THE NEED TO ESTABLISH CRITERIA

These are exciting times for the plant science community. Development and refinement of molecular biological techniques has proceeded simultaneously with an increased awareness of the role of diet in the development of chronic disease. These events have been the impetus for many botanical laboratories initiating projects with the objective of manipulating plant genes that control the accumulation of essential nutrients or potentially beneficial phytochemicals. The scientific and popular media have reported (and often sensationalized) how lycopene-enhanced tomatoes prevent prostate cancer, how anthocyanin-filled blueberries prevent aging, and how glucosinolate-enhanced broccoli helps remove carcinogens from the body. Other reports tell how the phytochemicals of tomorrow will prevent cancer, reduce cholesterol, improve memory, control blood sugar levels, promote weight loss, and keep the edge on our immune system. The public is literally buying the message, as “functional foods,” i.e. foods that contain compounds that promote health (and very noble!) effort to reduce poverty and improve human nutrition through agricultural/horticultural research and education. As a society of and for horticultural science, our key strengths are in our diverse array of international symposia, our publications, and our periodic Congresses. Through good leadership, sound management of Society resources, and strategic partnerships with other organizations involved in international development we can provide meaningful support to our colleagues in the developing world as they endeavour to conduct better research and be more effective educators. This is what your Board means when it talks about “capacity building” for international development.

Bioactive Compounds and Designer Plant Foods: The Need for Clear Guidelines to Evaluate Potential Benefits to Human Health

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Advances in molecular biological techniques have made it increasingly easy for scientists to manipulate genes involved in plant growth and development. Today many researchers are attempting to manipulate accumulation of so-called ‘bioactive compounds’, i.e. compounds that cause a specific biological response in animals that consume them, and so create foods from ‘super plants’ that can be marketed as functional foods. However, the ability of plant scientists to manipulate these compounds often exceeds the ability of medical scientists to understand what benefits, if any, they provide to the consumer. Consequently the plant food industry needs to establish their own set of criteria that will allow them to determine whether a specific compound is beneficial to human health when supplied as a chemical component of a plant food. Evaluation of scientific evidence for the biological benefits of supplemental β-carotene, lycopene, polyphenols, glucosinolates and selenocompounds by a proposed set of criteria finds major problems and deficiencies in all. The state of the knowledge should stimulate cooperation between plant scientists and human nutritionists that will allow development of plant foods that provide a real benefit to human health.

1 The views expressed are those of the author, and do not represent any official positions or policies of the USDA, Agricultural Research Service.
are ambiguous. This time the pictures in the
Like many other conflicts in the world today,
and in a relatively short period the images
scientists responded with breeding programs,
“Green Revolution,” in which plants are being
bells and emaciated limbs of young children in
However, this second green revolution is very
different from the first where the enemy was
clarity visible, and the objectives clearly defi-
The enemy was typified by the protruding
BBC and emaciated limbs of young children in
Africa and Asia suffering from kwashiorkor, or
improvement, and to increase “health”
the consumer. As in the first green revo-
plant scientists are in the forefront of the
beyond basic nutrition, are among the fastest
growing segments of the food industry with
sales of more than $10 billion worldwide in
2002. To some it may seem to be a second
“Green Revolution,” in which plants are being
altered not only to be more productive and
disease resistant, but also to increase “health”
to the consumer. As in the first green revo-
lution, plant scientists are in the forefront of the
battle.
Apart from demonstrating that a particular
compound improves health, additional criteria
are needed to prove that consumption of a
plant food containing enhanced quantities of
the compound will provide the same benefit.
For example, if there is evidence that lycopene
reduces the risk of prostate cancer, additional
criteria must be established to demonstrate that
consumption of lycopene-enhanced tomatoes
will also decrease the risk of prostate cancer.
There are virtually an unlimited number of
plant-based compounds that potentially benefit
human health; therefore criteria are needed to
limit the number of compounds that are given
even an initial assessment of health benefits.
The FDA Health Claim procedure requires of all
candidate compounds (a) that the compound
of interest be chemically identifiable, and (b)
the proposed health benefit have discrete and
measurable endpoints. For example, if an “anti-
oxidant” is promoted as beneficial, the antioxi-
dant activity should be attributable to discrete
compounds and not to the activity of uniden-
tified “factors.” Additionally endpoints used to
justify a benefit to health should not be based on
in vitro procedures such as the ability to
reduce an oxidized substrate (e.g. many of the
common antioxidant assays such as the Oxygen
Radical Absorbance Capacity (ORAC) or Ferric
Reducing Ability of Plasma (FRAP) assays). Only
compounds that meet these criteria should be
further evaluated for efficacy.

