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Influence of Production, Handling, and Storage on Phytonutrient Content of Foods

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The goals of agricultural production have traditionally been to try to accommodate needs for: 1) adequate and reliable yields to provide a sufficient food supply in a growing world; 2) food safety; 3) taste; 4) convenience; 5) profit; and 6) variety. Alternative strategies to enhance any of these outcomes are typically evaluated as to their probable effects on the key outcome: yield. However, with the burgeoning consumer interest in foods that optimize health, attention is shifting from concerns over quantity alone to concerns over the constituents of foods that may promote health, and thus to the agricultural practices that will protect, and perhaps enhance these constituents of the food supply. This shift in focus requires new thinking and new strategies across all segments of the food production system. This paper summarizes selected aspects of crop production that are pivotal to the nutrient value of foods for human consumption and suggests some strategies for establishing a new research and production paradigm that will embrace nutrient quality among the priorities of agricultural research.

Introduction

As explained in more detail in other papers from this same symposium, the term "phytonutrients" refers to those components of foods that are classically defined as nutrients, as well as those that may provide benefits beyond the prevention of dietary deficiencies. For the purposes of the symposium, even compounds that have unproven effects but that are under active investigation for their potential biologic effects on human health were included in a broad definition of phytonutrients and in the consideration of strategies for agricultural research for the future. Because this is an emerging field, neither nutritionists, agriculturists, nor the general public has adequate information concerning either the putative roles of "phytonutrients" or of the phytonutrient content of foods. Despite the lack of conclusive data on the roles of phytonutrients in maintaining human health, there is widespread optimism that agricultural practices could be modified to positively influence the phytonutrient content of foods.

In many cases, phytonutrients are secondary compounds generated through complex biosynthetic pathways, which are known to be subject to environmental influence. Thus, it is expected that significant amounts of variability in their content could be affected through agricultural production.

A small but growing body of information exists as to the effects of specific agricultural practices on food phytonutrient content. The symposium built on previous knowledge, such as that presented by the Tufts University School of Nutrition Science and Policy during a recent international conference on agricultural production and human nutrition.[1]

As more of this kind of information emerges, it will be important to place it in the context of a larger picture as to how such practices affect food composition and how to best communicate the findings to a broad audience. As a first step toward exploring the relationship between agricultural production factors and food phytonutrient content, our workshop addressed the following questions with regard to the influence of production, handling, and storage on the phytonutrient content of foods.

- What is the current status of scientific knowledge?
- What additional knowledge is needed to move the field forward?

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- What technical barriers exist to obtaining this additional information?
- What are the priority research topics?
- What is the unique role of the federal government in phytonutrient research of this kind?

The Issues

What Is the Current Status of Scientific Knowledge with Respect to Agricultural Production Factors and the Phytonutrient Content of Foods?

Agricultural production factors have been separated into categories by researchers interested in assessing their effects on vitamin content.[2] These categories may serve well in dividing agricultural production practices into separate areas for the purpose of phytonutrient investigation. In general, researchers broadly divide crop production into the pre- and the postharvest environments. Preharvest environmental factors include choice of germplasm, crop rotation practices, soil and seedbed preparation, fertilization, irrigation, pesticide application, maintenance of crop health, harvesting practices, etc. The postharvest environment may include field curing, temperature modification in controlled environment storage, packaging and processing, pesticide application, shipping and handling, and myriad additional factors. Because such a broad range of crop-dependent production practices is common, it has been difficult to place these practices into categories. It would be even more difficult to find systematic effects of particular practices on the relatively unknown area of phytonutrient content and composition.

Much of the published literature linking phytonutrients and agricultural production factors concerns the effect of agricultural production factors such as those mentioned above on ascorbic acid, pro-vitamin A carotenoids, minerals, and dietary fiber. Production, handling, and storage-induced changes in other phytonutrients such as anthocyanins and carotenoids have been noted particularly with respect to color and browning potential, but little work has been conducted on content or composition per se.

To further divide the pre- and postharvest production environments for the purpose of further discussion, we turn our attention to divisions provided by Kader,[2] including varietal selection, preharvest conditions, the cultural environment, postharvest handling and storage, and processing and culinary considerations. The sections below summarize findings to date on the roles of each of these classes of factors on some of the more widely studied phytonutrients.

