Testing the Nutrient Composition of Perennial Vegetables in Denmark, Sweden, and the United States



Eric Toensmeier, Perennial Agriculture Institute, USA

Karoline Nolsø Aaen & Tycho Holcomb, Myrrhis Forest Garden and Agroforestry Project, Rønde, Denmark

Aaron Guman & Graham Unangst-Rufenacht, Robinson Hill Beef's Perennial Vegetable Research Projects, Vermont, USA

Eva Johansson, Skillebyholm Research, Järna, Sweden

Aaron Parker, Edgewood Nursery, Falmouth, ME, USA

Annevi Sjöberg, Mångfaldsträdgården, Stjärnsund, Sweden

Rebecka Törnqvist, Puttmyra Forest Garden, Stjärnsund, Sweden

This publication is a citizen science collaboration between Edgewood Nursery (USA), Mångfaldsträdgården (Sweden), Myrrhis Forest Garden and Agroforestry Project (Denmark), the Perennial Agriculture Institute (USA), Puttmyra Forest Garden (Sweden), Robinson Hill Beef's Perennial Vegetable Research Projects (USA), and Skillebyholm Research (Sweden). It was intended for peer review but the number of samples of each species was not sufficient for peer-reviewed nutrition journals. Nonetheless we believe it is a valuable contribution to understanding the nutritional benefits of perennial vegetables.

Thanks to crowdsourced experiment.com, where more than 100 backers supported the Swedish research. The testing performed in the US, with extra funds for testing of *H. sieboldiana* and *H. tamnoides* in Sweden, was provided by USDA SARE and USDA Specialty Crop Block Grants. The testing performed by Myrrhis Forest Garden and Agroforestry Project was funded by Fonden for Økologisk Landbrug and Dyrenes Beskyttelse.

Keywords: perennial, vegetable, nutrition.

Abstract. Fourteen species of perennial vegetables were tested for levels of nine nutrients needed to address common deficiencies. Composition was compared to a set of widely grown and marketed reference vegetables. For eight of the nine nutrients, at least one species had higher content than any reference vegetable, and in the case of Zn, ten species did so. Ten species had more than one nutrient concentration higher than the reference vegetables, with the leaves of *Urtica dioica, Morus alba x M. rubra, Chenopodium bonus-henricus,* and *Rumex patientia* especially nutrient-rich. Based on their high content of key nutrients, perennial vegetables are worthy additions to gardens and cuisine, and warrant further exploration for commercial production. Perennial and biennial kale varieties were compared, and despite some variation were found to have identical nutrient concentration rank profiles.

1. Introduction

An estimated 33-56% of cultivated vegetable species are perennial, totaling over 600 species. They occupy only a small fraction of the world's vegetable production area, but offer an option to diversify agriculture. When compared to the world's most widely grown vegetables, many perennial vegetables are much higher in the nutrients needed to address the nutrient deficiencies that impact over 2 billion people.¹

Perennialization of agriculture offers many benefits to soil health, carbon sequestration, water quality, and other agroecosystem functions.² Despite this, perennial vegetables as a class have received little attention until recently, even though individual species like globe artichoke (*Cynara scolymus*) and asparagus (*Asparagus officinalis*) are well-researched.³ A 2020 article peer-reviewed by Toensmeier, Mamta, and Ferguson synthesized data on the nutritional content of 240 species of perennial vegetables. Many gaps were identified - for some species only partial data, or no data at all, were available.

This study reports the results of nutritional composition testing of fourteen perennial vegetable species from six sites in three countries. These species are perennial kale (*Brassica oleracea*), Turkish rocket (*Bunias orientalis*), good King Henry (*Chenopodium bonus-henricus*), sea kale (*Crambe maritima*), spinach vine (*Hablitzia tamnoides*), hosta "Big Daddy" (*Hosta sieboldiana*), orpine (*Hylotelephium telephium*), mulberry "Illinois everbearing" (*Morus alba x M. rubra*), sweet cicely (*Myrrhis odorata*), sochan (*Rudbeckia laciniata*), patience dock (*Rumex patientia*), scorzonera (*Scorzonera hispanica*), littleleaf linden (*Tilia cordata*), and stinging nettle (*Urtica dioica*). The focus of this study is on the nutrients that are needed to address deficiencies, though other nutrients are of course also important.