**CAREFULLY CONSIDER THE STRENGTH OF SUPPORTING RESEARCH**

The process of evaluating the efficacy of a can-
didate compound must consider the strengths
and limitations of the various types of suppor-
ting evidence. Studies that used animal and cell
culture models are important for describing potential effects. However cultured cells and
laboratory animals are not humans and the
scientific literature has numerous examples of
physiologic processes described in such models
that did not extend to humans. While epide-
miologic research is considered stronger than
investigational studies with non-human models,
there is an increasing awareness that
epidemiologic research is subject to biases that
can change the interpretation of reported
findings; such biases include ill-defined varia-
bles, inadequate attention to confounding interactions, inadequate sample size, and inadequately means of dietary assessment (Dennis et al., 2004). Interventional studies are the strongest evidence of efficacy, but all are not without limitations. The "gold standard" is the randomized, double blinded (blinded to the participant and the investigator), placebo-controlled study that examines the actual health end-point. Unfortunately such studies are very expensive and the literature is full of studies with much less scope and control. Evaluation criteria should address the limitations of intervention studies, especially in regards to using information from limited studies to make universal conclusions regarding human health in general.

Evidence that a particular chemically pure compound is bioactive (i.e. able to induce a biological response in the animal that consumes it) cannot be used to infer that the same compound is bioactive when consumed as a component of a plant food enriched in the same substance. Unfortunately, this often is assumed and the scientific literature has many examples of where the two measures of bioactivity were different (see discussion of β-carotene below).

Therefore additional criteria are needed to judge whether consumption of a bioactive compound as a component of a plant food also results in a benefit to human health. First, criteria should be established to judge whether the compound of interest is present in the food in an amount and chemical form that will result in bioactivity (given consumption of a reasonable amount of the food). The scientific literature contains reports of bioactivity that have been induced by feeding graded levels of a compound until a dose-threshold was reached; however such studies often do not take into account that such intakes would be impossible or even dangerous on a regular basis. Additional criteria also should evaluate whether enhancement of one phytochemical in a plant results in unintended negative interactions in the plant or in the animal that consumes it. Finally, the efficacy of the bioactive compound must be evaluated in the context of the food matrix, and the compound must be demonstrated to be bioavailable (i.e. able to move from the food matrix in the gut to the target tissue; this is a function of absorption and intermediary metabolism). The following examples summarize research concerning the bioactivity of several compounds of popular interest and the relevant research is used to illustrate misleading assumptions or knowledge gaps.

**Determination of Bioactivity, β-Carotene and Cancer: The Need for Critical Examination of All Data**

Carotenoids are pigmented phytochemicals found in almost all colored vegetables. One of the most common carotenoids, β-carotene, has been extensively studied for its purported ability to suppress cancer. Studies of β-carotene illustrate the importance of testing a compound within the context of the food matrix, and of critically examining all data, especially with regard to the strength of the models used.

Epidemiologic studies conducted before 1995, as well as some studies in animals and cell culture, seemed supportive of the hypothesis that β-carotene was the primary bioactive compound in fruit that reduced lung cancer risk (Willett, 1990). This idea was consistent with a hypothesis that explained β-carotene's function as an antioxidant that protected against oxidation-induced cellular damage and prevented DNA damage that could lead to mutations. To some, the evidence seemed sufficiently consistent to justify human intervention trials, but there was an important flaw in this conclusion in that the epidemiologic data were from studies of fruit and vegetable intake and not from studies of β-carotene per se. Thus in retrospect, perhaps it is not so surprising that a large randomized and blinded clinical trial found that supplementing 20 mg/day of chemically pure β-carotene to Finnish male smokers resulted in a slight, but statistically significant, increase in the incidence of lung cancer (The Alpha-Tocopherol, β-Carotene Study Group, 1994). These results were later confirmed by a second intervention trial conducted in the United States (Omenn et al., 1996).

