawberries under conventional and sustainable systems. They also analyzed the impact of three common post harvest processing treatments — freezing, freeze-drying, and air-drying.

The Folin-Ciocaltheu method was used to measure total phenolic levels. Ascorbic acid levels were also quantified, using HPLC (High Performance Liquid Chromatography). The conventional and sustainable corn and strawberries were treated with standard pre- and post-emergent herbicides, but no insecticides or fungicides were reported as applied. The apparent lack of insect and plant pathogen pressure in all plots might tend to minimize any differences in total phenolic levels. The experimental design also failed to account for differences in soil type on polyphenol levels.

Asami et al. (2003) found consistently higher levels of ascorbic acid (AA) in the organic and sustainable foods. A statistically significant decrease in AA levels was also observed in the freeze-dried and air-dried samples as compared to the frozen samples. The AA concentrations in organic and sustainably grown and frozen corn were 52.4 percent and 66.7 percent higher than in conventionally grown and frozen corn, respectively.

Total phenolic (TP) levels were also markedly higher in the organic samples compared to the conventionally grown crops. In the case of corn, TP levels ranged from 41 percent to 58 percent higher than conventional samples, depending on postharvest treatment. TP levels in frozen organic marionberries were 50 percent higher than in frozen conventional marionberries. Sustainably grown and frozen strawberries contained 19 percent higher TP levels than conventional, frozen berries.

The highest concentrations of TPs were consistently found in the frozen samples, followed by freeze-dried and then air-dried. The basic findings in frozen produce from the U.C.-Davis study are presented in Table 5. (There were no organic strawberries in the study; hence, only two of the study's three crops appear in Table 5).

Table 5. Ascorbic Acid and Total Phenolic (mg/100 g fresh weight) in Frozen Conventional and Organic Marionberries and Corn

	Marionberries			Corn		
	Conventional	Organic	Organic as a Percent of Conventional	Conventional	Organic	Organic as a Percent of Conventional
Ascorbic Acid	ND	ND	ND	2.1	3	152%
Total Phenolics	412	620	150%	24.7	39	158%

Note: Total pheolics is corrected for ascorbic acid content. Similar differences were observed for freeze-dried and air dried marionberries and corn.

Source: Table 3 and Firgure 1 in Asami et al., 2003

In a response to the U.C.-Davis study, Felsot and Rosen raised a number of issues with the way the authors interpreted and used published literature in forming their hypothesis and in drawing conclusions (Felsot et al., 2003). They note that published literature, including the above-mentioned studies, do show that polyphenol levels are sometimes higher in organic foods, but they take issue with the claim in the Asami et al. paper that consuming organic foods will improve health status.

Furthermore, they criticize the experimental design of the study and point out what would be required for a rigorous statistical comparison of polyphenol levels in organic versus conventionally grown foods. They note that differences in pesticide use between the organic, sustainable low-input, and conventional crops were modest to non-existent, and hence were unlikely to explain observed differences in total phenolic and vitamin C levels. While it is impossible to determine the causes of the differences in antioxidant levels in the organic and conventional samples tested by Asami et al. (2003), the analytical methods used support the conclusions that differences did exist. In this and many other studies, much more work will be needed to determine why antioxidants levels sometimes are elevated in organic plots, but in other circumstances, remain unchanged.

A team of Italian scientists studied differences in antioxidant levels in conventional versus organic peaches and pears. One of their goals was to identify biochemical markers that might one day be used to verify claims that fruit was grown according to the organic farming practices set forth in European Community regulations (i.e., EWG 24/6/91 no. 2092). They reasoned that levels of antioxidants might differ in organic versus conventional crops grown in the same area under similar production practices. They developed an experimental design to test this hypothesis. Their focus was on indicators of oxidative damage in the fruit. They also measured total polyphenols and polyphenoloxidase (PPO) levels, which serve as general indicators of total antioxidant capacity. Ascorbic acid, citric acid, and α- and λ-tocopherol levels were also assayed.

The pear and peach trees in the study were five years old and were growing on a government research station in Ciampino, Rome. The organic fruit was grown in compliance with EC regulations and the conventional fruit was treated with standard fertilizer and pesticide inputs; the same varieties were used in the organic and conventional plots. Organic and conventional fruit was picked at the same stage of maturity. Accordingly, this is one of very few studies where the experimental design eliminated most factors leading to variation in antioxidant and polyphenol levels, other than choice of organic versus conventional methods, and where there were major differences in pesticide use between organic and conventional blocks.

The authors found "a parallel increase in polyphenol content and PPO activity of organic peach and pear as compared with the corresponding conventional samples" (Carbonaro et al., 2002). Their basic findings are summarized in Table 6.

Table 6. Polyphenoloxidase (PPO) Activity and Total Polyphenol Content of Conventional and Organic Peaches and Pears (see notes)

	Peaches			Pears		
	Conventional	Organic	Organic as a Percent of Conventional	Conventional	Organic	Organic as a Percent of Conventional
PPO Activity (UE/100 grams)	2,053	2,655	129%	959	3,021	315%
Total Polyphenols	21.3	29	136%	58.4	64.5	110%

Note: PPO activity measured as chlorogenic acid as enymatic activity (UE). One unit of activity is the amount of enzyme that caused an absorbance increase of 0.001 unit per minute in the conditions of the assay. Total polyphenol concentrations measured as mg tannic acid per 100 grams fresh weight.

Source: Table 1 (Carbonaro et al., 2002)

Organic peaches contained about one-third higher concentrations of polyphenolic compounds than conventional peaches, while PPO activity in organic pears was more than three times higher than in conventional pears. The differences in polyphenol and antioxidant levels were all statistically significant and were the average of at least six matched-pair measurements. In addition, organic peaches had higher levels of ascorbic and citric acids than conventional peaches, and organic pears contained more alpha-tocopherol. The team concluded that:

> *"These data provide evidence that an improvement in the antioxidant defense system of the plant occurred as a consequence of the organic cultivation practice. This is likely to exert protection against damage of fruit when grown in the absence of pesticides."*

Another team of European scientists assessed the differences in the concentration of resveratrol, a key polyphenol thought to account in part for the so-called French Paradox, in organic and conventional wine grapes. A report on their findings appears in the proceedings of the Sixth Organic Viticulture Conference (Levite et al., 2000). The team selected farms growing organic and conventional grapes essentially side-by-side, minimizing variation linked to differences in weather, genetics, and soil type. Six sites were studied across western Switzerland.

Resveratrol levels were consistently higher in red grapes compared to white grapes. Organic wines contained higher concentrations of resveratrol in seven cases, and lower levels in two cases. Table 7 presents a summary of their findings. The organic grapes contained an average 32 percent higher concentration of resveratrol than the conventional samples.

Table 7. Resveratrol Conent of Conventional and Organic Wine Grape Varieties Grown in Western Switzerland (Vintage 1997)

Variety - Location	Resveratrol Content (ppm)		
	Conventional	Organic	Organic as a Percent of Conventional
Pinot Noir - Neuchatel	13.9	12.7	91%
Pinot Noir - Morges	13.5	17.6	130%
Pinot Noir - Peissy	8	11.0	138%
Pinot Noir - Ligerz	8	14.9	186%
Gamay - Morges	23.6	32.8	139%
Chardonnay - Neuchatel	0.2	0.3	150%
Chasselas - Aubonne	0.1	0.1	130%
Pinot Gris - Neuchatel	0.9	0.8	89%
Eight Comparisons			132%

Note: Data on Chasselas grown in Siere not include (0.0 ppm in conventional; 5.3 ppm in organic).

Source: Table 1 (Levite et al., 2002)

Organic vegetables had 30 percent to 10-times higher levels of flavonoids compared to conventionally grown produce in a study carried out in Japan. The team also demonstrated that the organic vegetables had heightened antimutagenic activity – an important step toward proving that consumption of organic food can lead to health benefits.

Five green vegetables were grown in organic and conventional production systems and tested for polyphenol content and antimutagenic potential in research carried out in Japan (Ren H. et al., 2001). The vegetables included qing-gen-cai, Chinese cabbage, Welsh onions, spinach and green pepper.

Organic vegetables were grown using water-soluble chitosan as a soil modifier and foliar spray. The conventional vegetables were produced on an adjacent farm and were treated with standard, intensive fertilizer and pesticide treatments. The antioxidant capacity of the organic spinach was 2.2-fold the level in the conventional spinach; 20 percent to 50 percent higher in the case of Welsh onions, Chinese cabbage and qing-gen-cai (Ren H. et al., 2001). The organic vegetables displayed heightened antimutagenic activity compared to the conventional vegetables in a number of cell assays. Juices extracted from the organic vegetables contained 1.3 to 10.4 times the flavonoid concentrations of conventional vegetables, suggesting a significant impact of production system on polyphenol content and antioxidant activity.

A second paper by the same team reported on three forward mutation and antimutagenicity assays, each carried out on 11 vegetables for a total of 33 assays comparing the antimutagenic potential of a matched pair of organically and conventionally grown vegetables. Eight out of 33 assays showed the organic produce had significantly greater antimutagenic activity than the conventionally grown vegetables; there were no significant differences in the other 25 cases (Ren et al., 2001a). The difference in antimutagenic activity was greatest in the case of spinach. The study also reported that the organic vegetables had much longer shelf life and tasted better than conventionally produced vegetables.

Flavonols and selected phenolic compounds in strawberries and blueberries were studied in Finland (Hakkinen et al., 2000). Significant differences were measured across varieties. In one location (eastern Finland), three direct comparisons across three cultivars were made of phenolic content in organic and conventional strawberries. No details are provided of the practices used in the organic systems, soil type differences, pesticide use in the conventional systems, nor differences in pest pressure.

Organic farming has elevated antioxidant levels in about 85 percent of the cases studied to date and on average, levels are about 30 percent higher compared to food grown conventionally.

Four phenolics were assayed: kaempferol, quercetin, ellagic acid, and ρ-coumaric acid. In two of the cultivars, the organic berries contained higher levels of two of the four phenolic compounds tested and there were no differences in any of the four phenolics in the third cultivar. One cultivar — 'Jonsok' — contained 12 percent higher total phenolics, mostly from higher levels of ellagic acid and kaempferol, which the authors suggest might have been a result of the higher level of pathogen pressure on the organic berries.

The authors note that "Unexpectedly, organic cultivation had no consistent effect on the levels of phenolic compounds in strawberries" (Hakkinen et al., 2000). Given the diversity of factors impacting polyphenol levels in plants, inconsistent differences in the levels of polyphenols between organic and conventionally grown plants should actually be expected.

Impacts of Specific Farming Practices on Antioxidant Levels

Hundreds of studies have analyzed the impacts of farming practices on antioxidant and polyphenol levels, but very few have focused specifically on applications of specific practices in organic farming systems in contrast to conventional systems. The studies discussed below demonstrate that farming practices can impact antioxidant levels, but shed no light on how such impacts might vary in organic versus conventional systems.