¹ Toensmeier, E, Ferguson, R, and Mehra, M. "Perennial vegetables: A neglected resource for biodiversity, carbon sequestration, and nutrition." *Plos one* 15, no. 7 (2020): e0234611.

² Pimental, D, Cerasale, D, Stanley, R, Perlman, R, Newman, E, Brent, L, Mullan, A, and Chang, D (2012) "Annual versus perennial grain production". *Agriculture, Ecosystems, and Environment* 161 (2012):1-9.

³ Toensmeier, E, Ferguson, R, and Mehra, M. "Perennial vegetables: A neglected resource for biodiversity, carbon sequestration, and nutrition." *Plos one* 15, no. 7 (2020): e0234611.

2. Materials and Methods

2.1 Sites

This study reports on research conducted in three countries: Denmark, Sweden, and the United States. Samples were collected from 5 sites. Several sites use the term "forest garden" to refer to themselves. A forest garden is a cold-climate analog of the tropical homegarden, a home-scale multistrata agroforestry system.⁴

East Hill Tree Farm in Plainfield, Vermont, USA, is a nursery for fruit trees and berry plants hardy to northern Vermont which provides planting, design and pruning—as well as research, propagation, and production of these plants. The farm is located at 472 meters above sea level. The plot where samples were taken from sits on moderately-drained Dummarston silt loam, a former dairy farm and hay field, and was newly worked in 2018 with tillage, mineral amendments, compost and wood chip.

Edgewood Nursery in Falmouth, Maine, USA, is a nursery that produces perennial vegetables, minor fruit crops and ecosystem support plants. The forest garden areas where samples were taken had very weak gravelly soil at the time of first planting 10+ years ago. Current soil conditions vary from patch to patch depending on what materials were used to amend soils over the past decade, but most areas are rich in most nutrients and soil carbon. Combined testing from 2019 shows a pH of 6.3, OM of 6.2%. Zinc was measured at 4.1ppm, which may be relevant to high zinc levels in sampled leaves.

The Farm Between in Jeffersonville, Vermont, USA, is a perennial production farm and nursery, managed with an ecological mindset for the last nearly 30 years. The sea kale bed where broccolis were taken for testing was established as a no-till bed in 2018 and sits in the midst of a perennial polyculture plot including adjacent rows of woody perennial shrubs. Prior to compost, lime and mineral amendments at the time of establishment in 2018, had an organic matter of 6.1%, had a cation exchange of 9.65, and a pH of 7.2, and were sampled at a depth of 6".

Myrrhis Permakulturhaven is located in Feldballe, Jutland, Denmark, approximately USDA zone 7. The 10,000m² site receives 650 mm of precipitation per year, spread throughout the whole year. The climate is classified as humid, semi-continental. The soil type is sandy loam with pH varying between 4.8 and 6.

Mångfaldsträdgården (The Diversity Garden) is in Stjärnsund, Sweden (60.43461, 16.20565). The Diversity Garden is a public forest garden of about 2700 m². The perennial vegetables that were analyzed were planted together in the same plot in spring 2018. In a previous study that was conducted in autumn 2018, soil samples (at a depth of 0-30 cm) for nutrient levels, pH and heavy

⁴ Toensmeier, E (2016) The Carbon Farming Solution

metal levels were measured at the study site⁵. The soil has a clay content of 16% and pH 6.8. The soil is fairly high in nutrient levels, e.g., nitrogen levels (total-N) of 22 mg/kg and phosphorus levels of 260 mg/kg. The carbon content is 16%. The carbon content is much lower, 2.9%, in the soil at a depth of 30-60 cm.