A primary lesson to be learned from the β-carotene experience is that extrapolation beyond the limits of data, or interpretation of data outside of the context of the experiment, may not only give erroneous results, but in fact may lead to dangerous conclusions. Epidemiologic data relating fruit and vegetable consumption to cancer cannot possibly ascribe an effect to a single compound, and it is in fact impossible to differentiate between the effects of a single compound and the synergy of multiple phytochemicals present in the plant. Despite these limitations many researchers focused exclusively on β-carotene and did not consider the complexity of the food matrix. In retrospect, it also appears that animal and cell culture studies may not have been nearly as supportive as claimed. Few cell culture studies were done in models of lung cancer, and much of the ‘antioxidant’ theory is based on emerging evidence. Certainly a lack of rigorous examination and questioning of the evidence may have at best impeded science, and at worst actually may have been a risk to public health.

**Determination of Bioactivity, Polyphenols and Cancer: The Need for Valid Endpoints**

"Antioxidant" is a ubiquitous term in food/nutrition science and industry, and many products claim to “improve antioxidant status” or “decrease oxidative stress.” Yet, for many antioxidants, there is disagreement as to the in vivo functional importance of the compound(s), as well as to the optimum level of intake or expo-
Sure. Despite these limitations, some researchers continue to promote supra-nutritional intakes of their favorite antioxidant compound. Polyphenols are an enormous general class of chemicals (more than 8000 described compounds), and many polyphenols are reported to have antioxidant ability; work with polyphenols illustrates some of the above problems.

The scientific literature published between 2001 and the present contains approximately 700 reports of the antioxidant potential of phenolic compounds in plants (excluding the reports of antioxidants associated with oils or oilseeds). Most of these reports have reported “antioxidant potential” based on in vitro methodology. For example it has been reported that in vitro antioxidant activity is well correlated to phenolic content of berries, tomatoes, nectarines, peaches and plums, grapefruit juice, and apple and yucca extracts. Although such results may add to our understanding of the chemical properties of such foods, they do not tell us anything definitive about their potential to promote health in humans; this is because many of the tests used to determine “antioxidant potential” have little or no relevance to human health. For example more than 200 studies reported the results of a common type of test that includes the Trolox Equivalence Assay or TEAC, the diphenyl-1-picrylhydrazly, or DPPH assay, and the 2,2'-anisobis-(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay. All of these tests measure in vitro ability of the plant extract to destroy a spontaneously formed radical (Arulom, 2003). There are multiple reasons why these tests do not give information relevant to human health. First, because the test is done in vitro there is no assessment of whether the compounds in the plant actually make it into the cell (or are even absorbed), and second the tests measure only the disappearance of a spontaneously formed radical and thus are only an indirect measure of ability to reduce the damage caused by oxidative stress. Thus the primary reason for the prevalence of such tests is their simplicity and not their relevance; such reports should be treated as preliminary information and not as evidence of a health benefit to humans.

These problems were summarized in a review in Mutation Research, which stated “… it is clear that not a single method can give a comprehensive prediction of antioxidant efficiency” and suggested that “the question of bioavailability and fate of metabolites of antioxidant components must be addressed,” and concluded that “we have to agree (to) governance on in vitro antioxidant methods based on an understanding of the mechanisms involved” (Arulom, 2003).

**A Plant Must Contain a Bioactive Compound in an Effective Concentration and Chemical Form: The Examples of Glucosinolates, Lycopene, and Cancer**

To prove bioactivity of a compound when consumed through a plant food, one must first demonstrate that the compound is found in the plant food in an amount and chemical form that provides bioactivity. Examples from two types of vegetables, cruciferous vegetables containing glucosinolates and tomatoes containing lycopene, illustrate the extent of the variation possible from genetic and environmental sources. They further illustrate that variation may be of a magnitude that makes it difficult to ascribe a health benefit to a particular compound in a plant.