The impact of soil type and irrigation was studied in grape vineyards. The focus was on carotenoid levels in grape berries. Soil A had higher water-holding capacity than Soil B. Both soils were managed with and without irrigation. As expected, non-irrigated berries had lower weight at harvest time (Oliveira et al., 2003) and the irrigated treatment contributed to higher sugar content in both soils. Carotenoid levels did vary across soil types. Levels in fruit grown in soil A did not vary as a function of irrigation, but were 60 percent lower in irrigated fruit grown in Soil B. The authors conclude that it is possible to grow a higher weight berry with higher sugar levels and comparable levels of carotenoids. In addition, they found that soil characteristics had a bigger impact on carotenoid levels than irrigation practices.

In a study of strawberry farming systems, the differences in polyphenol levels was measured between strawberries grown on raised beds with black plastic mulch, compared to strawberries planted in matted rows. Ascorbic acid levels were significantly higher in the raised bed system, as were levels of several measured polyphenols (Wang et al., 2002). The levels of specific polyphenols varied several-fold across varieties and production systems. The fruit grown on the raised bed system had higher levels of antioxidant capacity as measured by the ORAC assay (14.4 compared to 12.6 µmol of Trolox equivalent per gram of fresh weight). Total flavonoids were also about 14 percent higher in the raised bed system.

Impacts of Soil Quality and Weather

Strawberries are a major dietary source of antioxidants. A team of USDA scientists working at the Beltsville Fruit Laboratory analyzed the effects of compost and fertilization practices on phenolic and antioxidant concentrations in two strawberry cultivars. The majority of conventional strawberry growers still rely on methyl bromide fumigation to manage a range of soil-borne diseases, nematodes, and weeds, although some do apply compost. Most organic strawberry growers rely on compost to help sustain soil fertility, meet crop nutritional needs, and enhance soil microbial biocontrol. While several field studies have documented the benefits of compost in terms of strawberry fruit production, fruit quality, and soluble solids content, this is the first study assessing compost impacts on flavonoid levels and total antioxidant capacity (Wang et al., 2003a).

A Bug-Vac moves through a strawberry field. Aphids and other small, winged insects can move into organic fields and quickly reach damaging levels. Machines have been developed that use suction to capture and remove these insects.

The USDA scientists studied levels of a variety of antioxidants including ascorbic acid (AsA), glutathione (GHS), and total antioxidants (measured by the ORAC assay) in pots involving four soils: no compost or fertilizer (100 percent soil); 50 percent soil plus 50 percent sand; 50 percent compost plus 50 percent soil; and 100 percent compost. Each soil treatment was subdivided into three groups: control (no fertilizer); fertilized biweekly with full strength fertilizer; and fertilized biweekly with half-strength (50 percent) fertilizer. Plants grown in the 100 percent compost pots, as well as the 50 percent soil plus 50 percent compost pots, had higher levels of AsA and GSH. In the pots planted in compost and also treated with fertilizer, AsA and GSH levels increased marginally above levels in compost only pots. Levels of AsA in the compost only, no fertilizer pots were about 25 percent higher than in the soil only or soil plus sand pots. The magnitude of the differences did not materially change as fertilizer was added.

Total antioxidant capacity was measured in strawberry juices extracted from the harvested berries of both cultivars. For Allstar strawberries, the highest ORAC values were in the juices from berries grown in 100 percent compost plus fertilizer — 15.2 μmol of Trolox equivalents (TE) per gram of juice; the lowest level was in the 100 percent soil, no fertilizer plot, 9.8 TE/gram. Accordingly, compost treatment increased total antioxidant capacity about 50 percent. Juice from Honeoye berries had about 30 percent higher ORAC values in 100 percent compost pots, regardless of level of fertilization. Levels of some specific flavonoids were nearly 50 percent higher in pots grown in compost compared to soil.

In a study of muskmelons, fruit grown on fine sandy loam soils produced fruit with less beta-carotene than silty clay loam soil (Lester et al., 1996). Beta-carotene levels varied across cultivars and soil types by more than six-fold.

A study in New Zealand highlights the importance of soil selenium levels in determining the antioxidant capacity and health-promoting characteristics of certain foods. Soils deficient in selenium produce foods low in selenium, and lead to inadequate blood levels to support the formation of certain enzymes that play a role in preventing oxidative damage to DNA (Ferguson et al., 2004). A statistically significant association was found across New Zealand between the incidence of colon cancer and soil selenium levels. Other work showed that middle-aged men with blood selenium levels lower than 100 ng/ml (nanograms per milliliter) had depressed capacity to prevent oxidative cell damage. Ferguson et al. note that::

> *"Although there are a range of potentially antimutagenic fruits, vegetables and cereals available to these populations, current intake is generally below the level necessary to protect from dietary or endogenous mutagens."*

A study by USDA scientists focused on selenium levels in broccoli, a plant known to bioaccumulate levels of selenium to several-fold soil levels. The scientists grew broccoli in selenium-enriched soils. Harvested produce that contained more than 500 micrograms of selenium per gram of broccoli (Finley 2003). The broccoli was fed to rats with chemically-induced colon cancer. The incidence of tumors in the treatment group was 50 percent lower than controls. Selenium from enriched broccoli also decreased the incidence of rat mammary tumors. The authors conclude that methods to increase the selenium levels in broccoli could lead to significant improvements in its health-promoting properties.

In tomatoes, research has shown that the highest lycopene levels occur in fields with relatively low, but still adequate nitrogen concentrations in the soil, while increasing soil phosphorous levels generally increases lycopene content (Dumas et al., 2003). Increasing nitrogen levels in tomato production systems also consistently reduces vitamin C content, while yields typically rise. This relationship between nitrogen levels, crop yields, and vitamin C content has been labeled the "dilution effect" (Davis et al., 2004; Farrell 2003) and likely applies equally to conventional and organic systems.

Impacts of Plant Genetics

There is considerable variation in the levels of polyphenol secondary plant metabolites produced by plants within a given cultivar when it is grown under different conditions or over time. Likewise, levels differ markedly across varieties of a given fruit, vegetable or grain crop. Because of the magnitude of natural variation in antioxidant levels in foods triggered by varietal selection, it is challenging to definitively isolate the often-smaller impacts on antioxidant levels caused by changes in farming methods.

The total phenolic contents and antioxidant capacities of 11 wheat cultivars were studied, in addition to the levels of several specific flavonoids. Modest differences were observed in most measures of phytochemical content (Adom et al., 2003), although there were significant varietal differences in carotenoid and total ferulic acid content. Differences across varieties in lutein levels varied five-fold, zeaxanthin levels varied three-fold, and beta-cryptoxanthin levels differed 12-fold. The authors noted that such large differences across varieties in carotenoid levels open up the possibility of increasing the contribution of wheat to total carotenoid intake.

Most of the flavonoids found in tomatoes appear in the skin. Several teams have been exploring ways to increase the content of flavonoids in the flesh of tomatoes, thereby markedly increasing total phenolic content. One team developed a transgenic tomato cultivar engineered to express elevated levels of two maize transcription factors (Le Gall et al., 2003). The flesh of the engineered tomatoes was found to contain nine major flavonoids, six identified for the first time in tomatoes. One variety (Line 2059) had 10-fold higher levels of total flavonoids, compared to controls, with kaempferol glycosides accounting for 60 percent of the total. In addition, the presence of novel flavonol glucosides in the transformed varieties was highlighted by the authors as significant because the sugar moiety of flavonols have been shown to be more readily absorbed in the gut. As a result, genetically transformed tomatoes might both produce higher levels of flavonols, as well as more bioavailable forms of antioxidants.

This study demonstrates that transgenic techniques can both markedly increase total phenolic concentration and lead to the production of novel chemicals, which would trigger the need for extensive safety testing, since such tomatoes would not be "substantially equivalent" to conventional cultivars. ("Substantial equivalence" is a term of regulatory art adopted by the Food and Drug Administration to compare conventional food with the same food harvested from a genetically engineered (GE) cultivar. A GE food is deemed "substantially equivalent" to the unengineered variety if the levels of micronutrients and macronutrients are essentially the same).

A team of Spanish scientists worked with nine commercial tomato varieties and measured total phenolics, total antioxidants, and lycopene levels. Quercetin was the most abundant flavonoid and was found in concentrations varying from 7.2 to 43.6 milligram per kilogram of fresh fruit — a range of more than six-fold (Martinez-Valverde et al., 2002). Other flavonoids varied modestly or by up to a factor of two. Lycopene levels varied from 20 mg/kg to over 50 mg/kg.

The ORAC assay was used to measure the total antioxidant capacity of eight broccoli cultivars, isolating total antioxidants in the aqueous and fat-soluble (hydrophilic and lipophilic) extracts. The antioxidant capacity of aqueous extracts from the eight cultivars varied from 65 to 122 μmol Trolox equivalent (TE) per gram of tissue, whereas levels varied from 3.9 to 17.5 μmol TE per gram in lipid extracts (Kurilich et al., 2002).

Strawberries are known to have relatively high levels of the flavonoid ellagic acid. In a study of four strawberry cultivars, there was about a two-fold difference in the concentrations of ellagic acid across the cultivars (Williner et al., 2003), with the highest level recorded in immature fruit (2.07 mg/gram dry weight).

i

Significant differences in phenolic content were found in six pear cultivars in a study carried out in Portugal. The peel contained the highest concentrations of flavonoids, and only chlorogenic acid was found in the flesh of the fruit (Sanchez et al., 2003). In the peel, total phenolics ranged from 1,235 to 2,005 mg/kg and from 28 to 81 mg/kg in the flesh. In this study of pears, phenolic levels in the peel of the fruit were some 30-times higher than in the flesh of the fruit. Similar findings have been made in other studies, highlighting the importance of consuming fruit with the peel on (Daniel et al., 1999). Unfortunately, pesticide residue levels also tend to be highest on the peel of many fruits and vegetables (Benbrook, 2004), a factor which leads some consumers to wash and peel fresh fruit before serving it to their children, or eating it themselves.

University of Arkansas scientists assessed antioxidant activity and phenolic content in five commercial blueberry cultivars grown at the same location over two years. Variations in antioxidant (ORAC), total phenolic content (TPH), total anthocyanins (ACY), total hydroxycinnamic acids (HCA), and total flavonols (FLA) were measured. Flavonoid content was much greater across varieties than from one season to the next (Howard et al., 2003). The results led the team to conclude that environmental growing conditions have an impact on the levels of phenolics and antioxidant capacity that differs across genotypes, and that cultivars should be assessed over multiple growing seasons to take account of weather-genotype interactions. They report that:

> *"In general, genotypes with smaller berries had higher ORAC values and levels of TPH, ACY, HCA and FLA than large-berried genotypes."*

A team of Spanish scientists working with 18 pea varieties reports similar findings. A wide range of nutritional components was measured across the cultivars, including several flavonoids, different measures of protein, and sugar levels. They report that smaller peas had the highest concentrations of protein, verbascose, inositol pentaphosphate, and vitamins B_1 and B_2 (Vidal-Valverde et al., 2003).