Varietal Selection

Clearly the first production factor with the potential to influence phytonutrient content is genetic background, or cultivar. For many crops, a large variety of cultivars exist and thus, in principle, there is a substantial potential for genetic variability in phytonutrient content. On the other hand, genetic variability for performance traits among cultivars does not necessarily confer genetic variability for phytonutrient content. During the past 30 years, numerous authors have reported variability in nutrient value of plant cultivars, primarily with respect to vitamins and minerals. The fact that natural variability of vitamin content—as well as many traits of human interest—occur in the germplasm base of our crop plants is a fundamental principle now accepted by plant biologists and nutritionists. Little information, however, has been uncovered as to the patterns and underlying causes of such variation. Intervarietal variation in phytonutrient content per se has not been extensively studied across a broad range of germplasm. However, it is reasonable to hypothesize that the genetic basis for significant variation for these compounds is present in the germplasm base of these crops.

Much of what we have learned about varietal differences in nutrient content has come from plant breeding experiments. This is because the deliberate manipulation of plant populations for nutrient content often reveals the degree to which such traits are heritable. Naturally occurring variability and variability exploited either consciously or unconsciously through plant breeding is likely present for many phytonutrients. Many secondary compounds in plants also are amenable to manipulation by plant breeders. Perhaps one of the best examples of genetic variability for phytonutrient content among cultivars is the Illinois Long Term Selection Experiment, where the content of protein and oil in maize kernels has been increased approximately tenfold during the past 100 years.[3] One of the primary lessons from this experiment is that gain from selection for secondary compounds can still be realized even after nearly 100 generations in a closed population. This example provides evidence in support of the hypothesis that if given enough time, it might be possible to change phytonutrient content through targeted breeding strategies.

Another example of large phenotypic change in nutrient content through breeding is the case of high beta carotene carrots.[4] An average carrot in the United States contains from 70–120 μg per gram fresh weight of total carotenoids.[5] Simon et al.[6] measured values of up to 499 ppm total carotenoids in the variety HCM, which was developed by mass selection for increased carotenoid content. There are additional examples from other fruits and vegetables as well. For example, Patil et al.[7] have shown variability among onion cultivars for quercitin concentration. Variability among red beet cultivars for folic acid content has also been demonstrated.[8] Goldman et al.[9,10] have shown 50-fold variability in onion-induced antiplatelet activity among cultivated and wild species accessions in the genus *Allium*.

Other ways in which breeding can be used to improve phytonutrient content might include maturity groups, market classes or market types, and the production of defense compounds. Many phytonutrients are synthesized in parallel with the overall development and maturation of the plant or fruit. Thus, varietal differences in the degree of maturity, which is consistent with general marketing objectives, may provide a way to optimize stage of maturity at the time of harvest, and thus, the phytonutrient content of the product. Differences in market classes and market types could also be exploited to enhance phytonutrient content. For example, sweet onions comprise a market class that appeals to consumers because they can be eaten fresh in salads due to their low pungency. On the other hand, pungent onions comprise a different market class that is targeted primarily for cooking. Differences in health-functional properties exist between these two market classes of onions,[10] with pungent onions possessing more antiplatelet activity than mild, sweet onions. Lastly, because phytonutrients are associated with plant defense responses, the variability among varieties for relative pest resistance could also translate into phytonutrient content differences. An example of this latter situation is resveratrol, which is a phytoalexin produced in grape leaves and skins in response to stress, such as from ultraviolet light exposure. Resveratrol is thought to have significant health functionality, particularly with respect to cancer. Because this phytonutrient is associated with the production of plant defense compounds, the relative stress resistance among grape cultivars might be a predictor for the degree of phytonutrient content.[11] Likewise, the degree to which individual cultivars will respond to stress may also be a predictive factor for subsequent phytonutrient content. This phenomenon may not be limited to the grape and may also be generalizable to other species.

