Assessing Nutritional Changes in a Vegetable Over Time: Issues and Considerations

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Abstract. For many decades plant breeders have worked to improve vegetable crops for numerous economically important traits, like host plant resistance to disease, yield, and vegetable quality. Most improvements have been made with little knowledge as to how, or if, nutritional or phytonutrient concentrations might also be indirectly altered in the process. There have been some reports suggesting that concentrations of nutrients in vegetables have been reduced over time, possibly related to introductions of new cultivars. However, for most vegetables, current evidence indicating changes in nutrient concentrations, and specifically mineral concentrations, is circumstantial at best. To effectively test whether changes may have occurred over time as new cultivars replace older ones, appropriate field studies must be conducted wherein harvested produce from "old" vs. "new" crop cultivars is analyzed by appropriate methods and compared directly. Numerous considerations and issues such as 1) the set of cultivars to be used in field tests; 2) how nutritional concentration will be expressed; and 3) the evolution, history, and consumption changes of the crop under study must be addressed in making such direct comparisons and interpreting results.

Throughout the 20th century, there were concerted efforts in nearly all agronomic and vegetable crops to improve cultivated varieties for many different traits including crop yield, crop quality, disease resistance, adaptation to new climatic regimes as well as numerous other characters. Relatively few efforts were undertaken to alter the nutritional content of harvested grain or vegetables during this time period, and few, if any, breeding efforts monitored nutritional quality as a component of the broader breeding program. The result is that little is known about changes that may have occurred in the concentrations of various nutrients and whether such changes might be associated with the selection for other characteristics. With this in mind, a few recent research studies have sought to determine whether cultivars of crops, introduced during past decades, might yield harvested products with varying nutritive value and possibly demonstrate significant changes in the nutritional value over the course of long-term breeding.

STUDIES FOCUSED ON AGRONOMIC CROPS

Two primary types of studies evaluating nutritional components have been reported with agronomic crops. The first type involves assaying stored samples from historical plots

for components not previously tested as part of the original studies. We are aware of only one study like this conducted by Fan et al. (2008) in which they assayed the mineral concentration of archived wheat (*Triticum aestivum* L.) samples produced by the Broadbalk Wheat Experiment, first established in 1843 at Rothamsted and run continuously through the present. They found that concentrations of zinc (Zn), iron (Fe), copper (Cu), and magnesium (Mg) remained stable between 1845 and the mid-1960s and then decreased significantly after the 1960s coincident with the introduction of semidwarf, high-yielding cultivars. With Zn, Cu, and Mg, Fan et al. (2008) observed that both grain yield and the harvest index were highly significant variables explaining decreasing mineral concentrations.

A second type of study to discern potential changes in agronomic crops has been done by simultaneously growing cultivars released over a span of time in the same field(s) under the same growing conditions, assaying the respective harvested products. There are more examples of this second type of study documented; however, the total number is still relatively small. Using wheat as the test crop, Garvin et al. (2006) examined Zn, Cu, Fe and selenium (Se) concentrations in harvested grain of 14 cultivars spanning over a century of introduction. They observed significant increases in yield related to year of cultivar release that were also associated with significant decreases in Zn, Fe, and Se concentrations in harvested samples in at least one of two test locations. Monasterio and Graham (2000), also focusing on wheat, evaluated a historical set of cultivars from the CIMMYT breeding program (1950–92) and found a strong linear relationship between year of release and grain yield (e.g., $\approx 1\%$ yield increase per year). They also found a smaller but statistically significant negative trend in Fe, Zn, and phosphorus (P)

concentrations (e.g., $\approx 0.3\%$ per year) related to year of release. Monasterio and Graham (2000) noted that variation around the latter trend was substantial and that only $\approx 35\%$ of variability in mineral concentration was explained by year of release.

Scott et al. (2006) conducted a similar study with maize (*Zea mays* L.) in which they directly compared 45 cultivars that were widely grown from 1920 to 2002; measurements were made for protein, oil, and starch levels. They concluded that the development of modern maize hybrids was associated with reduced protein concentration of harvested grain; however, they observed no changes in protein quality. Scott et al. (2006) also observed that breeding efforts have led to a decline in maize oil concentration over time, whereas starch has increased over the same period. This study and the others described for agronomic crops lend support to the possibility that breeding for increased yield in grain crops over many decades has led to higher-yielding crops with lower nutritive value.