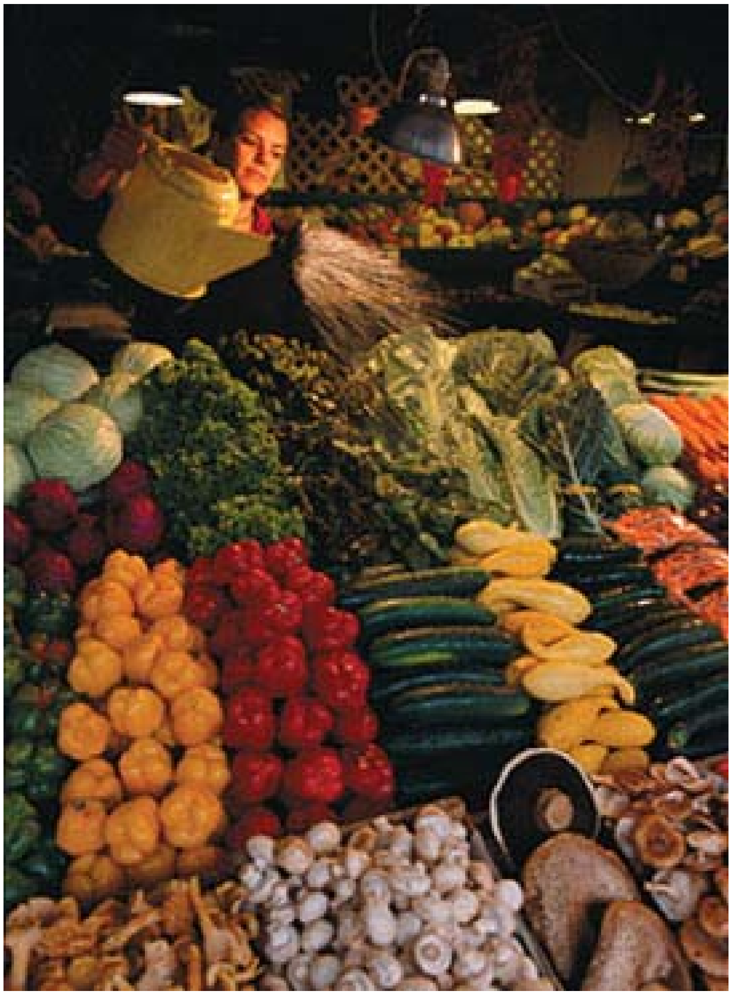
Impacts of Harvest Time and Ripeness

Three pepper cultivars were studied to assess the effects of fruit maturation on flavonoid and phenolic content. In general, the content of polyphenols increased with maturity, and the peppers contained levels of vitamin C above the Recommended Dietary Allowance based on typical serving sizes

(Howard et al., 2000). Levels of several phenolics varied substantially across the cultivars and changed relative to each other as a function of maturity. In another study, levels of 23 flavonoids in the pericap of peppers were studied at four maturity stages (Marin et al., 2004). Immature green peppers had a very high total phenolic content, while immature red and ripe red peppers had four- to five-fold lower levels. Ascorbic acid was the dominant antioxidant and its concentration increased with maturity.

The quantity and characterization of Cabernet Sauvignon grape flavonoids changed during the ripening process, with the level of all phenolics decreasing with maturity (Kennedy et al., 2000). Changes in vine water status were also found to alter polyphenol content. Another team focused on the total phenolics and levels of ellagic acid in eight muscadine grape cultivars at two levels of maturity (Lee et al., 2004). All phenolic concentrations generally increased with ripeness and were highest in the skin of ripe grapes. Hot-pressed juices contained markedly lower concentrations than whole grapes. Cultivar, maturity, and location significantly influenced antioxidant capacity.

Fruits and leaves of several thornless blackberry, red raspberry, black raspberry, and strawberry varieties were studied for total antioxidant activity using the ORAC assay, as well as total phenolic content. Blackberries and strawberries had the highest ORAC values in the green stage, whereas red raspberries had the highest ORAC values at the ripe stage (Wang et al., 2003b). Total anthocyanin content increased with stage of maturity in all berries tested. ORAC values in leaves ranged from 69.7 to 182.2 μmol of Trolox equivalents (TE) per gram fresh weight, whereas levels in fruit ranged from 7.8 to 33.7 μmol of TE per gram fresh weight. Levels declined in leaves as the plants matured. On the basis of freshweight of fruit, Jewel black raspberry and blackberries appear to be the richest source of antioxidants, whereas strawberries had the highest concentrations on the basis of dry weight. In a similar study, ellagic acid levels in strawberries were highest in green fruit, intermediate in ripening fruit, and lowest at maturity (Williner et al., 2003).



A study of nine blueberry cultivars assessed levels of antioxidant activity, total phenolic content, anthocyanin content and six other measures of fruit quality (Connor et al., 2002). All fruit was placed in cold storage. Antioxidant activity increased 29 percent in one cultivar and remained unchanged in the others. Antioxidant activity, total phenolics and anthocyanin levels were strongly correlated with one another.

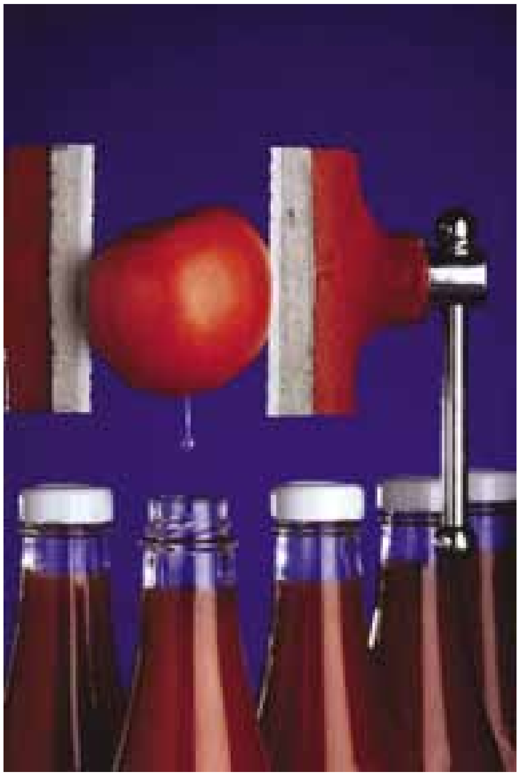
Several studies have shown that phenolic levels in tomatoes increase steadily from the green stage through full ripeness. Lycopene concentrations, in particular, rise sharply during the final days of ripening (Dumas et al., 2003).

Impacts of Food Storage, Processing and Preparation

Because of their growing importance in the diet, the stability of isoflavones in soy milk and soya products has been extensively studied. Concentrations of genistein and daidzein derivatives in soymilk declined at ambient temperature following first-order kinetics (Eisen et al., 2003) and accelerated approximately 40-fold at high temperatures (70 to 90 degrees Celsius). A study determined that the sun-drying of pears caused a 64 percent decrease in total phenolic content on a dry pulp basis, dropping from 3.7 grams per kilogram at harvest (Ferreira et al., 2002). Procyanidins accounted for about 96 percent of the total phenolic content. Storage of wheat flour for six months led to a 70 percent decline in phenolic acid concentrations (Sosulski et al., 1988).

A detailed study assessed the impact of various tomato processing methods on total polyphenol and vitamin C content and antioxidant capacity (Gahler et al., 2003). Tomato juice, baked tomatoes, tomato sauce, and tomato soup were tested. The vitamin C content of tomato products decreased during thermal processing, while total phenolics concentrations and water-soluble antioxidant capacity increased. Another team assessed three of the same processing techniques and found the largest reduction in naringenin concentrations, whereas chlorogenic acid levels were elevated (Re et al., 2002). Overall, the team judged that tomato processing leads to a general improvement in the levels of individual antioxidants.

An exception was documented in a study assessing the impact of tomato processing on carotenoid levels, and found modest reductions (Takeoka et al., 2001). A third detailed study focused on changes in vitamin C, lycopene and total antioxidant capacity in raw tomatoes and after thermal processing. Significant reductions in vitamin C were noted, whereas lycopene levels increased more than 2.5-fold after 30 minutes of processing and total antioxidant capacity rose about 50 percent (Dewanto et al., 2002). The authors of this third study concluded that:



The processing of tomatoes into ketchup actually leads to an increase in lycopene.

> *"These findings indicate that thermal processing enhanced the nutritional value of tomatoes by increasing the bioaccessible lycopene content and total antioxidant activity and are against the notion that processed fruits and vegetables have lower nutritional value than fresh produce."*

Large-scale orange juice processing and pasteurization techniques have also been studied intensively. Five industrial processes used in juice-making were studied, along with hand squeezing, a "domestic" technology (Gil-Izquierdo et al., 2002). Commercial squeezing removed 22 percent more total phenolics than hand squeezing. Freezing caused a dramatic drop in phenolics and pasteurization of pulp led to the degradation of several phenolic compounds, with losses around one-third of pre-pasteurization levels. Pasteurization increased vitamin C levels but did not markedly change total antioxidant capacity in juice. L-ascorbic acid accounted for 77 percent to 96 percent of the total antioxidant capacity of orange juice. Pasteurization did reduce total antioxidant capacity in citrus pulp by 47 percent (Gil-Izquierdo et al., 2002). In a study of apple juice, the variety of apple pressed had the biggest impact on polyphenol concentrations (Guyot et al., 2003).

A number of studies have documented significant reductions in antioxidant capacity in apple juice made with conventional processing techniques. By applying an alcohol extraction step either on the pulp or the pumace, apple juice fortified through this added step contained 1.4 to 9 times the levels of selected flavonoids and 5 times higher total antioxidant capacity (van der Sluis et al., 2004). Apple juice fortified with this novel approach retained 52 percent of the total antioxidant capacity of the fresh fruit used to extract it. The novel method, however, also changed the taste and color of the juice.

In a study of olives, the type of processing had no impact on polyphenol content in oil, but did affect levels in olive juice (Romero et al., 2004a). Colored olives had about six times the levels of polyphenols as oxidized olives. Plain olives across all cultivars tested had higher concentrations than pitted olives, and stuffed olives had the lowest levels.

The impact of cooking vegetables on polyphenol levels is highly variable. Thermal processing of tomatoes and sweet corn can increase total antioxidant capacity despite lowering vitamin C content (Jiratanan et al., 2004a). The thermal processing of beets resulted in marginally higher total antioxidant capacity despite an 8 percent loss in vitamin C content. In beans, vitamin C and folate remained constant, but there was a 32 percent reduction in total phenolics content (Jiratanan et al., 2004b). The authors conclude that:

> *"...optimal health benefits may be achieved when a wide variety of plant foods (fruits, vegetables and whole grains) and preparation methods are incorporated into the diet."*

Onions and tomatoes lose three-quarters or more of their quercetin content after boiling for 15 minutes, 65 percent after cooking in a microwave, and 30 percent after frying (Aziz et al., 1998). Steam cooking of vegetables is the preferred method of preparation to preserve polyphenol content, since it avoids leaching. Essentially all fruits and vegetables should be consumed with the skin to maximize polyphenol intake; processing and food preparation that entails removal of skin and outer portions of leafy vegetables typically reduces polyphenol intake dramatically, often by 90 percent or more (Manach et al., 2004).