Crucciferous vegetables such as broccoli, ponytail, Brussels sprouts, Chinese cabbage, radish, horseradish, wasabi, white mustard, watercress, and cauliflower are a dietary source of glucosinolates, compounds that may reduce the risk of several cancers. Glucosinolates in plants are chemically converted by bacteria in the gut or enzymes in the plant tissue to isothiocyanates, and it is the isothiocyanate that causes a biological response (Keck and Finley, 2004). One of the most important reactions is the conversion of the glucosinolate glucoraphanin to the isothiocyanate sulforaphane. In an animal, sulforaphane has several important biological actions including activation of cell signals that upregulate detoxification enzymes and thus rid the body of potential carcinogens, and regulation of cell division and cell death in irreparably damaged cells. There is limited epidemiologic evidence that glucosinolates reduce the risk of cancer, and although very few human feeding studies have been conducted, the animal, cell culture, and epidemiologic evidence has been used as the basis for production of glucosinolate-enhanced plant foods.

The primary problem with marketing foods based on glucosinolate content is that we are just beginning to understand the factors that affect the amount and form of glucosinolates in cruciferous foods. Plants are not produced under the same rigorous conditions as pharmaceuticals, and variations in production and post-harvest processing conditions may induce substantial variation. In fact the variation in the glucosinolate content of crucifers can be so great as to cast doubt on whether the cancer inhibitory effects of crucifers really can be ascribed to glucosinolate intake. A recent study used a modeling procedure to introduce estimated variation in the glucosinolate content of crucifers reported in cancer studies (Dekker and Verkerk, 2003). When glucosinolate intake was assumed to be a constant function of crucifer intake, high crucifer consumption cut the relative risk of cancer by as much as half.
However when estimates of glucosinolate variation resulting from cultivation, processing and domestic cooking were introduced into the model, glucosinolate consumption did not significantly reduce cancer risk.

The chemical form of the predominant glucosinolate varies between different cruciferous species. For example glucobrassicin and glucoraphanin may account for as much as 95% of the total glucosinolate concentration in broccoli, whereas Brussels sprouts, cabbage and cauliflower contain little or no glucoraphanin. Crucifers other than broccoli generally contain high concentrations of sinigrin, and glucoraphanin is abundant in Chinese cabbage, radishes, and watercress. Researchers of the University of Illinois have studied the variation of glucosinolates in cruciferous vegetables and have reported that the concentration of total glucosinolates in a plant is not predictive of a specific glucosinolate compound as Brussels sprouts contained twice the total glucosinolates but only ~15% of the glucoraphanin content of broccoli (Kushad et al., 1999). The same researchers also reported that the glucoraphanin content of different broccoli varieties varied more than 25-fold. Thus one cannot ignore the variation inherent in the production system or inherent in genetic diversity. In fact the variation may be so substantial as to call into question whether a compound in a plant actually does benefit health.

Lycopene and tomatoes illustrate that substantial variation in the form and concentration of a bioactive compound can be introduced by post-harvest and processing conditions. The media has given much attention to the benefits of lycopene and many people assume it to be essential especially for protection against prostate cancer in men (this perception has been enhanced by the inclusion of lycopene in several brand name vitamins). Tomatoes are the richest plant food source of lycopene, and those seeking to market the benefits of lycopene-containing tomatoes need to ensure that they contain adequate lycopene in a bioavailable form. Similar to cruciferous vegetables, genetic variation greatly affects the lycopene content of tomatoes (lycopene content of deep red varieties is much greater than in yellow varieties). However, harvest, post-harvest and processing conditions may have an even greater effect on lycopene concentrations. Tomatoes become enriched in lycopene as the fruit ripens, and vine-ripened tomatoes contain more lycopene than tomatoes picked green and ripened in storage; likewise tomatoes produced outdoors in the summer contain more lycopene than tomatoes produced in a greenhouse. Post-harvest processing also affects lycopene bioavailability, and cooking in general causes physio-chemical changes that increase the bioavailability of lycopene (Shi and Le Maguer, 2000; Bramley, 2002). Consequently marketing tomatoes on the basis of lycopene requires that production conditions are carefully monitored and standardized, otherwise one consumer may receive a product with an effective dose, whereas another consumer may receive a product with a much lower dose and/or perhaps in a less bioavailable form.