STUDIES FOCUSED ON HORTICULTURAL CROPS

No studies like that of Fan et al. (2008) evaluating archived samples have been reported for horticultural crops. This is likely the result of a lack of any long-term field studies with vegetables like the Broadbalk Wheat Experiments. Such studies with vegetables would also be more difficult to accomplish considering the highly perishable nature of horticultural compared with agronomic products. Until recently, there were also no reports wherein horticultural crop cultivars developed over a span of time were compared directly for nutritional content when grown in the same field trials. To examine the possibility of change over time in a vegetable crop, we (Farnham et al., 2011) undertook a study with

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broccoli (*Brassica oleracea* L. var. *Italica*) wherein we grew 14 cultivars (released over a 50-year period) in two separate field tests and assayed harvested florets for an array of minerals. The two field tests of broccoli were conducted in two fall seasons (2008 and 2009) in South Carolina using current recommended practices of fertilization, irrigation, and pest management for broccoli production. We also used relatively modern population densities in the conduct of these trials.

We observed that the oldest cultivar, Waltham 29, which is a relatively unimproved open-pollinated population, typically exhibited some of the highest concentrations (on a fresh weight basis) of most minerals when compared with all other studied cultivars (all of which were hybrids). Moreover, when 'Waltham 29' was included in the linear correlation analysis of mean mineral concentrations with year of cultivar release, P, S, Mg, manganese, Fe, and Zn concentrations exhibited significant negative correlations with year of release. On the contrary, when 'Waltham 29' was excluded from the regressions, none of the minerals were significantly correlated with release year. Based on these observations, we stated that "if 'Waltham 29' is included, one could conclude that there has been a decline in certain mineral concentrations in broccoli from 1950 to the present." However, we also made the point that "from 1975 to the current time, the data do not indicate any significant linear increases or declines." This latter timeframe is especially important because it represents a span of time in the United States when broccoli consumption increased ≈ 8 -fold, transforming it from a vegetable of minimal importance to one of the top 10 vegetables in the country.

We are unaware of any other studies to date wherein vegetable cultivars released over a span of time were compared directly. There are some additional but different studies (Davis et al., 2004; Mayer, 1997) that have examined historical data in food composition databases [e.g., including and similar to the USDA National Nutrient Database for Standard Reference (U.S. Department of Agriculture, Agricultural Research Service, 2009)]. The USDA National Nutrient Database is the major source of food composition data in the United States, providing a foundation for most food composition databases in the public and private sectors. As information is updated, new versions of the database are released and in many cases concentration values change. In general, use of the database for comparing changes over time is not recommended because many of the recorded values do not represent uniform or systematic analyses, and older data in particular were mostly taken from the literature and listed only as means lacking SE. In some cases, data are inferred from comparable foods, thus having a sample size of zero for the listed nutrient.

Davis et al. (2004) produced an oft-cited study wherein they examined the nutrient content data published in 1950 and 1999 for 13 nutrients in 43 vegetable crops to determine if nutrient levels in test samples

changed as numbers in the database were replaced over time, the assumption being that new values represented more recent samples. These authors compared all vegetables as a group by calculating median and geometric mean R values with $R = (1999 \text{ concentration} / 1950 \text{ concentration})$ to determine any general trend for vegetables as a group. They also evaluated individual vegetables by calculating hypothetical R values with confidence intervals dependent on SE. They concluded that as a group, the 43 vegetable foods showed apparent, statistically reliable declines between 1950 and 1999 ($R < 1$) for protein, calcium (Ca), P, Fe, riboflavin, and ascorbic acid. When examining individual vegetables, Davis et al. (2004) had to use estimates of SE for the 1950 concentration data because these parameters were not included at that time. Depending on whether they used low or high SE estimates for the 1950 means, 43% or 26%, respectively, of apparent R values were found to be "reliably < 1 ." This indicated the possibility of declines in concentrations between 1950 and 1999. Contrary to that result, 16% or 11%, respectively, of the vegetable/nutrient comparisons were found to have R values that were "reliably > 1 ," indicating possible increases between 1950 and 1999. The remaining vegetable crop/nutrient comparisons did not appear to show nutritional differences between the 2 years of sampling.

Comparisons of old and new data in food composition databases are imperfect, because these comparisons use values from the databases in ways for which they were never intended and also because the values often lack important statistical parameters (e.g., SE). Any test that makes comparisons based on data gathered in different decades, using samples from unknown sources or cultivars and sometimes assayed using different methods, can only provide circumstantial evidence, at best, for a particular outcome (i.e., decline or increase). Only side-by-side comparisons of old and new vegetable cultivars grown in the same field trial(s) can adequately address any question of possible changes that may have occurred over time related to the release of new cultivars. When such direct comparisons are made, there are a number of issues that should be considered when interpreting results and determining how they may impact the nutritional value of a commodity for consumers.