The production of fruit juices or jams in large-scale facilities often includes steps designed specifically to remove flavonoids responsible for hazing or discolorization of fruit.

Flavonoid, catechins, procyanidins, and resveratrol content in grapes and the grape mash left after pressing for wine or juice have been studied across a wide range of storage, ripeness and processing parameters. One study found that the method used to press grapes had the greatest impact of flavonol levels, followed by cultivar, pasteurization, and vintage (Fuleki et al., 2003). Pasteurization increased the concentration of catechins in cold-pressed juices, but pasteurized, hot-pressed juices had depressed levels. The production of fruit juices or jams in large-scale facilities often includes steps designed specifically to remove flavonoids responsible for hazing or discoloration of fruit.

A Norwegian study of total antioxidant capacity using the FRAP assay showed that dried apricots and prunes contained relatively high concentrations of antioxidants, at levels two to six times higher than in fresh fruit (Halvorsen et al., 2002). Raisins, however, contained substantially lower levels of antioxidants compared to fresh grapes, an impact that might be caused by the drying process, varietal differences, or the markedly different cultural practices typically used in vineyards growing grapes for the fresh market versus for raisins.

OPTIONS AND OPPORTUNITIES TO INCREASE ANTIOXIDANT INTAKES IN THE TYPICAL AMERICAN DIET

Increasing the polyphenol and antioxidant intake of the average American's diet will require progress on four fronts:

- eating more fruits and vegetables;
- choosing fruits and vegetables that contain high levels of relatively bioavailable antioxidants and polyphenols;
- growing, processing, and cooking foods in ways that retain or increase antioxidant and polyphenol content; and
- consuming fruits and vegetables with their skin on at an optimal stage of ripeness.

For consumers motivated to do so, it is fairly easy to increase the size and/or number of servings of fruits and vegetables and preferentially seek out fruits and vegetables that are relatively high in antioxidant content. Looking for deeply and brightly colored fresh produce in supermarkets is a reliable strategy to select high-antioxidant foods. Switching from highly processed to less-processed or fresh foods will also make a big difference in total antioxidant intake in most cases and is an option most consumers can pursue.

There are important exceptions. For example, antioxidant levels increase significantly in most common processed tomato products compared to levels in raw, fresh, and ripe tomatoes (Wu et al., 2004).

Because many people are trying to cut caloric intake in order to lose weight, a recommendation to eat more fruits and vegetables high in antioxidants must be accompanied by eating less of some other food group (unless, of course, people increase their activity level and burn more calories). The health benefits of eating more of foods that promote good health can be negated when, and to the extent that, caloric intake and excess body fat creep upward. For this reason, consumers who want to increase intake of antioxidants without increasing calories need to take into account the “antioxidant bang-per-calorie” of different foods. Figure 1 reports the average H-ORAC antioxidant units per calorie for foods in each of four groups established by USDA researchers, based on the antioxidant content of food per serving and per gram (see Table 3 for source data and more details).

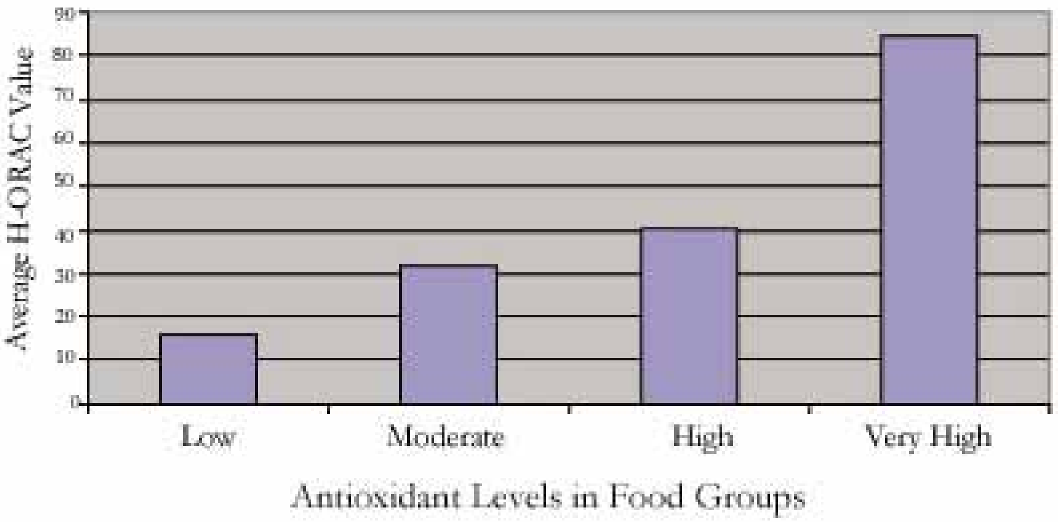


Figure 1. Average Antioxidant Capacity for Foods per Calorie in Antioxidant-Content Food Groups Established by USDA

A consumer that selects foods from the “Very High” antioxidant group will, on average, consume 84.5 H-ORAC units of antioxidant capacity per calorie of food consumed, compared to just 16 H-ORAC units in the “Low” group. For this reason, one serving of food in the “Very High” antioxidant group is likely to deliver over five times the antioxidants per calorie.

Tufts University scientists have recommended that people seeking to optimize the benefits of antioxidants in the diet consume 3,000 to 5,000 ORAC units per day. Based on the high-end of this range, a consumer that seeks out foods in the “Very High” group would need to devote only about 60 calories daily to the consumption of foods high in antioxidant potential, or just three percent of a typical 2,000 calories per day diet. If the consumer chose only “Low” antioxidant foods, it would require about 312 calories of these foods to reach the 5,000 ORAC unit goal, or over 15 percent of daily caloric intake.

Antioxidants in Organic and Conventional Foods

Relatively few well-designed studies have compared the antioxidant levels in a given crop grown under conventional and organic systems, where confounding variables are largely eliminated or controlled. The characterization of conventional and organic farming systems in published studies is too sparse to reach more than tentative conclusions. Still, the available evidence is encouraging.

There are 15 quantitative comparisons of antioxidant levels in organic versus conventional fruits and vegetables in Tables 5, 6, and 7. Organically grown produce had higher levels in 13 out of 15 cases. On average, the organic crops contained about one-third higher antioxidant and/or phenolic content than comparable conventional produce. Several studies have found levels of specific vitamins, flavonoids or antioxidants in organic food to be two or three times the level found in matched samples of conventional foods.

Dr. Preston Andrews (left) and Dr. John Reganold (right) select strawberries for antioxidant and sensory testing. These Washington State University (WSU) scientists are carrying out this research with funding from the Organic Center.

Another set of studies has documented often significant and sometimes dramatic impacts on antioxidant levels from adoption of farming methods that are typically used by organic farmers and are only occasionally used on conventional farms. Studies reviewed in this SSR provide evidence that several core practices on organic fruit and vegetable farms — use of compost, cover crops, slow release forms of nitrogen — can increase antioxidant and polyphenol content compared to conventional practices that depend on commercial fertilizers and pesticides. The number of research teams exploring the links between farming practice and food quality is growing rapidly, especially in Europe.

The weight of the evidence reviewed in this SSR shows that a shift toward organic production methods could play an important role in increasing the total supply of antioxidants in the U.S. diet. The state of science points to some, but surely not all the reasons why.

There clearly is a connection between plant stress levels and the production of secondary metabolites, including many polyphenols and antioxidants. There is substantial agreement among plant pathologists, physiologists, and entomologists that:

- relatively higher levels of antioxidant secondary plant metabolites are produced by plants in response to biotic and abiotic stress; and
- levels produced are a function of genetics, farming methods, and plant health.

These well-established facts have led many scientists to hypothesize that plants on organic farms produce higher levels of polyphenols and antioxidants because they are under more pest pressure, and hence stress, because applications of synthetic pesticides are prohibited. As a whole, studies that have explored this hypothesis have either supported this basic premise or at least not contradicted it.

Pest-induced plant stress is almost certainly not the only factor leading to generally higher antioxidant levels in organic foods, because pest pressure is in fact often reduced on well-established organic farms. Indeed, the operational goal of pest management on successful organic farms is the prevention and avoidance of damaging levels of pest pressure, more so than planting and producing crops with "super-strength" defense mechanisms capable of repairing the damage caused by heavy pest populations. Thus far, research on organic farming and polyphenols-antioxidants has focused on the connections between pest pressure, plant stress, and polyphenol-antioxidant levels. It is time to broaden the focus of ongoing research to include the role of polyphenol- and antioxidant-driven plant defense mechanisms in promoting healthy plant growth and warding off pests, responses that can prevent plants from having to contend with the wounds and stress induced by successful pest attacks.

Some sort of linkage between plant stress and antioxidant levels is almost surely a piece of a complex, variable, and dynamic puzzle. But plants produce secondary plant metabolites and polyphenol antioxidants for physiological and metabolic reasons in addition to response to stress and pest attack.

Science has also hardly begun serious exploration of other ways that organic farming methods might elevate polyphenol and/or antioxidant levels. Organic systems tend to deliver nutrients to plants, trees, and vines more slowly and more in line with needs, changing the pace of a crop's physiological development and often the size of the fruit or grain upon harvest. The superior taste and higher vitamin levels in many organic foods is likely linked to these differences in physiological patterns of growth. Could the form, source, and levels of plant nutrients in the soil also quantitatively impact antioxidant levels in plants and produce, or qualitatively change the mix and distribution of specific antioxidants?

Organic farming systems support relatively higher levels of soil microbial diversity and activity compared to conventional farms (Letourneau et al., 1996; Reganold 2004; Reganold et al., 2001). This can lead to more complex plant-microbe interactions, higher levels of nutrient cycling, and changes in plant metabolism and biochemistry. How might these factors affect polyphenol-antioxidant levels in the crops harvested from organic farms? In all likelihood, the humus-rich and biologically-active soils on many organic farms have the potential to change both the levels and mix of antioxidants in harvested produce compared to soils that are periodically fumigated, always heavily fertilized, and generally less biologically active as a result of intensive, conventional tillage, planting, and pest management practices.

Humus-rich and biologically-active soils on organic farms have the potential to change both the levels and mix of antioxidants in harvested produce.

Nutritional Quality Differences Likely to be a Moving Target

Over time the nutritional quality and antioxidant differences between organically and conventionally grown foods have, and will continue to, vary as a function of trends in conventional and organic production methods and technology. In several regions of the United States, the differences are narrowing between the farming methods and techniques relied upon on well-managed organic farms and those used on nearby conventional farms that have chosen to adopt more biologically-based methods to manage pests and sustain soil fertility levels.

In particular, an increasing number of conventional growers are adopting prevention-based pest management systems that include many of the core methods used by organic farmers. Many conventional fruit growers, for example, are now relying predominantly on "softer" pesticides and pest population suppression through biological and management-based approaches. To the extent that conventional fruit and vegetable farmers continue to move away from hard-chemical-based pest management systems, pest management-related differences in antioxidant levels in conventional

versus organic foods may become less frequent and significant.

