



Study review

## Variation in content of bioactive components in broccoli

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### Abstract

The discovery of bioactive components in foods is exciting, suggesting the possibility of improved public health through diet. Yet the content of bioactive components in plant food varies, making quality control and intake recommendations problematic. Variation in content of bioactive components in fruits and vegetables depends upon both genetics and environment, including growing conditions, harvest and storage, processing and meal preparation. Cruciferous vegetables, which contain both anticarcinogenic and antioxidant properties, are excellent examples to illustrate the problem in assessing health benefits of foods that vary in content of bioactive components. In broccoli, the content of both glucosinolates and their bioactive hydrolysis products varies with genotype, environment and processing. Antioxidant vitamins and flavonoid content varies also. Here we review the influences of genetics, environment and post-harvest processing on content of bioactive components in broccoli, an area that is presently only partly understood. Reporting a range for bioactive component content can help the public to make informed choices about diet. For the future, research into the mechanisms behind this variation can lead to an understanding of genetic regulation of these variations, resulting in the generation of a consistent supply of nutritionally enhanced plant foods on the market.

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### 1. Introduction

Interest in food composition has broadened past nutrients to include bioactive components that may prevent chronic disease. Some nutrients, such as the antioxidant vitamins carotenoids, tocopherols and ascorbic acid, appear to play a dual role in metabolism. At one dose they are

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required for normal growth and development, and at a higher dose they appear to provide antioxidant protection against chronic diseases, including chronic heart disease, arthritis and cancer (Krinsky et al., 2000). Numerous plant metabolites, including polyphenolics, allyl sulfides and glucosinolates, that have no known role in normal intermediary metabolism, appear to play a role in prevention of chronic disease (Kris-Etherton et al., 2002). The term functional food has been coined to describe foods that, as normal dietary constituents, can provide sufficient amounts of bioactive components to prevent or delay chronic disease and thereby improve the public health (Milner, 2002). In aiming to provide sufficient information for the general public to make informed choices and reap the full benefit of functional foods, it is necessary to know the natural variation in content of antioxidant vitamins and other bioactive food components (Dekker & Verkerk, 2003). Such variation might be regulated genetically, or result from changes in the growing environment or from differences in post-harvest handling, processing, storage or food preparation (Fig. 1).

The USDA food composition tables show that a cup of raw carrots contains 28 000 IU vitamin A. Since the color of carrots varies with variety, and since carotenoids, including  $\beta$ -carotene the precursor to vitamin A, are responsible for much of the color, it follows that the value shown above is a representative value;  $\beta$ -carotene levels have been reported to vary by 2–3-fold across varieties (Heinonen, 1990). Other vegetables, such as corn, have also been shown to vary with variety in their  $\beta$ -carotene content (Kurilich & Juvik, 1999). Furthermore, genetic improvement of food crops is no longer constrained by traditional hybridization techniques. Genetic modification via transformation can produce varieties that fall outside the nutrient range found among traditional varieties. For example, “golden rice” is genetically modified to contain  $\beta$ -carotene, which is not normally present in rice (Ye et al., 2000). While other food plants are being modified to provide elevated  $\beta$ -carotene levels also, this product presently provides approximately one fourth the daily requirement of  $\beta$ -carotene in a serving (Mackey, 2002). These data and many other studies indicate that  $\beta$ -carotene content of vegetables is primarily under genetic control.

In addition to genetic regulation, there are many indications that the environment can play a role in regulating expression of plant metabolites (Falconer & Mackay, 1996). Research is uncovering the fact that not only climactic conditions, but availability of plant nutrients in the soil and extent of pest control can be important factors in determining the extent of nutrient synthesis within a plant. For example, glucosinolate levels in rapeseed (*Brassica napus* L.) have long been known to vary in response to soil sulfur and nitrogen availability, water stress and mammalian

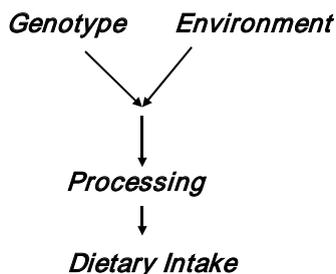


Fig. 1. Genotype, environment and processing affect the content of bioactive components in functional foods, resulting in unpredictable intake from fresh produce.

and arthropod feeding (Zhao, Evans, Bilsborrow, & Syers, 1994; Guistine & Jung, 1985; Champoliver & Merrien, 1996; Smith, Griffiths, & Boag, 1991). It is necessary to consider the relative roles that genetics and environment play on levels of any bioactive component before one can plan strategies for improving the health benefit of functional foods. Only with this knowledge can one determine, for example, whether to develop a new cultivar or to grow an existing variety under specific fertilization, irrigation and/or pest control regimens to stimulate synthesis of bioactive components.