ISSUES IN MAKING DIRECT COMPARISONS OF CULTIVARS RELEASED OVER TIME

To test the possibility that nutrient concentration of a vegetable has changed over time, it is important to examine an array of cultivars released over some defined period. For certain vegetable crops, gathering a useful set of new and old cultivars is relatively straightforward. This is especially true for self-pollinated crops like tomato or beans for which good collections of germplasm have been established and maintained with the intrinsic nature (e.g., homozygosity) of new and old strains remaining unaltered as they

are handled in curation. These crops have collections similar to the agronomic crops, like wheat, which have been more readily tested in side-by-side comparisons (Garvin et al., 2006; Monasterio and Graham, 2000).

With crops like broccoli and sweet corn that have been sold as hybrids for many decades, the varietal makeup is a trade secret and the seed is only available as long as a commercial company continues to generate the F_1 combination. Old hybrids are often replaced by new ones and at some point companies stop regenerating seed for them. Old seed may be available for many years after such a hybrid is no longer sold, but eventually the seed loses germinability. If saved as part of a germplasm collection, hybrids cannot be regenerated like inbreds or open-pollinated populations. Thus, it is not uncommon for seed of old hybrids to become unviable and then lost and, ultimately, unavailable for testing. In our broccoli experiments (Farnham et al., 2011), we were able to obtain seed of one hybrid developed in the 1970s and a few released in the early 1980s, but we were unable to obtain seed of any of the earliest hybrids developed in the 1960s, some of which were very prominent as some of the first hybrids. Some older open-pollinated populations of broccoli like 'Waltham 29' and 'DeCicco' can still be found in some catalogs, but these old cultivars are never used in commercial production anymore, and they have been perpetuated simply because it can be done inexpensively and easily. The availability of seed for broccoli hybrids limited the time period that we (Farnham et al., 2011) were able to investigate for potential changes in nutritive value, and this in turn affected the conclusions we were able to make from our results.

At the outset, it is important to establish how nutritive value will be reported. Although many vegetables are consumed in a fresh or fresh frozen state, it is more common to analyze heat-dried tissue samples for minerals or lyophilized tissue samples for various organic phytonutrients. Moisture content may not vary significantly for the same tissues from different cultivars of certain crops (Farnham et al., 2011; Farnham and Kopsell, 2009), but in other cases, significant variation in moisture content will occur (Farnham et al., 2012). In these latter cases, it will be important to take into account the variable moisture concentrations and to convert concentrations on a dry mass basis to a fresh weight basis.

Depending on how the nutrient value is expressed, the comparison of cultivars over time may yield different outcomes. This is well illustrated by the data set we presented in Farnham et al. (2011), wherein we examined results on a fresh weight basis. In that study, we found that six of 11 minerals assayed were negatively and significantly correlated with release year of 14 cultivars, including 'Waltham 29', which was released in 1950 (Table 1, column 1). When data from that same study, expressed on a dry mass basis, are correlated with release year, P exhibits a significant negative relationship with release year, sodium exhibits a significant

Table 1. Correlation coefficients (*r*) resulting from linear correlation analysis of the mean concentrations of 11 separate minerals in 14 broccoli cultivars (released between 1950 and 2004) expressed on a fresh weight (fm), dry mass (dm), and per-head basis with year of release.^z

	With year of release (14 cultivars) μg or mg/100 g fm	With year of release (14 cultivars) μg or mg·g ⁻¹ dm	With year of release (14 cultivars) μg or mg/head
	Coefficient of linear correlation 'r'		
Phosphorus	-0.75**	-0.61*	0.69**
Potassium	-0.38 NS	0.38 NS	0.68**
Sodium	0.30 NS	0.52*	0.67**
Calcium	-0.20 NS	-0.06 NS	0.62*
Sulfur	-0.69**	-0.44 NS	0.66**
Magnesium	-0.64*	-0.50 NS	0.76**
Manganese	-0.58*	-0.28 NS	0.66*
Molybdenum	-0.51 NS	-0.21 NS	0.59*
Copper	-0.44 NS	-0.03 NS	0.81*
Iron	-0.60*	-0.38 NS	0.68**
Zinc	-0.65*	-0.45 NS	0.70**

^zStatistical significance of correlation coefficient is indicated below the coefficient.