New technologies and innovation in organic production methods and systems are also likely, and some of these are bound to alter polyphenol and antioxidant profiles in organic produce, at least under some circumstances. Innovations in the handling, packaging and marketing of fresh-cut and other fresh forms of produce, both conventional and organic, are also destined to impact antioxidant levels and intake. Food manufacturers are experimenting with a number of antioxidant-friendly ways to press, freeze, preserve, partition, cook and combine foods. Some of these new technologies are bound to offer American consumers and the food industry cost-effective ways to increase total antioxidant intake, but it is remains to be seen whether current market forces and food quality standards will place a premium on such innovations.

The tools of molecular biology also are helping plant breeders identify the genes that are responsible for the production of specific secondary plant metabolites. Many teams are working to isolate the genes that produce the enzymes and hormones that control the physiological and biosynthetic pathways that produce plant antioxidants. Genetic breakthroughs have great potential to help plant breeders using traditional plant breeding methods, coupled with marker-assisted breeding techniques, develop new varieties that have higher antioxidant levels under most production systems. How and whether the more aggressive and invasive tools of biotechnology will bring about a quantum leap in the breeding of transgenic, high-antioxidant plant varieties remains to be seen and will depend on many factors beyond what is technically feasible.

Impacts of Food Processing and Manufacturing Methods

The way foods are processed, mixed together with other foods, cooked, preserved and prepared for final consumption can dramatically affect polyphenol-antioxidant levels. In the near term, increasing the retention of antioxidants in foods as they are processed and prepared is likely to offer the greatest potential to increase average antioxidant intakes, especially if consumer interest and purchasing patterns makes the retention of antioxidants a priority for the food processing and manufacturing industries.

There are some significant differences in the food processing and manufacturing technologies that are allowed and typically used in companies manufacturing processed organic foods in contrast to conventional foods. Some of these differences are known to have a significant and consistent impact on polyphenol-antioxidant levels. For example, the synthetic chemical hexane is one of several chemicals used to promote extraction of oils from crops in conventional, high-temperature and high-pressure oil processing plants, but this synthetic chemical may not be used in the extraction of organic oils. Hexane is known to promote removal of antioxidants.

Some production plants extracting organic oils use cold-pressing methods that are generally thought to yield oils that are richer in flavor and retain more of the nutrients in the foods being processed. More research is needed to quantify the degree to which organically approved oil extraction methods may be leading to higher antioxidant levels.

Water-soluble antioxidant levels tend to fall when fruit juices or other processed foods are treated with water, steam blanching or other thermal processes, including pasteurization. Non-thermal processing techniques, or methods than involve lower temperatures, should be studied in more detail to determine the degree to which they might help preserve the antioxidants that are in fresh foods prior to processing.

Understanding Antioxidants and Diet-Health Connections

There is direct evidence from a Danish study that an organic diet does substantially increase flavonoid intake and antioxidant activity in the body, by as much as 60 percent to 100 percent for some major flavonoids like quercetin and kaempferol (Grinder-Pedersen et al., 2003). As noted throughout this State of Science Review, hundreds of studies have suggested or documented that increases in antioxidant intake can help delay the onset of diseases and aging, lessen the severity of disease, especially those in which inflammation plays a role, and promote sustained neurological health.

It is far harder to prove that increasing the consumption of antioxidant X or flavonoid Y will reduce a specific person's risk of a given disease, which is why most scientists continue to emphasize the need for diversity in food forms and types as people increase fruit and vegetable intake. In addition, new research is continuously adding new layers of complexity to our understanding of how antioxidants can have an impact on health.

Two examples follow. A team of scientists has shown that the secondary plant metabolite and antioxidant resveratrol mimics the impacts of caloric restriction and can prolong life through a mechanism that has nothing to do with the anti-inflammatory benefits of most antioxidants (Wood et al., 2004). It does so by activating enzymes known as sirtuins, which play a key role in gene silencing, DNA repair, and the aging process at all levels of the tree of life. A recent study on antioxidants in apples presents evidence suggesting that the increase in plasma antioxidant capacity in humans following

The planting of certain flowers and shrubs in and around organic strawberry fields helps attract beneficial insects and provides habitat for populations to grow.

ingestion of apples reflects the impact of fructose in the apples on urate, rather than antioxidants in the apples (Lotito and Frei, 2004).

Several studies have shown that current analytical methods are measuring only a portion of the biologically active antioxidants in foods. Assays that measure total antioxidant capacity of a given food often produce values double what can be accounted for by the levels of individual, known antioxidants in the same food.

Recall that secondary plant metabolites, including many antioxidants, play key roles in triggering or amplifying complex plant biosynthetic pathways that govern a plant's physiology and development, its efforts to ward off insects or prevent infections from plant pathogens, and how it responds to the damage caused when a pest attack is successful. Most biosynthetic processes within plants require many compounds, in the right proportions, to function optimally. Excesses of certain nutrients and bioactive chemicals can be as damaging as inadequate levels of particular nutrients and chemicals. The same is likely true in people after consumption of plant-based antioxidants. These chemicals go to work quickly, playing a variety of roles in cell signaling and cascades of biological responses. The degree of response brought about by an antioxidant, and the health benefits flowing from that response, vary enormously as a function of a person's age, total diet, health status, and whether they smoke, as well as whether a person is taking medication or has recently been exposed to potentially toxic environmental pollutants.

Accordingly, today's methods for measuring the total antioxidant capacity of foods are inadequate and do not capture fully the potential of foods to deliver health benefits. In addition, that potential is contingent upon many factors and differs across people. For this reason, much more sophisticated, circumstance-specific dietary guidance will be needed to help consumers identify the most important ways to promote good health through changes in food consumption patterns.

Conflicting claims are often encountered about the health benefits associated with increased intake of specific antioxidants or total antioxidants. Some human dietary intervention trials and epidemiological studies conclude that increased consumption of a specific antioxidant leads to health benefits, while other studies show no effect, or instead, point to the mix of antioxidants in a given food. There are many reasons why seemingly similar studies reach different conclusions:

- differences in experimental design and the selection of study groups;
- imprecise indicators of antioxidant intake and uptake;
- less-than-perfect diagnosis of disease incidence and severity;
- inability to control for confounding factors also affecting health status; and
- practical limitations in controlling all aspects of a person's diet.

In summary, more and better studies are needed to determine whether and how organic farming methods and organically-acceptable food processing technologies can help increase average antioxidant intake. Much more research is also needed to better quantify the human health benefits that might be expected as a result. The widely accepted need for more research, however, does not undermine the breadth and depth of scientific consensus that fruit and vegetable consumption promotes good health, and that antioxidants in fruits and vegetables play complex and sometimes-important roles in bringing about this outcome.

Need for New Research Strategies

Today, most nutritional and biomedical research is striving to isolate the specific ingredients in foods that confer health benefits, on the assumption that there must be a roughly linear relationship between intake of "good" constituents in foods and some positive health outcome. While this sort of reductionist research remains important, we are not confident it is going to provide the breakthroughs needed to explain why whole foods are better for people than processed foods, why fruits and vegetables are so beneficial, or what lies behind well-established mysteries like the French Paradox.

Our reading of the scientific literature suggests that the roots of food quality are more complex than the levels of proteins, fats, carbohydrates, vitamins, minerals, and antioxidants in foods.

First, we strongly suspect that there are as-yet-unidentified phytochemicals in foods, including some with unique and specific biological properties. Likewise, some polyphenols in foods that have been identified are likely to affect health through mechanisms not yet recognized. Many will be antioxidants. Some constituents in foods may trigger harmful, or beneficial, biological responses only under certain specific sets of circumstances.

Second, there is considerable evidence suggesting that the health benefits of a given food or diet arise from the totality of what is in a person's diet. In particular, health outcomes may be linked more strongly to the relative levels of fats, fiber, certain vitamins, minerals, antioxidants and other chemicals in a person's diet, than to whether a given nutrient or vitamin or antioxidant is present at levels sufficient to satisfy our contemporary understanding of a "Recommended Dietary Allowance."

Food quality may have more to do with the balances among, and relationships across the constituents of foods than the levels of any individual constituent. If this is true, fundamentally different ways are needed for scientists to isolate what gives rise to "food quality."

ORGANIC CENTER ANTIOXIDANT RESEARCH AND POLICY RECOMMENDATIONS

The impacts of conventional and organic farming methods on the vitamin, mineral, protein and antioxidant content of food clearly warrants further study, especially in light of research in both the United States (Davis et al., 2004) and abroad (Davis et al., 2004; Meyer 1997) showing that food quality has declined over the last four decades. For the most part, there is little research underway, especially in the United States, exploring the ways that farming methods can and have altered food quality. Work on polyphenol antioxidants is a welcomed exception.

Factors affecting polyphenol antioxidant levels in foods, and possible human health implications, are under active exploration by dozens of labs in the U.S. and thousands of scientists around the world. Most of the ongoing work is narrow in scope and reductionist in nature. Levels of a specific flavonoid, or total antioxidant capacity in a food are measured as a function of one or more of several parameters thought to alter concentrations. Some studies go on to test the biological impacts of various levels of antioxidant intake in cell assay systems. Other studies are carried out with laboratory animal models, again testing biological responses to various levels of antioxidant intake. Several human nutrition intervention trials have been conducted testing for links between antioxidant intake levels and indicators of health status.

Progress is being made in understanding some pieces of the puzzle, but the puzzle's overall shape and how the pieces fit together over time and across the population remain a mystery for the most part. Still, a substantial body of evidence supports four conclusions:

- Fruit and vegetable intake promotes good health;
- Fruits and vegetables are the primary sources of antioxidants that the body needs and in some cases cannot manufacture;
- Polyphenol antioxidants in foods can lead to health benefits in multiple ways, including some which are not yet understood, or even recognized; and
- It is rarely possible to determine the capacity of a given food to promote health by analyzing the impacts of the individual nutrients and chemicals in the food.

Research Recommendations

The Organic Center has identified research priorities in three areas: Increasing Antioxidant Intake, Retaining Antioxidants and Nutrients in Processed Foods, and Understanding the Nature of Food Quality.

Increasing Antioxidant Intake

A logical way to identify where and how organic farming can help the nation increase average antioxidant intake is to focus research efforts on foods that are simultaneously:

- consumed regularly;
- serving for serving, a significant dietary source of important antioxidants;
- currently grown by organic farmers, with potential to become more widely grown on certified organic operations;

- consumed by people in fresh and processed forms that do not sacrifice a high degree of antioxidant content; and
- contain antioxidants in forms that are reasonably bioavailable to people when consumed in common food forms.

Another criterion should be considered in targeting research – the frequency and levels of pesticides on or near the skins of fresh produce. The skins of fruits and vegetables are major sources of antioxidants in the diet; pesticide residues are also most commonly found on the skin and outer layers of conventionally grown produce.