Preharvest Conditions: Climate, Temperature, and Light

Both temperature and light intensity have been shown to influence vitamin content of fruits and vegetables.[2] Vitamins or pro-vitamins such as the carotenoids, thiamin, ascorbic acid, and riboflavin are strongly influenced by temperature and light intensity during crop production. In particular, ascorbic acid is influenced by changes in light intensity, with increased light intensity shown to result in elevated ascorbic acid content in strawberries.[2] Lee and Coates[12] have shown month-to-month variability in vitamin C content of processed Florida citrus products such as orange and grapefruit juices. They have also demonstrated significant year-to-year fluctuations in vitamin C content of up to 24% in these products. Because the citrus crop is highly susceptible to damage from very cold temperatures and cultivars differ in their ability to tolerate these temperatures, significant fluctuations in vitamin C content can be affected by freezing temperatures. Patil et al.[13] have shown that growing environments, (including factors such as temperature and rainfall patterns) more so than soil types or plant maturity, play a significant role in relative quercitin concentration among onion cultivars. Thus, it is likely that the aspects of the production that are more difficult to control, such as temperature and rainfall, may be the most critical factors for development of flavonoids like quercitin.

Little research has been conducted to determine the effects of temperature and light on most phytonutrients. Because phytonutrients such as the flavonoids and carotenoids are associated with plant pigments, it would not be surprising to learn that factors such as light intensity play a major role in the expression of phytonutrient content in crop plants. The assessment of effects of climatic factors on phytonutrient content necessitates large-scale field trials over years and locations. The dearth of information in this area illustrates the fact that little multi-environment agricultural research has been conducted on phytonutrient content of crop plants. In part because phytonutrients are often challenging to measure, both in terms of labor and technical barriers, the collection of information in this area will require real interdisciplinary collaboration over a period of years between crop production specialists and analytical specialists.

Cultural Practices

Most of the research in this area has shown that fertility practices, such as soil amendments designed to deliver excess or luxuriant levels of nutrients to crop plants, do not have a very large impact on vitamin content of crop plants.[2] Although luxuriant fertility levels can certainly influence mineral content, there is little information (other than work with selenium presented below) as to their impact on phytonutrient content or composition. Furthermore, because soil-plant interaction systems are complex, it has been difficult to assess general soil type effects on phytonutrient content. Despite this lack of information, some observations of soil type effects have been made. Lester[14] reviewed data on cultural practices affecting carotenoid content of muskmelon (cantaloupe) fruit; beta carotene content in muskmelon fruits was higher when plants were grown on silty clay loam soils as compared with sandy loam soils.[15]

Perhaps the best examples of fertility practices with a potential impact on crop plant phytonutrients are in the areas of sulfur and selenium accumulation. Selenium is an essential micronutrient for animals because of its role in protein synthesis. Selenium is obtained via a food web that ultimately rests on its inadvertent uptake by plants, for which it is neither essential nor beneficial. The chemistry and biochemistry of selenium is more easily expressed in terms of the better known sulfur chemistry because of the great similarities in chemical properties they share by virtue of being adjacent Group VIA elements. This leads to significant interactions from both a chemical and biological standpoint. The vegetable *Allium* and *Brassica* species—which include onion, garlic, and broccoli—are the most widely studied sulfur-containing foods because they are relatively ubiquitous in the human diet and appear to possess cancer chemopreventative properties related to their unique organosulfur compounds. Recent investigation has demonstrated the efficacy of selenium-enriched garlic and onion in reducing tumor growth in animals,[16,17] suggesting that organoselenium compounds may offer additional chemopreventative benefits.

The biosynthesis of organosulfur and organoselenium compounds is complicated by competition for sulfate and selenate uptake by plants. Increasing sulfur levels in solution culture was shown to increase the concentration of organosulfur compounds in onion tissue, including those responsible for pungency.[18] Barak and Goldman[19] found that increasing selenium levels in a hydroponic system reduced sulfur uptake in onion but not sulfur concentration. Although high selenium levels increased selenium concentration in plant tissue, the plants had a low selenium content, ostensibly due to selenium-limited growth. Selenium content was not appreciably increased in onion tissues beyond solution culture levels of 2 mg/L. These findings support the conclusion that a competitive relationship exists between these two elements.

Orvis[20] found that increasing sulfate in a nutrient solution to supra-optimal levels (from 2 mM to 12 mM SO_4^{2-}) did not affect onion-induced antiplatelet activity, perhaps because most of the added sulfur was not transported from roots to bulb tissue. It is possible that beyond a fairly low level of sulfate, the sulfur assimilation pathway is saturated and no additional sulfur uptake is realized. Manipulation of fertility for certain elements may therefore not result in modification of phytonutrient content if uptake is already maximized.