*, **Coefficient significant at *P* = 0.05 and *P* = 0.01, respectively. NS indicates coefficient is nonsignificant.

positive relationship with year, but the remaining minerals exhibit non-significant correlations (Table 1, column 2). Thus, when examined on a dry mass basis the nutritional value of new cultivars appears to have changed little over time. It is also possible to consider mineral content of broccoli on a per head basis, which is very relevant because broccoli is now often priced and sold by the head. When examined on this basis, all mineral concentrations in sampled heads are significantly and positively correlated with release year (Table 1, column 3). This adds a new dimension to the question of change over time, clearly demonstrating that significantly more mineral content is being supplied to consumers in every head of broccoli they consume today than was previously supplied in the past. Again, all of this serves to illustrate that any possible change in concentration or content of nutritional components over time must consider the way that these criteria are being expressed.

Any given vegetable crop to be studied must also be viewed in light of its respective crop evolution and history. In general, vegetable breeders have worked to improve their respective crops with a paramount focus on quality and much less effort on yield, especially compared with the focus of agronomic crop breeders (Luby and Shaw, 2009). Janick (2005) argued that agronomic crop improvement in the 20th century was grower-directed, relying on improving yield by increasing yield stability under high population density. Janick (2005) contrasted agronomic crops with horticultural crops for which breeding objectives have and continue to be consumer-directed because consumers make individual decisions about which vegetables to consume, choosing among products of different cultivars of a crop and also among products of different crops. Most such consumer decisions made in the produce section of a store are largely based on fruit and vegetable quality. Janick's premise is supported by the fact that the intrinsic nature and quality of a maize or wheat kernel changed little during the 20th century, when yield of these grain crops dramatically increased. On the contrary, harvested vegetables from modern cultivars are often markedly different from those harvested

from predecessors produced just a few decades ago. Janick (2005) cited several examples, including modern seedless watermelon cultivars and sugary-podded pea cultivars that produce a harvested product nearly unrecognizable from their predecessors.

Broccoli cultivars introduced during the 20th century also illustrate the unique nature of vegetable improvement; modern broccoli cultivars produce a vegetable that is dramatically different from that consumed in the United States 40 to 50 years ago. Until the 1960s, all broccoli cultivars grown in the United States were open-pollinated populations that were highly heterogeneous. Many improved populations were released in the 1940s and 1950s (Farnham, 1999), but these cultivars were grown at a time when broccoli consumption was very low (e.g., less than 1 pound per person per year) in the United States. Many researchers began to investigate hybrid broccoli in the 1960s when heterosis was documented in the crop (Legg and Souther, 1968) and the use of self-incompatibility was recognized as a means to hybridize inbreds (Pearson, 1932). Once introduced commercially, the adoption of hybrids was relatively rapid for this vegetable with nearly all commercial producers using hybrids exclusively by the mid-1970s. Hybrid broccoli heads were dramatically different from the heads

of open-pollinated cultivars, exhibiting greater density and mass and smaller, more compact flower buds (also called beads). Hybrid broccoli heads were much better suited for longer-term storage and long-distance shipping. Interestingly, consumption of this crop began to increase dramatically after the adoption of hybrids in the 1960s and 1970s and this increasing trend has continued into the new century (Fig. 1). We postulate that the introduction of hybrid broccoli heads that were amenable to long-distance shipping allowed this vegetable to reach all points of the U.S. produce market, and consumers increasingly made a deliberate choice that this was a quality product for the dinner table. Numerous reports (Fahey et al., 1997; Zhang et al., 1992) in the 1990s and later regarding the nutritional and chemoprotective attributes of broccoli likely helped to sustain increased consumption of this vegetable, but the introduction of hybrid broccoli was likely a watershed moment for this crop.

Any possible changes in nutrient concentration of a vegetable over time need to be considered in light of any changes in consumption that may have occurred for that vegetable. The increasingly popular broccoli crop again illustrates this point well. As indicated earlier, we reported in our previous work (Farnham et al., 2011) that if 'Waltham

Fig. 1. U.S. per-capita consumption (pounds per person per year) of broccoli from 1950 to 2012. Data were compiled from the USDA, National Agricultural Statistics Service Annual Reports.

Table 2. Estimation of U.S. per-capita intake of zinc (Zn), iron (Fe), calcium (Ca), and potassium (K) from broccoli in 1962 and 2012 based on reported USDA consumption data for those 2 years and concentrations measured by Farnham et al. (2011) in florets of an important cultivar grown in 1962 ('Waltham 29') and an important cultivar (Green Magic) widely grown at present.