Puttmyra Forest Garden in Stjärnsund, Sweden, is a 10 year old private forest garden of about $15,000 \text{ m}^2$. The analyzed leaves of *M. alba* 'Illinois Everbearing' were grown in an unheated greenhouse in the forest garden. Soil samples were analyzed in autumn 2018 at a depth of 0-30 cm. The soil has a clay content of 26% and pH 6.0. The soil has nitrogen levels (total-N) of 19 mg/kg and phosphorus levels of 160 mg/kg.

2.2 Selection of species

This study uses the following definition of perennial vegetables: living for three years or more with more than one year of harvest; including both woody and herbaceous species, terrestrial and aquatic; with edible leaves or other vegetative parts and/or reproductive structures (flower buds, flowers, fruits, and unripe seeds) consumed in salads and main dishes (as opposed to sweet or tart dessert fruits); and excluding root crops and starchy staple fruits like bananas and breadfruit.⁶

Data for many species was unavailable, or only available for a few nutrients.⁷ Species for this study were chosen for several reasons: to test species currently being marketed; to test species with commercial potential; to test species identified in gardens as having excellent flavor; and/or to fill gaps identified in the article by Toensmeier, Mehra and Ferguson.

Both perennial and biennial kales were sampled by Myrrhis Permakulturhaven to compare their nutritional profiles.

2.3 Sampling

Species were harvested at the ideal stage for home consumption or marketing as identified by the growers. The time of harvest of the plants was at the peak of ripening. The time of harvest varied between plants and was decided based on when the growers considered it to be optimal. The vegetables were picked, weighed for fresh weight, and photo-documented. The whole leaves were then vacuum packed and kept chilled during the transport to the accredited laboratories. Swedish standard analysis methods for minerals and vitamins in food were used. Two exceptions were the Swedish leaf harvest of *H. tamnoides* and *M. alba,* which were later in the season than ideal, as new time-limited funding appeared for these two extra species. These Swedish *H. tamnoides* were from younger plants than ideal as well.

⁵ Törnqvist, R. (2019) Förstudie: Fleråriga grönsakers tillväxt, näringsinnehåll och upptag av tungmetaller

⁶ Toensmeier, E, Ferguson, R, and Mehra, M. "Perennial vegetables: A neglected resource for biodiversity, carbon sequestration, and nutrition." *Plos one* 15, no. 7 (2020): e0234611.

⁷ Toensmeier, E, Ferguson, R, and Mehra, M. "Perennial vegetables: A neglected resource for biodiversity, carbon sequestration, and nutrition." *Plos one* 15, no. 7 (2020): e0234611.

2.4 Testing

The Swedish and Danish samples were harvested, kept away from sunlight in plastic bags and slightly vacuum packed in cooler bags. They were driven to the laboratory within a few hours where they were kept at 5°C. in the case of Denmark and Sweden, and shipped to testing facilities. The US samples were harvested, vacuum packed, kept away from sunlight and shipped overnight with cooler packs, and kept under refrigeration in the lab until sampled. *B. oleracea, B. orientalis, C. bonus-henricus, H. tamnoides* (#2), *H. telephium, M. odorata, R. laciniata, R. patientia,* and *S. hispanica* (#2) were tested at Eurofins Steins Laboratorium in Vejen, Denmark. *H. sieboldiana, M. alba, S. hispanica* (#1), *T. cordata,* and *U. dioica* were tested at ALS Scandinavia, Lulieå, Sweden and ALS Laboratory Group, Prague, Czech Republic. *M. alba* and *H. tamnoides* (#1) were tested at EMSL Analytical in New Jersey, United States.

The ALS Laboratory Group laboratory uses the following methods. Determination of minerals, analysis with ICP-SFMS was according to ISO (2005) and EPA (1994). Dissolution took place in a microwave oven in closed Teflon containers with HNO3/H2O2/HF. Determination of vitamin C (ascorbic acid) and ascorbyl-6-palmitate by liquid chromatography according to CEN (2003). Determination of beta-carotene by HPLC with UV detection according to SOP: AM / V / 906. The reporting limit is 0.05 mg/kg and the measurement range is 0.05-3000 mg/kg. Determination of water content at 105°C by gravimetric method. Determination of folate, determination of the sum of folic acid + folate according to BVL (2006).