Maturity at Time of Harvest and Postharvest Handling

Although harvest maturity is one of the primary factors affecting vitamin content of crop plants,[2] little is known regarding its effects on phytonutrient content. Despite the fact that most fruits and vegetables reach their maximum vitamin content when mature, harvest usually takes place at an earlier stage in order to facilitate handling and transportation of the commodity. For this reason, full vitamin content often is not realized and in some cases can be significantly compromised.[2] This may be true for certain phytonutrients, although little information exists.

Price et al.[21] demonstrated a 50% loss in quercitin monoglucoside—a bioactive flavonoid produced in onion tissues—during the initial curing process following harvest. A common practice in onion production is field curing, whereby freshly harvested onion bulbs are placed in windrows or mesh bags and left in the field to dry for several days. The curing process allows for enough drying to permit proper storage of onion bulbs prior to marketing. Despite this initial loss in quercitin monoglucoside, sixth months of cold storage did not significantly affect quercitin content, suggesting that this phytonutrient is retained for a long period during the standard postharvest treatment for storage onions.

Ferreres et al.[22] showed that the wounding associated with minimal processing of lettuce decreased the anthocyanin content in green and red tissues and increased the phenolic acids and anthocyanins in the midrib. One of the key factors requiring additional study is the functional significance of phytonutrients. If anthocyanins are involved in wound responses, it is likely that newer and more "invasive" techniques such as minimal processing have the potential to modify the phytonutrient content of foods. Ockenden et al.[23] studied the stability of phytate (phytic acid), an antinutritional factor that complexes iron, calcium, magnesium, and zinc. Storage decreased phytate in both barley and common bean; however this decrease was much larger in bean. If standard crop storage practices can reduce "antinutritional" factors such as those that complex essential minerals, these may provide low-cost and low-technology alternatives to modification of phytonutrients in these crops.

The ability of onion extracts to inhibit blood platelets is thought to be mediated by organosulfur compounds produced in onion bulbs. Debaene[24] studied changes in onion-induced human antiplatelet activity from harvest to more than 200 days in postharvest cold storage. During the storage period for bulbs produced over several years in several locations, antiplatelet activity consistently increased from 0 to 90 days postharvest, suggesting that storage may promote the formation of such compounds in the onion bulb. Finally, as storage conditions become increasingly controllable, it may be possible to manipulate these conditions to improve phytonutrient content. Barth and Zhuang[25] found that modified-atmosphere packaging (MAP) retained carotenoids in broccoli florets, but that both vent packaging and automatic misting decreased carotenoid content by up to 57%. MAP was also effective in retaining vitamin C in broccoli but vent packaging and automatic misting decreased vitamin C by up to 46%. Gill et al.[26] found that controlled atmosphere (CA) storage decreased anthocyanin content of internal strawberry tissues but did not affect anthocyanin content in external tissues. Curry[27] reported up to tenfold increases in antioxidant content of "Delicious" and "Granny Smith" apples during the first two months of storage at –1° C and significant decreases during the following 4 months of storage.

What Additional Knowledge Is Needed to Move the Field Forward?

Perhaps one of the primary objectives for moving this field forward is compilation of a list of primary phytonutrients for further research. Since at present it is not known which phytonutrients are most affected by agricultural production, the nutrition and medical research communities will likely make the choice of phytonutrients for further study. Once such a list is compiled, however, it will be important to begin dialogue with agricultural scientists to learn which crop production systems contain these phytonutrients and what kinds of production factors might be most important in producing these crops. To this end, we suggest that model crop plants be chosen for further study to represent a range of plant organs. It may also be useful to make the choice of model crop plants based in part on the types of phytonutrients they contain. Thus, the two groups should work together to choose the best model systems for further study.

At present there are very little data available about the extent to which agricultural production systems can affect phytonutrient content, so additional knowledge will be very useful in shaping future research objectives. Once model systems are chosen, some of the suggested pieces of information to seek include: 1) comparison of relative amounts of phytonutrient synthesis and degradation during crop production and postharvest handling; 2) comparison of food processing methods for phytonutrient retention and degradation; 3) evaluation of correlated response to manipulation of one particular phytonutrient to determine if other traits have been affected positively or negatively; 4) identification and then manipulation of production factors that contribute most significantly to changes in phytonutrient content; 5) evaluation of phytonutrient content throughout plant development, regardless of whether immature or mature organs are the item of commerce; and 6) consideration of the entire food system prior to manipulating agricultural production factors because certain phytonutrient-crop combinations like cereals may be less amenable to manipulation but may represent larger portions of the human diet.