Mineral	U.S. per-capita amount derived from broccoli 1962 ² (amount per person/yr)	U.S. per-capita amount derived from broccoli in 2012 ³ (amount per person/yr)
Zn	4.1 mg	28.8 mg
Fe	3.7 mg	27.8 mg
Ca	154 mg	1,530 mg
K	1,600 mg	14,510 mg

²The per-capita consumption of each mineral in 1962 was estimated by multiplying reported per-capita consumption of broccoli in 1962 (U.S. Department of Agriculture, National Agricultural Statistics Service, 1963, 1978, 1984, 1994, 2005, 2012) with the concentration of each mineral in 'Waltham 29' reported by Farnham et al. (2011).

³The per-capita consumption of each mineral in 2012 was estimated by multiplying reported per-capita consumption of broccoli in 2012 (U.S. Department of Agriculture, National Agricultural Statistics Service, 1963, 1978, 1984, 1994, 2005, 2012) with the concentration of each mineral in 'Green Magic' reported by Farnham et al. (2011).

29' were included as part of the comparison of cultivars, one could conclude that there has been a decline in certain mineral concentrations in broccoli from 1950 to the present. However, if examined in light of a more than 8-fold increase in consumption that has occurred since the time 'Waltham 29' was grown commercially, any potential concentration differences are relatively unimportant between 1950 and the present day, when calculating the annual mineral intake derived by consumers from consumption of this vegetable (Table 2). Indeed, using USDA per-capita consumption data (Fig. 1) and mineral concentrations measured in our previous work (Farnham et al., 2011), we calculate that U.S. consumers currently derive ≈ 28.8 mg Zn, 27.8 mg Fe, 1,530 mg Ca, and 14,510 mg of potassium (K) from broccoli annually (represented by the current popular hybrid 'Green Magic') compared with only 4.1 mg Zn, 3.7 mg Fe, 154 mg Ca, and 1600 mg K from broccoli (represented by 'Waltham 29') in 1962 (Table 2). Any changes in concentration have had minimal impact on consumer nutrition in light of the dramatically increased consumption of this vegetable. This observation shows the significant impact that can be garnered if consumers simply increase their consumption of a relatively nutritious vegetable.

Although these annual intake differences are noteworthy, they should also be viewed with regard to human nutrient requirements on a daily basis. The Institute of Medicine of the National Academies (2006) provides Recommended Dietary Allowance (RDA) and Adequate Intake (AI) values for individuals of different life stage and gender groups. The RDAs and AIs represent average intakes for each mineral that would be expected to meet or exceed the daily needs of most healthy individuals in that group. For instance, the Zn, Fe, Ca, and K recommendations for a 14- to 18-year-old teenage girl are RDAs of 9 and 11 mg·d⁻¹ for Zn and Fe, respectively, and AIs of 1300 and 4700 mg·d⁻¹ for Ca and K, respectively. Thus, for a teenage girl today, who is consuming the average annual per-capita amount of broccoli (≈ 9 lbs); she would only obtain 1 to 3 day's

worth of these minerals over the course of a year. This again reinforces the point that elevated consumption patterns for a vegetable are much more significant than minor differences in cultivar mineral concentrations.

CONCLUSIONS

Vegetable breeders have worked for decades to improve vegetable crops for numerous economic and quality traits. Vegetable breeding has always been a balancing act in which breeders have tried to combine attributes like host plant resistance to disease with yield and vegetable quality, all at the same time. Most improvements made in the modern era were accomplished with little knowledge as to how, or if, nutritional or phytonutrient concentrations might also be indirectly altered in the process. However, any evidence indicating changes in nutrient concentrations, and specifically mineral concentrations, of most vegetables is circumstantial at best.

Studies that directly compare nutritional concentrations in harvested vegetables from cultivars released over time are retrospective studies, trying to assess changes that may or may not have occurred as a consequence of selecting for other traits. As a result of the increased awareness of phytonutrients as health-promoting constituents and the added value that such constituents may confer to a crop, future vegetable breeders are more likely to assess nutritional components as another trait to consider in the process of developing new cultivars. Indeed, several modern examples like 'Beneforte' broccoli with high glucoraphanin (Traka et al., 2013), 'Tasti-Lee' tomato with high lycopene (Scott et al., 2008), other tomatoes with high anthocyanin concentration (Mes et al., 2008), and other examples like these underlie the current existence of specific programs led by vegetable breeders to select for enhanced levels of health-promoting compounds. These new breeding efforts are forward-looking in approach and could likely result in increased consumption of targeted vegetables. As illustrated by broccoli, any efforts that result in greater consumption of a vegetable may do

more to increase nutritional intake from vegetables than most changes in nutrient concentrations that might be made through breeding.

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