The Eurofins Food and Feed Testing laboratory uses the following methods. Determination of calcium, magnesium, iron and zinc, method reference in accordance with ISO (2016). Vitamin A samples are extracted one time in a mixture of ethanol and hexane and then two times in hexane. The separation is performed by RP HPLC on a C30 column and detected by a diode array detector (DAD) at 450 nm. For quantification a 3-point calibration curve is used. β-carotene is calculated as the sum of all-trans β -carotene, 9Z β -carotene and 13Z β -carotene, 9Z and 13Z β-carotene are calculated using a response factor. Folate samples treated with amylase. Folic acid is extracted, SPE cleaned-up and quantified in LC/MS. For vitamin C, the sample is extracted in an aqueous solution containing trichloroacetic acid (TCA) and the antioxidant tris(2carboxyethyl)phosphine (TCEP). Vitamin C is degraded in a basic environment, which the presence of TCA prevents. TCA contributes additionally to trap dissolved proteins. TCEP reduces dehydroascorbic acid (DHAA) to ascorbic acid (AA), so DHAA is also determined and also prevents the degradation of vitamin C. The final extract is analyzed by RP-HPLC with UV detection at 265 nm. The isoascorbic acid will be seen as a peak after the ascorbic acid peak. Vitamin E is measured as the content of dl-alpha-tocopherol. Result is expressed in mg dl-alpha-tocopherol /kg.

2.5 Analysis

This study classifies perennial vegetable nutrient concentrations using a scale developed by Toensmeier, Mehra and Ferguson. That study compared perennial vegetables to a group of "reference vegetables" which are globally marketed. The reference vegetable species are okra (Abelmoschus esculentus), leek (Allium ampeloprasum), scallion (Allium fistulosum), asparagus (Asparagus officinalis), broccoli (Brassica oleracea Italica group), cabbage (B. oleracea Capitata group), cauliflower (B. oleracea Botrytis group), kale and collard greens (B. oleracea Acephala group), pak choi and Chinese cabbage (*Brassica rapa*), pepper (*Capsicum annuum*), cucumber (*Cucumis sativus*), summer squash and zucchini (*Cucurbita spp.*), winter squash (Cucurbita spp.), globe artichoke (Cynara scolymus), lettuce (Lactuca sativa), avocado (Persea americana), green bean (Phaseolus vulgaris), pea (Pisum sativum), tomato (Solanum lvcopersicum), eggplant (Solanum melongena), spinach (Spinacia oleracea), and sweet corn (Zea mays). For each nutrient a scale is used. The reported range for reference vegetables was broken into thirds: "low" for the lowest third, "medium" for the intermediate concentrations, and "high" for the highest. In addition a "very low" category includes results lower than the lowest reported for reference vegetables, "very high" for those higher than the highest reference vegetables, and "extremely high" for those more than twice as high as the highest reference vegetables. See Table 2.1. All nutrient concentrations are presented on a fresh weight basis.

	Fiber	Fiber Ca		Mg	Zn	Α	folate	С	Е	
	%	mg/100g	mg/100g	mg/100g	mg/100g	mgRAE	mcg/100g	mg/100g	mg/100g	
Very low	0.00-0.39	0.00-11.94	0.00-0.46	0.00-11.24	0.00-0.15	0.00	0.00-13.49	0.00-5.64	0.00-004	
Low	0.40-1.45	11.85-86.71	0.47-1.01	11.25-35.75	0.16-0.29	0.00-0.18	13.50-73.07	5.65-43.33	0.05-0.73	
Medium	1.46-2.50	86.72-161.57	1.02-1.55	35.76-60.26	0.30-0.42	0.19-0.37	73.08-132.63	43.43-79.01	0.73-1.42	
High	2.51-3.85	161.58-238.7 0	1.56-2.11	60.27-85.50	0.43-0.56	0.38-0.55	132.64-194.00	79.02-116.80	1.43-2.54	
Very high	3.86-7.15	238.71-477.4 0	2.12-4.21	85.51-171.00	0.57-1.12	0.56-1.12	194.01-388.00	116.81-233.59	2.55-5.08	
Extremely high	7.16+	477.41+	4.22+	171.01+	1.13+	1.13+	388.01+	233.60+	5.09+	

Table 2.1 Nutrient concentration classes based on reference crop nutrient levels
From Toensmeier et al (2020). All are on a fresh weight basis.