## 2. Content of bioactive antioxidants and glucosinolates in broccoli

Inclusion of cruciferous vegetables in the diet decreases the risk for a number of different cancers (Verhoeven, Verhagen, Goldbohm, van den Brandt, & van Poppel, 1997). In estimating the amount required for this effect, epidemiological studies have revealed that the ingestion of three or more half-cup servings of cruciferous vegetables such as broccoli, Brussels sprouts, or cabbage, per week significantly lowered the risk for prostate cancer by 40% compared to ingestion of one or fewer servings per week (Cohen, Kristal, & Stanford, 2000). Cruciferous vegetables are an excellent dietary source of both antioxidant vitamins and glucosinolates, precursors to a group of isothiocyanates shown to be anticarcinogenic (Jeffery & Jarrell, 2001). Dietary antioxidants, vitamins and non-nutrient components such as flavonoids, are present in crucifers and may decrease the risk for certain cancers (Lindsay & Astley, 2002). Glucosinolates are not directly bioactive, but their hydrolysis products are thought to protect against cancer. For example, sulforaphane, the hydrolysis product of the glucosinolate glucoraphanin, is highly potent at upregulating detoxification enzymes in cell culture and is reported to prevent mammary cancer in rodents (Zhang & Talalay, 1994). Indole-3-carbinol, an hydrolysis product of the glucosinolate glucobrassicin, has proven successful in National Cancer Institute clinical trials against breast cancer (Telang, Katdare, Bradlow, Osborne, & Fishman, 1997) and respiratory papilloma (Rosen, Woodson, Thompson, Hengesteg, & Bradlow, 1998).

Since the content of these broccoli components varies significantly, it may not be easy to advise the general public on how much vegetable to include in their diet. An examination of 50 broccoli varieties showed that  $\beta$ -carotene levels varied over a six-fold (Table 1; Kurilich & Juvik, 1999).  $\alpha$ -Tocopherol and ascorbate also varied, but not in concert with the  $\beta$ -carotene (Table 1; Kurilich et al., 1999). The content of glucosinolates in the same 50 varieties of broccoli varied approximately 20-fold, with the variation being far greater for aliphatic than for indolyl glucosinolates (Table 1; Kushad et al., 1999). Similar glucosinolate variation has been reported for both broccoli and other cruciferous vegetables (Fenwick, Haeney, & Mullin, 1983; Farnham, Stephenson, & Fahey, 2000; Branca, Li, Goyal, & Quiros 2002). Cruciferous vegetable types each exhibit a characteristic profile of glucosinolates, differing substantially, even though they are all members of *Brassica oleracea* (Fenwick et al., 1983; Kushad et al., 1999). All of the different cruciferous vegetable types contain glucobrassicin, and most contain substantial amounts of sinigrin. In broccoli, sinigrin levels are comparatively low and the predominant glucosinolate is glucoraphanin, frequently making up greater than 50% of the total glucosinolates. When crucifers are chopped or crushed, a hydrolyzing enzyme, myrosinase, comes into contact with the

Table 1  
Variation in content of bioactive nutrients among broccoli varieties

Component	Mean value	Range	Reference
$\beta$ -Carotene	0.89 <sup>a</sup>	0.37–2.42	Kurilich et al. (1999)
$\alpha$ -Tocopherol	0.89 <sup>a</sup>	0.46–4.29	Kurilich et al. (1999)
Ascorbate	74.71 <sup>a</sup>	54.0–119.8	Kurilich et al. (1999)
Glucoraphanin	7.1 <sup>b</sup>	0.8–21.7	Kushad et al. (1999)
Aliphatic glucosinolates	10.5 <sup>b</sup>	3.0–31.4	Kushad et al. (1999)
Indolyl glucosinolates	1.9 <sup>b</sup>	0.4–6.2	Kushad et al. (1999)

<sup>a</sup> mg/100 g fresh wt.

<sup>b</sup>  $\mu$ mol/g dry wt.

glucosinolates, releasing glucose and leaving an unstable thiono compound that can rearrange to form the active isothiocyanate or less active nitriles or thiocyanates (Matusheski & Jeffery, 2001).