**Enhancing the Content of One Phytochemical in a Plant May Result in Unforeseen Interactions with Other Bioactive Compounds: The Example of Selenium in Broccoli**

Selenium (Se) is an essential trace element that is used by animals as a component of various selenium-containing enzymes. However, Se also may suppress cancer by mechanisms completely unrelated to its role as a nutrient. Nutritional requirements for selenium are satisfied by an intake of 55 micrograms/day, but a study conducted in the eastern US demonstrated that supplementation of 200 micrograms of selenium/day dramatically reduced overall cancer incidence and mortality and specifically reduced prostate and colorectal cancer (and there was some indication of reduced lung cancer incidence) (Clark et al., 1996). Because the results of the human intervention study are supported by a multitude of epidemiologic studies and mechanistic studies with animals and cultured cells, a strong argument may be made for enhancing foods with selenium.

Broccoli is easily enhanced with selenium and may accumulate selenium in an especially beneficial chemical form. Selenium-enriched broccoli has been reported to inhibit development of several types of cancer in laboratory animals (Finley, 2003). However, studies with high-selenium broccoli illustrate another potential problem with the production of plants enhanced with a specific bioactive compound - enhancement of one bioactive compound (selenium) interferes with production of another important phytochemical (sulforaphane). Further, the interaction between selenium and sulforaphane also causes unexpected changes in the animal that consumes selenium-enhanced broccoli.

Selenium-enriched broccoli is easily produced by fertilization with selenium during maturation of the plant inflorescence. However, compared to unfertilized broccoli, selenium fertilization may decrease the total content of sulforaphane by as much as 75% (there also is evidence that it may decrease specific phenolic acids by as much as 50%) (Finley et al., 2005). The interaction of selenium and sulforaphane extends to the animal that consumes broccoli. Thiooxidon reductase is a protein that needs selenium for activity, and thiooxidon reductase activity is controlled in part by the availability of dietary selenium. However studies in cultured cells have demonstrated that sulforaphane and/or broccoli induces transcription of thiooxidon reductase mRNA and increases the activity of thiooxidon reductase enzyme activity beyond the maximum normally induced by Se alone (Hintze et al., 2003). The functional consequences of such a change in thiooxidon reductase regulation are unclear since the enzyme is both a powerful antioxidant (potentially protective against cancer) as well as a potent inducer of many growth genes (potential induction of cancer). But regardless of the overall impact on cancer, these studies demonstrate a completely unforeseen interaction by two phytochemicals with very different metabolic pathways. Such interactions must be characterized and the impact on health determined in order to assess the overall value of the food.

**SUMMARY**

Advances in molecular biology have opened the door to the development of “super plants” that contain greatly enhanced concentrations of presumed beneficial compounds and can be marketed as Functional Foods. The health and welfare of the consumer, as well as the future of the industry, depend on development of strict standards and criteria that will ensure such foods are safe and efficacious for the desired health benefit. A multi-step evaluation process has been proposed and may be summarized as follows:

1. Determine whether the compound of interest is chemically defined and whether the proposed health benefits have measurable endpoints. If either of these criteria are lacking, then the compound should not be considered until the basic science is more complete.

2. Carefully consider the totality of evidence (especially human interventional and epidemiologic studies) relating a compound to a proposed health outcome - collaborations with departments of nutrition and/or medical schools will greatly facilitate this step. Compounds with proven efficacy become candidates for manipulation in plant systems.

3. When a plant food is developed, determine whether production and/or processing systems introduce unacceptable variation in the final product. This may take considerable experimentation and refinement of techniques.

4. Determine whether consumption of the plant food results in the same biological outcome as consumption of the chemically pure compound. For many compounds, this may have been already accomplished in step 2, however for other products, this may require additional human studies.

5. Finally, carefully consider interactions induced by the manipulation of the plant. Some interactions may affect the function of the plant, whereas other interactions may occur in the human that consumes the plant food. A product that improves one functional measure of health but decreases another may be at best a waste of the consumer's
improving health through improvement of plant foods; rather it should stimulate further research to resolve these inadequacies. Improvement of health care has become a national political debate and priority. Our top three problems are heart disease, cancer and the host of problems associated with obesity, all of which have nutritional problems at their core. Thus by logical extension the solution to these problems must have nutrition at its core. Plants hold great promise for becoming ‘designer’ or ‘super’ foods that can be targeted at specific health problems, but the development of plant foods that make a real difference will depend on extensive cooperation between plant scientists and medical researchers to ensure that marketed products actually do provide the purported health benefits.

References