3. Results

Table 3.1 presents the nutrient concentration data for the 14 species tested, notes the part tested and the location of the sampling site. All data are presented on a fresh weight basis. Not all species were tested for all nutrients. Additional nutrients, tested for some species, are shown in the Supplemental Materials.

Latin Name	Edible part tested	Sample Location	Fiber %	Ca mg/100 g	Fe mg/100 g	Mg mg/100 g	Zn mg/100g	Vitamin A mg RAE	Folate mcg/10 0g	Vitamin C mg/100g	Vitamin E g/100g
Brassica oleracea	leaf	Djursland, Denmark	4.00	220.00	1.10	31.00	0.63	0.31	103.00	66.30	0.31
Bunias orientalis	leaf	Djursland, Denmark	2.70	170.00	3.70	37.00	0.97	0.20	86.50	83.20	1.26
Chenopodium bonus-henricus	leaf	Djursland, Denmark	4.40	110.00	1.40	57.00	0.71	0.58	239.00	40.90	1.59
Crambe maritima #1	inflorescence	Vermont, USA	low	96.20	low	31.20	low	0.35	60.20	24.40	
Crambe maritima #2	inflorescence	Vermont, USA	3.31	125.00	0.81	21.20	0.38	1.22	99.10	55.70	
Hablitzia tamnoides #1	leaf	Stjärnsund, Sweden		66.00	0.59	71.00	0.23	0.25	<5.00	33.90	
Hablitzia tamnoides #2	leaf	Maine, USA	2.74	79.00	1.46	127.00	1.90	0.00	41.20	2.31	
Hosta spp.	shoot	Stjärnsund, Sweden		39.70	0.66	21.90	0.66	0.03	30.20	191.00	
Hylotelephium telephium	leaf	Djursland, Denmark	1.60	84.00	<1.00	14.00	<0.50	0.14	61.90	47.40	1.12
Morus alba	leaf	Stjärnsund, Sweden		780.00	7.70	130.00	1.10	0.27	<5.00	8.13	
Myrrhis odorata	leaf	Djursland, Denmark	4.30	160.00	4.30	50.00	0.90	0.20	130.00	44.30	0.85
Rudbeckia laciniata	leaf	Djursland, Denmark	3.10	160.00	2.30	40.00	1.10	0.30	96.70	33.50	1.02
Rumex patiencia	lead	Djursland, Denmark	3.20	53.00	2.30	33.00	<0.50	0.29	74.30	94.40	1.53
Scorzonera hispanica	leaf	Stjärnsund, Sweden		66.90	0.38	16.3	0.28	0.15	62.40	<0.80	
Scorzonera hispanica #2	leaf	Djursland, Denmark	4.10	110.00	1.20	33.00	0.73	0.37	89.30	31.90	0.58
Tilia cordata	leaf	Stjärnsund, Sweden		83.00	1.28	44.10	0.67	0.15	33.40	<0.80	
Urtica dioica	shoot	Stjärnsund, Sweden		357.00	1.77	56.20	0.73	0.05	157.00	<0.80	

Table 3.1: Perennial vegetable nutrient concentrations from 5 sites. All are per 100g fresh weight.

Table 3.2 shows which species ranked high to extremely high in each nutrient. Some results were provided as <X, which in some cases did not permit assigning a concentration level for that nutrient.