Some consumers peel produce before serving it to their families in order to limit pesticide exposure. For these consumers, the selection of fresh organic produce can deliver dual benefits — health promotion from increased intake of antioxidants and lessened pesticide risk from reducing dietary exposures. (For an analysis of pesticide residues in organic versus conventional foods, see the Organic Center's State of Science Review entitled "Minimizing Pesticide Dietary Exposure Through the Consumption of Organic Food" accessible at: http://www.organic-center.org/science.htm?groupid=4&articleid=25).

Ten commonly consumed foods meet all or most of these criteria and should be among the foods studied in the near-term:

Blueberries
Plums
Blackberries
Strawberries
Red cabbage
Sweet cherries
Leaf lettuce
Spinach
Apples
Tomatoes

Despite falling into the "Low" antioxidant group in their raw form, tomatoes warrant priority attention because antioxidant levels increase substantially in most processed tomato products — about four-fold in the case of ketchup. In addition, tomato-based products are major components of the diets of many people.

Research Priority 1:

Improved strategies and methods are needed to carry out rigorous field studies comparing the impacts of farming methods on antioxidant levels in harvested organic versus conventional crops.

Properly designed studies will allow scientists to establish whether there are differences in antioxidant levels between organically grown crops and conventionally grown crops produced in the same area on the same type of soil, using the same crop varieties. The challenge in designing comparative studies is to control for all other possible sources of variation in antioxidant levels, other than the farming practices unique to organic production.

Determining whether there are differences, while important, is just a first step. The next and likely more difficult step is exploring why differences exist or do not exist.

Research Priority 2:

Refined methods are needed to produce accurate, replicable laboratory results when antioxidant levels are tested in foods, and when markers of antioxidant status are measured in animal models and the human body.

Improved test methods should be coupled with better and more standardized ways to report, share and compile empirical findings on the influence of various factors on antioxidant levels in foods, animals and people. Today, differences in test methods and the way results are reported by different laboratories make it difficult to compare and compile test results across laboratories and over time. Such variation in analytical results makes it more difficult to identify the impacts of various factors on antioxidant levels, especially factors that might typically alter levels 10 percent to 20 percent.

Retaining Antioxidants and Nutrients in Processed Foods

Antioxidant levels in many processed foods are a fraction of the levels in fresh foods. In some cases, food processing actually concentrates antioxidants (i.e., lycopene in most processed tomato products). Novel food processing methods and technologies could markedly lessen the loss of antioxidants in a variety of processed foods, and in particular, in oils and juices.

Research Priority 3:

The impact of organically acceptable food processing methods and technologies on antioxidant retention should be systematically studied and compared to standard food processing technologies and methods.

This work should help isolate where and to what extent organically acceptable food processing technologies and methods are superior or inferior to conventional methods in terms of retaining antioxidants. This information will help in two ways: first, in documenting the size of the organic food quality premium in cases where organically processed foods retain a greater portion of antioxidants than similar conventional foods; and second, in targeting research toward those organically accepted food processing methods that need further refinement or replacement in order to increase antioxidant retention.

Technologies used to extract oils from oilseed crops, olives, grains, cotton and other commodities vary dramatically, both within the conventional and organic food sectors. Differences in methods are known to have substantial impacts on antioxidant retention and need to be better understood in order to guide investments in new technology and manufacturing processes.

Research Priority 4:

Cost-effective options need to be developed to maximize the retention of antioxidants in the process of extracting and purifying oils for cooking, baking and direct consumption, and in extracting juices and other food processing systems and technologies.

Companies in the business of manufacturing oils need better information on antioxidant and nutrient retention to guide capital expenditures on oil pressing technologies and equipment. Organic food companies buying oils to use in their manufacturing processes need to better understand how different vendors' extraction processes affect antioxidant levels.

Likewise, some consumers will welcome information on the extent to which the purchase of certified organic oil products can help them increase their average daily antioxidant and nutrient intakes.

Understanding the Nature of Food Quality

Research might unlock the secrets of organic farming systems with the potential to produce food with predictable, unique nutritional qualities and health-promoting attributes. As a result, the term "custom farming" might someday take on a whole new meaning.

The promotion of healthy plant development and the prevention of plant stress are the hallmarks of successful organic farming systems. Is it possible that the biochemical signals, interactions among organisms, and metabolic pathways that organic farmers have learned to manage and take advantage of in the course of producing a crop might significantly alter antioxidant levels and/or the mix of antioxidants in food when it is harvested? Will research eventually unlock the secrets that organic farmers need to understand to manage organic farming systems that produce food with predictable, unique nutritional qualities and health-promoting attributes?

> *Research might unlock the secrets of organic farming systems with the potential to produce food with predictable, unique nutritional qualities and health-promoting attributes. As a result, the term "custom farming" might someday take on a whole new meaning.*

Research Priority Five:

Development and application of new methods to assess the levels and relationships among the nutrients and phytochemicals produced by plants during the normal course of development, and as a function of the levels and nature of pest pressure.

Research in this area should focus on differences in the levels and distribution in nutrients and phytochemicals produced on different organic farms. It could also assess differences on matched sets of conventional and organic farms producing the same crop under comparable conditions.

Key insights might emerge when two fields producing the same crop, under similar conditions, turn out to produce crops that differ markedly in yield, quality, or resistance to pest attacks. Consistent empirical differences may be found in the mix of phytochemicals and nutrients in the foods harvested from such fields, and those differences may, in turn, reflect differences in soil chemistry, microbial activity, and plant-soil interactions.

A long-term goal in this area of research will be to identify critical thresholds in the ratios of various plant nutrients and phytochemicals above which, or below which, plant health, productivity, or crop quality is likely to suffer.

As key ratios are identified that appear to be correlated with plant health, these same ratios can be tested in cell culture assay systems and laboratory animals to determine whether the relative presence of nutrients that promotes health in plants also does so in mammals.

Research Priority Six:

Greater investment is needed in novel approaches to explore the attributes and characteristics of foods that lead to nutritional quality and health promotion, so that more sensitive and accurate experimental models can ultimately be developed and applied.

Vitamin and mineral content and total antioxidant capacity are clearly important pieces of the diet-health puzzle, but they do not fully explain why or how a given food or diet can promote health or undermine it. In order to more precisely predict when and to what extent increased antioxidant intake might promote health, the totality of factors and attributes of foods and diets affecting health must be better understood.

Work in this priority area will begin to create the scientific concepts, models and tools to test whether the animal and plant physiological processes that unfold under organic farming methods, in the presence of soils that support high levels of microbial activity, enable plants to produce phytochemicals and nutrients in relative proportions to each other that are uniquely healthy for people and farm animals.

Years down the road, expanded effort in these six priority areas of research might provide the foundation for answering organic farming's $64,000 question: "Do healthy soils produce healthier plants, and in turn, promote good health in the animals and people consuming the plants?"

The science needed to work toward an answer to this question is complex and will take many years, even for a single crop and location. Absolute and widely applicable answers are unlikely, yet practical methods are bound to emerge that will tip the scales toward healthy plants and hopefully also, healthier people.

The potential benefits from a focused commitment to research in these six areas are enormous and justify a quantum leap in public research investment in the United States. The scope of work needed is such that only the government can marshal the resources needed, especially to tackle all six areas systematically across the many important foods in the American diet.

Policy Recommendations to USDA

The Organic Center strongly supports government and private sector efforts to increase the average number of daily servings of fruits and vegetables. Clearly, achieving the eight or more daily servings of fruits and vegetables now recommended by USDA is a necessary first step and will require a major change in dietary patterns, given that the average person consumes about 4.8 servings today. The doubling of average fruit and vegetable intake will likely deliver pronounced health benefits for several reasons including increased antioxidant and vitamin intake.

The science supporting such a recommendation is strongest for individuals suffering from diseases rooted in, or made worse by inflammation. Such individuals should also consider switching their choice of fruits and vegetables toward those that are high or moderate in fiber and in antioxidants per calorie and typical serving (see Tables 1 and 3). For the same reason, people also need to become aware of fruits and vegetables that tend to retain a high percentage of their antioxidant content during storage, freezing, cooking or food processing.

Given the variation in antioxidant levels in foods, and the substantial impacts of processing on antioxidant retention, USDA needs to support more internal and extramural research on the most cost-effective ways to increase antioxidant intake in the near-term.

Policy Priority 1:

USDA should carry out a quantitative assessment of the increases in antioxidant intake that might be achieved through various options, along with the pros and cons of alternative options and strategies to achieve them.

At a minimum, the alternatives studied should include those highlighted throughout this State of Science Review:

- increasing the average number of servings of fruits and vegetables, or fruit juices;
- reducing the negative impact of storage, processing, and cooking on antioxidant capacity;
- promoting the uptake and bioavailability of antioxidants consumed in food; and
- increasing antioxidant levels through changes in genetics and farming methods, including the adoption of organic systems.

Policy Priority 2:

USDA should couple internal analyses of options to increase average antioxidant intake with substantial competitive grant funding for research on the most promising strategies to increase antioxidant content through the National Research Initiative, new organic research programs, and the Small Business Innovation Program.

The scientific challenges ahead are enormous but could lead to profound new insights on the links between food production, diet and health. As USDA identifies ways to increase antioxidant levels, or to retain antioxidant content as food is processed, it should provide extramural research grant support to teams and companies interested in developing cost-effective systems and processes that will increase average antioxidant intakes.

In addition, USDA should leverage change underwritten by its own research programs by encouraging other government agencies and research programs to initiate new, and expand existing, food and dietary antioxidant-focused research.

Provide More Diversified Dietary Guidance

USDA has just announced revised dietary guidelines for the nation. In developing consumer education materials and resources, more attention should be directed to explaining the factors that a person should take into account when deciding whether and how to alter dietary antioxidant intakes. These factors certainly include age, weight, plans for having children, health status, and activity level.

Policy Priority 3:

USDA should issue antioxidant-focused dietary guidance, and possibly revised food pyramids, for people at different stages of life and for pregnant women.

Millions of people will benefit from better information and more direct guidance in selecting foods high in antioxidant capacity that also meet other dietary needs and preferences. New antioxidant-focused dietary guidance should be accompanied by consumer-friendly data and educational information on the total antioxidant capacity per typical serving of food and per calorie consumed.

Tufts University has already developed and made available a food pyramid for older Americans (accessible at http://nutrition.tufts.edu/consumer/pyramid.html). Other populations hoping to sustain health or slow the progression of disease will appreciate access to dietary guidance customized in response to their special needs.

Managing diets for optimal health and to slow the progression of disease is neither simple nor risk-free, yet is a challenge that millions of Americans now face. Providing solid antioxidant-focused dietary guidance tailored to the needs of major segments of the population with specific health promotion goals is a good place for USDA to start in meeting this challenge.

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GLOSSARY

Aglycone. The non-carbohydrate portion of a molecule that is left after the glycoside is stripped off via hydrolysis; the form of a molecule left after the sugar moiety has been enzymatically removed.

Alpha-lipoic acid (lipoic acid). A powerful antioxidant that is readily absorbed and utilized within the cell where it is capable of regenerating glutathione. As such, lipoic acid supplementation results in increased glutathione levels. Lipoic acid is also involved in energy production. Lipoic acid works together with vitamin E and vitamin C to protect the entire cell from oxidative stress.