### 3. Role of genotype and environment in determining broccoli glucosinolate content

The cause for the reported variation in glucosinolate (or other nutrient) content across a number of broccoli varieties might be related to differences in genotype, or might include effects of the environment, changing with the farm soil, season or harvest conditions. Evaluation of a subset of 10 of these varieties grown over 4 seasons allows one to determine the extent to which glucosinolate content varies with genotype and with environment (Brown et al., 2002). Synthesis of indolyl glucosinolates (those derived from tryptophan), such as glucobrassicin the parent to indole-3-carbinol, was regulated very differently to the aliphatic glucosinolates (derived from methionine), such as glucoraphanin the parent to sulforaphane. Synthesis of aliphatic glucosinolates was clearly regulated by genotype (60%), with environmental and environment  $\times$  genotype components exerting smaller effects (5% and 10%, respectively). In contrast, the effects of genotype (12%), environment (33%) and environment  $\times$  genotype (21%) on the content of indolyl glucosinolates appeared reversed, with regulation being primarily environmental (Brown et al., 2002). Thus, one might hypothesize that organic crops, undergoing greater browsing stress than pest-free, highly fertilized conventionally grown crops, may develop greater indolyl glucosinolate levels. To date there are no reports comparing organic and conventionally grown broccolis for their indolyl glucosinolate content, although herbivory has been shown to greatly increase total glucosinolate content in radish (Agrawal, Conner, Johnson, & Wallsgrove, 2002). There is one report evaluating indolyl glucosinolate levels in stored chopped broccoli, showing a significant, 2–4-fold increase in indolyl glucosinolate levels after 48 h (Verkerk, Dekker, & Jongen, 2001). This suggests that not only conditions in the field, but conditions during harvest and handling also may alter indolyl glucosinolate synthesis. These data also allow one to conclude that genetic transformation of broccoli to develop high-glucosinolate hybrids is possible for aliphatic glucosinolates such as glucoraphanin, but to develop a hybrid with a defined content of indolyl glucosinolates, it may be necessary to determine the mechanism whereby the stress of

browsing and handling causes upregulation of indolyl glucosinolates, so that one might successfully manipulate associated genes for regulation.

#### **4. Role of processing in maintaining and optimizing content of bioactive components in a broccoli meal**

There are a number of reports of post-harvest handling and storage resulting in loss of glucosinolates and/or the hydrolysis products (Rangkadilok et al., 2002; Howard, Jeffery, Wallig, & Klein, 1997). Similarly, there are reports of loss of glucosinolates and/or hydrolysis products following various cooking methods (Czyska, Waszczuk, & Kozłowska, 1994; Vallejo, Thomas-Barberan, & Garcia-Viguera, 2002; Howard et al., 1997). Harvesting, handling, storage and cooking methods that provide minimal loss of bioactivity have yet to be determined.

An understanding of glucosinolate hydrolysis appears key to determining post-harvest, processing and food preparation methods for optimizing dietary sulforaphane. Although the major alkyl glucosinolate in broccoli, glucoraphanin, can form bioactive sulforaphane, unfortunately the most common product of hydrolysis is a nitrile, which exhibits orders of magnitude less activity than sulforaphane (Matusheski & Jeffery, 2001). Frequently greater than 80% of the product of glucoraphanin hydrolysis is the nitrile, rather than active sulforaphane. The fraction of glucoraphanin converted to sulforaphane varies with variety, suggesting that the pathway directing product formation may be under genetic regulation. Furthermore, there are a number of studies showing that nitrile formation from glucosinolates in *Crambe abyssinica* varies with the season and other environmental factors (Tookey & Wolff, 1970). Identification of genetic and environmental influences on this pathway remains to be determined. However, it is worth noting that the improved bioactivity reported for a common broccoli cultivar when crossed with a wild *Brassica* species could not be accounted for simply by an increase in glucoraphanin content (Faulkner, Mithen, & Williamson, 1998). The authors hypothesized that the hybrid hydrolyzed a greater proportion of glucoraphanin to sulforaphane, and more recently showed this to be the case (Mithen et al., 2003). Research is underway to identify a myrosinase-associated protein in broccoli that might act as a cofactor in specifying nitrile formation, similar to a protein that has been identified in rape seed (Bernardi, Negri, Ronchi, & Palmieri, 2000; Foo et al., 2000). Breeding for low or null expression of the gene product might be expected to enhance the yield of sulforaphane relative to the nitrile, and thus enhance bioactivity.

#### **5. Future directions for research**

If research is to successfully identify the “recommended dietary intake” of broccoli that may offer protection from cancer, not only does more basic research on cancer prevention needs to be carried out, but the composition of fresh broccoli needs to be better characterized and optimized. Excitingly, the USDA nutrient database has very recently been updated to include food values for flavonoids, a group of non-nutritive bioactive food components (USDA, 2003). It will surely not be long before values for glucosinolates are included. However, if we are to ensure health benefits for people who include cruciferous vegetables in their diets, a vegetable product with standardized

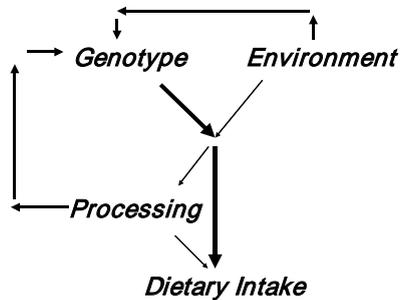


Fig. 2. Understanding the mechanisms by which environment and processing affect content of bioactive components in functional foods can lead to genetic control of these variables, resulting in a consistent supply of bioactive components in foods.

content of glucosinolates is needed. Research is needed to improve our understanding of environmental, post-harvest and processing causes of variability in production of bioactive glucosinolate hydrolysis products. Once the environmental, post-harvest and processing systems controlling production of the bioactive components are understood, these pathways can be brought under genetic control and a high-quality broccoli product with a consistent health-promoting activity can be developed (Fig. 2).

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