	Fiber	Calcium	Iron	Magnesium	Zinc	Vitamin A	Folate	Vitamin C	Vitamin E
Extremely high		Morus alba x M. rubra	Morus alba x M. rubra, Myrrhis odorata		Hablitzia tamnoides (#2)	Crambe maritima, Scorzonera hispanica (#1)			
Very high	Brassica oleracea, Chenopodium bonus-henricus, Myrrhis odorata, Scorzonera hispanica (#2)	Urtica dioica	Bunias orientalis, Rudbeckia laciniata, Rumex patiencia	Hablitzia tamnoides,(#2) Morus alba x M. rubra	Brassica oleracea, Bunias orientalis, Chenopodium bonus-hemricus, Hosta sieboldiana, Morus alba x M. rubra, Myrrhis odorata, Rudbeckia laciniata, Scorzonera hispanica (#2, Tilia cordata	Chenopodium bonus-henricus	Chenopodium bonus-henricus	Hosta sieboldiana	
High	Bunias orientalis, Crambe maritima, Hablitzia tamnoides (#2), Rudbeckia laciniata, Rumex patiencia	Brassica oleracea, Bunias orientalis	Urtica dioica	Hablitzia tamnoides (#1)			Urtica dioica	Bunias orientalis, Rumex patiencia	Chenopodium bonus-henricus, Rumex patiencia

 Table 3.2 Species ranked high to extremely high in concentration for each nutrient

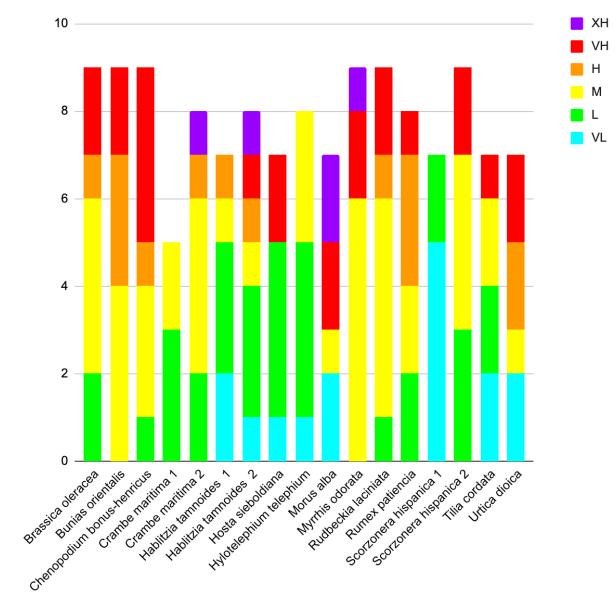
Figure 3.1 shows the results by nutrient concentration rank for each species.

Species with over 50% of tested nutrients with levels high or higher include: *Urtica dioica* and *Morus alba x M. rubra* (57%); *Bunias orientalis* and *Chenopodium bonus-henricus* (56%); and *Rumex patiencia* (50%).

Species with 25-39% of tested with nutrients testing high or higher are: *Brassica oleracea*, *Hablitzia tamnoides* #2, *Myrrhis odorata* and *Rudbeckia laciniata* (33%); *Hosta sieboldiana* (29%); and *Crambe maritima* #2 (25%).

Species with less than 25% of levels high or higher are: *Scorzonera hispanica* #2 (22%); *Hablitzia tamnoides* #1 and *Tilia cordata* (14%); and *Crambe maritima* #1, Hylotelephium telephium, *and* Scorzonera hispanica #2 (0%).

Figure 3.1 Results by nutrient concentration rank per species.



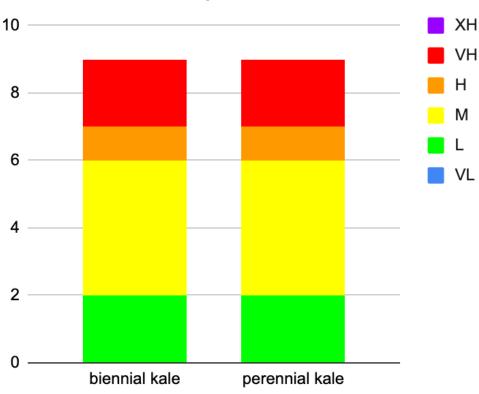
Results by nutrient concentration rank per species

This study also compared the nutrition of perennial and biennial kale grown in the same soils. While there was some variation (see Table 3.3), the overall proportion of nutrients in different concentration classes is the same. Folate was notably higher in the perennial kale, and calcium higher in the biennial. See Table 3.3. and Figure 3.2.