Amino acids. The building blocks of proteins. There are non-essential amino acids, those we can make in our bodies, and essential amino acids, those we can not make but must get from our diet. Amino acids are not only important in the synthesis of protein, but also function in transmission of neural pulses, and are part of the antioxidant front battling oxidative stress.

Antioxidant. An enzyme or other organic molecule that can counteract the damaging effects of oxygen in tissues. Although the term technically applies to molecules reacting with oxygen, it is often applied to molecules that protect from any free radical (molecule with unpaired electron). The term antioxidant is used to describe a dietary component that can function to decrease the tissue content of reactive oxygen. Common antioxidants include vitamins C and E, beta-carotene, N-acetylcysteine, selenium, zinc and alpha-lipoic acid.

Aromatic compound. An organic molecule containing a benzene ring, or characterized by one or more planar rings.

Autoimmune disease. Autoimmune diseases occur when the body tissues are mistakenly attacked by its own immune system. The immune system is a complex organization of cells and antibodies designed normally to destroy pathogens, particularly viruses and bacteria that cause infections. Individuals with autoimmune diseases have antibodies in their blood which target their own body tissues.

Beta-carotene, Lutein, and Lycopene. These three carotenoids make up 70-80% of the major carotenoids found in foods, and 70-80% of the carotenoids found in human serum. Carotenoids are found in higher plants and function to protect the plant from the oxyradicals produced during the absorption of light, and to act as pigments to aid in light absorption.

Bioavailable. The portion of a nutrient (or other chemical) that can be absorbed, transported, and utilized physiologically.

Calorie. A unit of thermal energy equal to 4.184 joules.

Carboxylation. The introduction of a carboxyl group (-COOH) or carbon dioxide into a compound.

Carotenoids. Yellow, orange and red pigments in plants, often masked by chlorophyll and thought to function as protective antioxidants. There are hundreds of carotenoids in nature, both in the food supply and in other plants; for example, the bright red color of tomatoes is attributable to the carotenoid lycopene. The orange color of carrots is due to the presence of large amounts of beta-carotene. Some carotenoids can be converted to vitamin A in the body.

Case-control study. A study in which the risk factors of people who have been diagnosed with a disease are compared with those without the disease. Because the risk factor (e.g., nutrient intake) is generally measured at the time of diagnosis, it is difficult to determine whether the risk factor was present prior to the development of the disease. Another potential draw back is the difficulty in obtaining well-matched control subjects.

Catalyst. A substance that increases the rate of a chemical reaction by reducing the activation energy, but which is left unchanged by the reaction.

Cation. An ionic species with a positive charge.

Central nervous system (CNS). The brain, spinal cord, and spinal nerves.

Chelate. The combination of a metal with an organic molecule to form a ring-like structure known as a chelate. Chelation of a metal may inhibit or enhance its bioavailability.

Cholesterol. A lipid used in the construction of cell membranes and as a precursor in the synthesis of steroid hormones. Dietary cholesterol is obtained from animal sources, but cholesterol is also synthesized by the liver. Cholesterol is carried in the blood by lipoproteins (e.g., LDL and HDL). In atherosclerosis, cholesterol accumulates in plaques on the walls of some arteries.

Clinical trial. A research study, generally used to evaluate the effectiveness of a new treatment in human participants. Clinical trials are designed to answer specific scientific questions and to determine the efficacy of new treatments for specific diseases or health conditions.

Coenzyme. A molecule that binds to an enzyme and is essential for its activity, but is not permanently altered by the reaction. Many coenzymes are derived from vitamins.

Cohort study. A study that follows a large group of people over a long period of time, often 10 years or more. In cohort studies, dietary information is gathered before disease occurs, rather than relying on recall after disease develops.

Cross-over trial. A clinical trial in which at least two interventions or treatments are applied to the same individuals after an appropriate wash-out period. One of the treatments is often a placebo. In a randomized cross-over design, interventions are applied in a randomized order to ensure that the order of treatments did not contribute to the outcome.

Cross-sectional study. A study of a group of people at one point in time to determine whether a risk factor or a level of a risk factor is associated with the occurrence of a disease. Because the disease outcome and the risk factor (e.g., nutrient intake) are measured at the same time, a cross-sectional study provides a “snapshot” view of their relationship. Cross-sectional studies cannot provide information about causality.

Density. Mass per unit volume (i.e., milligrams of a vitamin per gram of food)

Daily Value (DV). A term used in food and supplement labeling in the U.S. The amount of a vitamin or other nutrient in a serving of a food or supplement is expressed as the percentage of the total Daily Value of that nutrient, based on a daily 2,000 or 2,500 calorie diet.

Diabetes (diabetes mellitus). A chronic condition associated with abnormally high levels of glucose (sugar) in the blood. The two types of diabetes are referred to as insulin-dependent (Type I) and non-insulin dependent (Type 2). Type I diabetes results from a lack of adequate insulin secretion by the pancreas. Type 2 diabetes (also known as NIDDM) is characterized by an insensitivity of the tissues of the body to insulin (insulin resistance).

Diabetic ketoacidosis. A potentially life-threatening condition characterized by ketosis (elevated levels of ketones in the blood) and acidosis (increased acidity of the blood). Ketoacidosis occurs when blood glucose levels are not adequately controlled.

Dietary supplement. A product (other than tobacco) intended to supplement the diet that bears or contains one or more of the following dietary ingredients: a vitamin; mineral; herb or botanical; amino acid; any other dietary substance for use by man to supplement the diet by increasing the total dietary intake.

Double blind. Refers to a study in which neither the investigators administering the treatment nor the participants know which participants are receiving the experimental treatment and which are receiving the placebo.

DRI. Dietary Reference Intake. Refers to a set of at least four nutrient-based reference values (RDA, AI, UL, EAR) each with a specific use in defining recommended dietary intake levels for individual nutrients in the U.S. The DRIs are determined by expert panels appointed by the Food and Nutrition Board of the Institute of Medicine.

Electrolyte. A substance which forms ions in an aqueous (water) solution. Major electrolytes in the body include sodium, potassium, magnesium, calcium, chloride, bicarbonate, phosphate.

Endocrine system. The glands and parts of glands that secrete hormones that integrate and control the body's metabolic activity. Endocrine glands include the pituitary, thyroid, parathyroid, adrenal, pancreas, ovary, and testes.

Endogenous. Arising from within the body. Endogenous synthesis refers to the synthesis of a compound by the body. Secondary plant metabolites are also endogenous within plants.

Enzyme. A protein that catalyzes a chemical reaction. A substance that increases the speed of a chemical reaction without being changed in the overall process. Enzymes are vitally important to the regulation of the chemistry of cells and organisms.

Epidemiologic study. A study examining disease occurrence in a human population.

Etiology. The causes or origin of a disease.

Fat soluble vitamins. Nutrients that dissolve in fats or oils but not in water. These vitamins are often found in foods that contain fat, and fat may be necessary for their absorption from the digestive tract into the bloodstream. People who eat very little fat may have difficulty getting enough of the fat-soluble vitamins A, D, E, and K.

Fatty acid. An organic acid molecule consisting of a chain of carbon molecules and a carboxylic acid (COOH) group. Fatty acids are found in fats, oils, and as components of a number of essential lipids, such as phospholipids and triglycerides. Fatty acids can be burned by the body for energy.

First order kinetics. The rate of change of physical or chemical systems as it pertains to an equation measuring a chemical reaction.

Folic acid. Water-soluble B complex vitamin. Plays an important role in cell division and thus is important to the development of the nervous system of the fetus. Folic acid can also reduce levels of homocysteine, preventing damage to the artery walls, and ultimately, atherosclerosis. Folic acid is easily absorbed directly from the digestive tract into the bloodstream. Folates, however, must be processed by enzymes in the intestinal lining before they can be absorbed.

Folin-Ciocaltheu. An assay designed to measure total antioxidant capacity.

Fortification. The addition of nutrients to foods to prevent or correct a nutritional deficiency, to balance the total nutrient profile of food, or to restore nutrients lost in processing.

FRAP. The "Ferric Reducing Ability of Plasma" antioxidant assay.

Free radical. An atom or a molecule with an unpaired electron. Because they have a free electron, such molecules are highly reactive with nearby molecules. By interacting with cellular components, free radicals may cause cellular and genetic damage, and their involvement has been implicated in several diseases. Free radicals are generated by smoking, environmental pollutants, exposure to UV radiation, and also occur naturally in the body as a result of metabolic processes. Free radical damage may be countered with antioxidants.

Fructose. A sweet 6-carbon sugar abundant in plants. Fructose is increasingly common in sweeteners such as high-fructose corn syrup, found naturally in fruit.

Gastrointestinal. Referring to or affecting the stomach and intestines (small and large bowel).

Glucose. A 6-carbon sugar which plays a major role in the generation of energy for living organisms.

Glucoside. A compound in which the sugar moiety is a glucose residue. Formerly glycoside

Glutamate. An excitatory neurotransmitter. Under certain circumstances glutamate may become toxic to neurons. Glutamate excitotoxicity appears to play a role in nerve cell death in some neurodegenerative disorders.

Glutathione. In its reduced form, GSH, glutathione protects cells against various oxyradicals. Glutathione levels in humans have been shown to decrease with age. Glutathione cannot be absorbed in the stomach and therefore levels of this cellular protector cannot be increased with dietary supplementation. Instead, alternate antioxidants (i.e. alpha-lipoic acid) and precursors to glutathione (i.e. N-acetyl cysteine) must be taken in order to increase glutathione levels.

Glycoside. A compound in which the sugar moiety is a glucose residue. An obsolete term for glucoside.

HDL. High density lipoproteins. HDL transport cholesterol from the tissues to the liver where it can be dismantled and eliminated in bile. HDL-cholesterol is considered good cholesterol, because higher blood levels of HDL-cholesterol are associated with lower risk of heart disease.

Heterocycle. Any compound or molecule that is heterocyclic.

Heterocyclic. Any cyclic molecular structure containing atoms of at least two different elements in the ring or rings.

H_2O_2 (hydrogen peroxide). An unstable compound that is readily broken down in to water and oxygen and that is capable of reacting with cellular components. This interaction can be very damaging as seen with lipid peroxidation and the development of atherosclerosis.

Huntington's disease. An inherited degenerative disorder of the brain. Its symptoms include movement disorders and impaired cognitive function. Symptoms of Huntington's disease, previously known as Huntington's chorea, typically develop in the fourth decade of life and progressively deteriorate over time.

Hydrophilic. The tendency to dissolve in water; having a strong affinity for water.

Inflammation. A response to injury or infection, characterized by redness, heat, swelling, and pain. Physiologically, the inflammatory response involves a complex series of events, leading to the migration of white blood cells to the inflamed area.

Intervention trial. An experimental study (usually a clinical trial) used to test the effect of a treatment or intervention on a health- or disease-related outcome.

In vitro. Refers to studies and/or phenomena that take place outside the body (e.g., in test tubes).