What Are the Technical Barriers to Obtaining This Information?

One key limitation to learning more about these effects is that analytical techniques are not well established for many phytonutrients, and in many cases appropriate standards are not yet available. Because research to elaborate the biologic basis for enhancing the quantity or quality of phytonutrients in the food supply and the agricultural strategies to do so will require an interdisciplinary approach, significant financial and human resource investment will be necessary. For example, agricultural production specialists do not have a long history of collaboration with nutritionists or with analytical biochemists. In order to advance research in this area, it will be of primary importance to identify the primary phytonutrients of biological significance. Moreover, existing and potential sources of such compounds will have to be identified and priorities established for agricultural research. Guidelines should be established to coordinate efforts among research groups and should include identification of key information about all plant material including cultivar name, production area, maturity at harvest, date of harvest, duration between harvest and analysis, and handling conditions. To facilitate these efforts, phytonutrient composition data compiled in numerous research reports could be added to the United States Department of Agriculture (USDA)–Agricultural Research Service (ARS) Nutrient Data Laboratory Database as they become available over time. This will increase the size of the database and identify areas of need for phytonutrient composition.

What Are the Priority Research Topics?

One approach to research that garnered significant support among workshop participants was to choose a set of "model" crops from which to begin a systematic research approach. Because many different phytonutrients are present in a wide range of plant organs, it will be important to select a representative crop plant from among the relevant botanical groups. These would include root crop, leaf crop, immature flower crop, immature fruit crop, mature fruit (climacteric), mature fruit (non-climacteric), seed crop (cereal), seed crop (legume), and seed crop (nut). Model crops from each group should be analyzed for the particular phytonutrient(s) in question under a range of production systems as a basis for predicting the magnitude of improvement that could be achieved, as well as the potential significance of this degree of improvement given dietary habits and food marketing opportunities.

As an example, the workshop was able to identify variables such as varietal selection; preharvest environmental conditions such as temperature and light; cultural practices such as soil fertility; harvest maturity; and storage duration as factors that have the potential to influence the phytonutrient content of crop plants. Putting this list of variables together with the model crops and specific phytonutrients would then serve as a framework from which research could be designed. It would also be important to choose model systems that are amenable to molecular techniques, as these approaches continue to provide some of the clearest insight as to mechanisms of phytonutrient biosynthesis and efficacy.

Particularly interesting research questions might include learning more about the following:

- Which of the identified variables are of the greatest consequence in phytonutrient content?
- Are there similarities and differences in a particular phytonutrient and its content across different crop plants from different plant organs, and do any patterns emerge regarding the accumulation and distribution of this phytonutrient for these crops?

- What is the relationship between phytonutrient content and other consumer-oriented variables such as appearance and flavor during the post-harvest period?

What Is the Role of the Federal Government in Phytonutrient Research in This Area?

Workshop participants identified several areas in which the federal government could facilitate the research process of food crop-based phytonutrients. One area would be the addition of data from the many published reports on phytonutrients that appear in the scientific literature to the USDA-ARS Beltsville Human Nutrition Center's Nutrient Data Laboratory's database. In addition, it would be valuable to include information on variety and, whenever possible, production environment in this database. In this way, the food composition database could serve to identify areas of research need and prevent duplicative efforts.

Many people working in the area of food phytonutrients are interested in nutrition labeling. Crop producers, wholesalers, and retailers have wondered whether they might be able to label and market a product based on its phytonutrient content. In order to facilitate gathering the necessary information to answer such questions, it might be useful to have USDA work closely with the U.S. Food and Drug Administration in developing guidelines for nutrition labeling with respect to phytonutrients. Furthermore, these two organizations might play a role in assisting with the development of standards for testing phytonutrients. With a diverse research community spanning many disciplines, it will be particularly important to develop clear and unambiguous standards for phytonutrient testing in both research laboratories and in the marketplace.

Finally, it is recognized that the federal government may play a significant role in enhancing research on agricultural production systems and phytonutrients by increasing the level of grant support for this area. New granting programs that combine interdisciplinary approaches—where agricultural production is integrated with phytonutrient assessment and perhaps even clinical testing—should be sought to enhance the level of funding for phytonutrient research. In order to establish such programs and provide new ideas in this area, it might be useful to appoint an interdisciplinary task force that also could focus on the development of model systems and model phytonutrients for further research.

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