Latin Name	Common Name	Part	Fiber %	Ca mg/100 g	Fe mg/100 g	Mg mg/100 g	Zn mg/100g	Vitamin A mg RAE	Folate mcg/10 0g	Vitamin C mg/100g	Vitamin E g/100g
Brassica oleracea	Perennial kale	leaf	4.00	220.00	1.10	31.00	0.63	0.31	103.00	66.30	0.31
Brassica oleracea	Biennial kale	leaf	4.30	310.00	1.10	39.00	0.55	0.24	58.90	48.70	0.41

 Table 3.3 Nutrition of perennial and biennial kale compared.

Figure 3.2 Nutrient concentration rank of perennial and biennial kale compared.



biennial kale and perennial kale

by category

4. Discussion

For all but one of the nine nutrients studied, at least one perennial vegetable had nutrient concentrations higher than the reference vegetables (ranked very or extremely high). In the case of Zn, ten perennial vegetables were ranked very or extremely high. See Table 3.2.

Many perennial vegetables are high in the nutrients needed to address nutrient deficiencies. Ten out of the 12 species tested ranked high or higher in multiple nutrients. See Figure 3.1. The leaves of *Urtica dioica, Chenopodium bonus-henricus, Morus alba x M. rubra,* and *Rumex patientia* are especially notable in this regard.

No species ranked above "high" for either Vitamin E. This may be due to low sample size, as only 5 of the 13 species were tested.

It is interesting to note that the same species often sowed different nutrition in different samples. This may be due to different soils or growing techniques, different genetics (e.g., cultivars), harvest times, or other variables. This points to the need for additional data collection.

In a somewhat reversed situation, both perennial and biennial kales were found to have rather similar nutrient profiles. This suggests that perennializing kale production would have no nutritional drawbacks.

5. Conclusions

This study is a contribution to an understanding of perennial vegetable nutrition. The high levels of key nutrients found in some perennial vegetables make them an important resource to address nutrient deficiencies. The high concentrations of these nutrients found in some of these crops provide an additional argument for commercialization.

6. References

BVL (2006) "BVL L 00.00-97:2006-12. Analysis of food - determination of vitamin B6 (including glycosidically bound compounds) in food - HPLC method (adoption of the identical standard DIN EN 14663, March 2006 edition). Accessed November 22, 2021. https://www.beuth.de/de/technische-regel/bvl-l-00-00-97/96447033

CEN (2003) "CEN EN 14130:2003. Foodstuffs - Determination of Vitamin C by HPLC"

ISO (2005) "ISO 17294-1:2004. Water quality - Application of inductively coupled plasma mass spectrometry (ICP-MS)". Accessed November 22, 2021. https://standards.iteh.ai/catalog/standards/cen/15d21027-d891-4ab0-aaea-5d87c7cc39b7/en-1413 0-2003

ISO (2016) "ISO 17294-2:2016. Water quality - Application of inductively coupled plasma mass spectrometry (ICP-MS) - Part 2: Determination of selected elements including uranium isotopes". Accessed November 22, 2021. https://www.iso.org/standard/62962.html

Pimental, D, Cerasale, D, Stanley, R, Perlman, R, Newman, E, Brent, L, Mullan, A, and Chang, D (2012) "Annual versus perennial grain production". *Agriculture, Ecosystems, and Environment* 161 (2012):1-9.

Toensmeier, E. (2016) *The carbon farming solution: A global toolkit of perennial crops and regenerative agriculture practices for climate change mitigation and food security.* Chelsea Green Publishing, White River Junction.

Toensmeier, E, Ferguson, R, and Mehra, M. "Perennial vegetables: A neglected resource for biodiversity, carbon sequestration, and nutrition." *Plos one* 15, no. 7 (2020): e0234611.

U.S. EPA. 1994. "Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry," Revision 5.4. Cincinnati, OH. Accessed November 22, 2021.

https://www.epa.gov/esam/epa-method-2008-determination-trace-elements-waters-and-wastes-in ductively-coupled-plasma-mass