In vivo. Refers to studies and/or phenomena that take place in animals or humans.

Ion. An atom or group of atoms that carries a positive or negative electric charge as a result of having lost or gained one or more electrons.

Ionic bound. A chemical link between two atoms caused by the electrostatic force between oppositely-charged ions in an ionic compound.

Isomers. Compounds that have the same numbers and kinds of atoms but that differ in the way the atoms are arranged.

Joule. The basic SI (System International) unit of energy. A joule is equal to the kinetic energy of a two-kilogram mass moving at the speed of one meter per second.

Kinetics. The study of the rates of chemical reactions.

LDL. Low Density Lipoprotein. Lipoproteins (particles composed of lipids and protein) are the form in which fats are transported throughout the body, in the bloodstream. LDL transport cholesterol from the liver to the tissues of the body. A high proportion of cholesterol carried in LDL (LDL-cholesterol) is associated with an increased likelihood of developing cardiovascular diseases (heart disease and stroke). Oxidized LDL appear to play an important role in the development of atherosclerosis.

Lipophilic. Possessing the tendency to dissolve in, having a strong affinity for, or readily mixing with lipids or fats.

Membrane potential. The electrical potential difference across a membrane. The membrane potential is a result of the concentration differences between potassium and sodium across cell membranes which are maintained by ion pumps. A large proportion of the body's resting energy expenditure is devoted to maintaining the membrane potential, which is critical for nerve impulse transmission, muscle contraction, heart function, and the transport of nutrients and metabolites in and out of cells.

Meta-analysis. A mathematical or statistical analysis, used to pool the results of all studies investigating a particular effect (e.g., the effect of folic acid supplementation on homocysteine levels) and provide an overall estimate of that effect.

Macronutrients. Nutrients that the body needs in relatively large amounts. The major macronutrients are protein, carbohydrate, fat, and water.

Metabolism. A general term for the complex biochemical processes by which the body generates energy from food, manufactures substances that it needs, and breaks down substances in food into simpler components for incorporation into the body or detoxification and excretion from the body.

Metabolite. A compound derived from the metabolism of another compound is said to be a metabolite of that compound.

Micronutrients. Nutrients that the body needs in small amounts. Vitamins and minerals are micronutrients.

Methylation. A biochemical reaction resulting in the addition of a methyl group ($-CH_3$) to another molecule.

Minerals. Nutritionally significant elements. Elements are composed of only one kind of atom. Minerals are inorganic, i.e., they do not contain carbon as do vitamins and other organic compounds.

Moiety. Either of two, usually distinctive, component parts of a complex molecule; e.g. the steroid and saccharide moieties of a cardiac glycoside.

Mole. The SI (System International) base unit for the amount of a substance. Equals the amount of substance that contains as many elementary entities as there are atoms in 0.012 kilogram of carbon-12. One mole of a compound has a mass equal to its relative molecular mass in grams.

N-acetyl cysteine (NAC). A powerful antioxidant made from the amino acid cysteine and is found naturally in foods. It is a precursor for glutathione, an important antioxidant.

Neurodegenerative diseases. Progressive diseases of nervous system, such as Alzheimer's disease and Parkinson's disease, that are characterized by the loss or degeneration of neurons.

Nutraceutical. A product isolated or purified from foods, and generally sold in medicinal forms not usually associated with food and demonstrated to have a physiological benefit or provide protection against chronic disease.

Observational study. A study in which no experimental intervention or treatment is applied. Participants are simply observed over time.

Optimum health. In addition to freedom from disease, the ability of an individual to function physically and mentally at his or her best.

ORAC. The "Oxygen-Radical Absorbance Capacity" assay of total antioxidant capacity.

Oxidation. A chemical reaction that removes electrons from an atom or molecule.

Oxidative stress. An organism is said to experience oxidative stress when the effects of prooxidants (e.g. free radicals, reactive oxygen and reactive nitrogen species) exceed the ability of antioxidant systems to neutralize them.

Oxyradical (reactive oxygen). A free radical derived from molecular oxygen.

Parkinson's disease. A disease of the nervous system caused by degeneration of a part of the brain called the basal ganglia, and by low production of the neurotransmitter dopamine. Symptoms include muscle rigidity, tremors, and slow voluntary movement.

Pathogen. A disease causing agent, such as a virus or a bacteria.

Peptide. A chain of amino acids. All proteins are made up of one or more peptides.

Peptide hormones. Hormones that are proteins, as opposed to steroid hormones, which are made from cholesterol. Insulin is an example of a peptide hormone.

Pericarp. The fruit wall which has developed from the ovary wall; sometimes used for any fruit covering. From the Greek, peri (peri), "around;" and karpos (karpos), "fruit".

pH. A measure of acidity or alkalinity.

Pharmacologic dose. The dose or intake level of a nutrient many times the level associated with the prevention of deficiency or the maintenance of health. A pharmacologic dose is generally associated with the treatment of a disease state and considered to be a dose at least 10 times greater than that needed to prevent deficiency.

Phospholipids. Lipids (fat molecules) in which phosphoric acid as well as fatty acids are attached to a glycerol backbone. Phospholipids are found in all living cells and in the bilayers of cell membranes.

Phosphorylation. The creation of a phosphate derivative of an organic molecule. This is usually achieved by transferring a phosphate group ($-PO_4$) from ATP to another molecule.

Physiologic dose. The dose or intake level of a nutrient associated with the prevention of deficiency or the maintenance of health. A physiologic dose of a nutrient is not generally greater than that which could be achieved through a conscientious diet, as opposed to the use of supplements.

Phytochemical. Substance derived from a plant. The term is generally reserved for molecules with biological activity.

Prooxidant. A atom or molecule that promotes oxidation of another atom or molecule by accepting electrons. Examples of prooxidants include free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS).

Prospective cohort study. An observational study in which a group of people—known as a cohort—are interviewed or tested for risk factors (e.g., nutrient intake), and then followed up at subsequent times to determine their status with respect to a disease or condition of interest.

Protein. A polypeptide or molecule made up of polypeptides. A complex, nitrogen-containing substance that is found in food and is essential for the functioning of the human body. Protein molecules consist of long chains of building blocks called amino acids. Some of these amino acids can be manufactured in the human body. Others must be supplied by the diet. The body breaks down food proteins into their amino acid constituents and then reassembles the amino acids into the proteins needed for normal functioning.

Provitamin. A compound that the human body can convert into a vitamin. For example, beta-carotene is a provitamin because the body can convert it into vitamin A, as needed.

Randomized controlled trial (RCT). A clinical trial that involves at least one test treatment and one control treatment, in which the treatments administered are selected by a random process (e.g., coin flips or a random-numbers table).

Randomized design. An experiment in which participants are chosen for the experimental and control groups at random, in order to reduce bias caused by self-selection into experimental and control groups. This type of study design can provide evidence of causality.

RDA. Recommended Dietary Allowance set by the Food and Nutrition Board of the Institute of Medicine. The RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97-98%) healthy individuals in a specific life stage and gender group (e.g., women from 19-50 years of age). It is intended as a goal for daily intake of specific nutrients by individuals.

Reactive nitrogen species (RNS). Highly reactive chemicals, containing nitrogen, that react easily with other molecules, resulting in potentially damaging modifications.

Reactive oxygen species (ROS). Highly reactive chemicals, containing oxygen, that react easily with other molecules, resulting in potentially damaging modifications.

Receptor. A protein on or protruding from the cell surface to which select chemicals can bind. Binding of a specific molecule (ligand) may result in a cellular signal, or the internalization of the receptor and the ligand.

Redox reaction. Another term for an oxidation-reduction reaction. A redox reaction is any reaction in which electrons are removed from one molecule or atom and transferred to another molecule or atom. In such a reaction one substance is oxidized (loses electrons) while the other is reduced (gains electrons).

Retinol. The chemical name for vitamin A.

Scavenge (free radicals). To combine readily with free radicals, preventing them from reacting with other molecules.

Selenium. A component of the antioxidant enzyme, glutathione peroxidase. Glutathione peroxidase works with vitamin E in preventing free radical damage to cell membranes. In addition, selenium appears to have antioxidant properties on its own and plays a role in cancer, cardiovascular disease, enhancing immune function, inflammatory conditions, and cataracts.

Signal transduction. The transfer of a signal from the outside of a cell to the inside of the cell by means other than the introduction of the signal molecule itself into the cell. Typically, entails the interaction of a hormone or growth factor with a specific membrane receptor that triggers synthesis within the cell of one or more second messengers, or to the activation of downstream cascades.

Tannins. Any of a large group of plant-derived compounds. Tannins tend to be bitter tasting and may function in pigment formation and plant protection.

Taurine. A sulfur-containing amino acid that is derived from the amino acids methionine and cysteine. It is the most abundant free amino acid in muscle. Taurine is involved in the synthesis of bile salts, in many metabolic processes, and in maintaining the health of the retina.

TEAC. "Trolox Equivalent Antioxidant Capacity" assay of total antioxidant capacity.

Transcription factor. A regulator in the process of the synthesis of either RNA on a template of DNA or DNA on a template of RNA.

Tryptophan. One of the amino acids that makes up proteins. Tryptophan is of special importance in nutrition because it can be converted into the B vitamin niacin.

Vitamin. The name that is given to 13 organic substances that are essential in the diet because they cannot be manufactured by the body. Vitamins are needed in very small amounts, but they are essential to life.

Vitamin A. A fat soluble vitamin involved in the maintenance of healthy skin, eyes, bones, hair and teeth and is essential to proper immune function. Vitamin A can be synthesized from the antioxidant /provitamin beta-carotene.

Vitamin B2. Riboflavin.

Vitamin B6. Is important in the formation of proteins, structural compounds, messengers in the nervous system, red blood cells, prostaglandins, proper functioning of a large number of enzymes and in maintaining proper immune function. Low levels of Vitamin B6 result in high levels of homocysteine. Homocysteine damages the cells that line the arteries, which can eventually result in atherosclerosis. Vitamin B6 can inhibit platelet aggregation, lower blood pressure, can protect against the development of diabetic neuropathy and enhances the immune system.

Vitamin C. Important as an antioxidant but also in its ability to regenerate the antioxidant form of Vitamin E. With acute viral infections (flus, colds), vitamin C is thought to reduce symptom severity and shorten illness time at high levels. Important in the maintenance of bones, teeth, blood vessels and connective tissue as well as enhancing the immune system, and in decreasing the risk of death from heart attacks, strokes, and cancer.

Vitamin E. A fat soluble antioxidant playing an important role in protecting the cell membrane, fats, the immune system and vitamin A from oxidative stress. Studies suggest that vitamin E supplementation may improve immune function and reduce the risk of chronic diseases such as heart disease, cancer, strokes.

Water-soluble vitamins. Nutrients that dissolve in water. These include vitamin C and the B vitamins. Water-soluble vitamins can easily be lost in cooking if they are allowed to leach into the cooking water, which is then discarded. This problem can be avoided by serving foods raw, cooking foods in as little water as possible, or including the cooking water in the finished dish (e.g., in a soup or stew).