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Micronutrient Content of Wild Vegetable Species Harvested in Forested and Non-Forested Areas in Southwest Burkina Faso

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Abstract

Generation of improved data on biodiverse foods for nutrition required that nutrient composition data be paired with proper botanical identification of species. This study assessed the nutrient content of ten wild vegetable species within and outside forested area in Burkina Faso. The ten-vegetable species included: *Adansonia digitata* L., *Balanites aegyptiaca* (L.) Del., *Boerhavia diffusa* L., *Ceiba pentandra* (L.) Gaertn., *Cerathoteca sesamoides* Endl., *Crataeva religiosa* Sieber, *Ficus ovata* Vahl, *Moringa oleifera* Lam., *Strycnos spinosa* Lam. and *Vitex doniana* Sweet. Additionally, the nutrient content for each of the species was compared for specimens collected within and outside forested areas. The iron levels ranged between 3.9-107.9 mg/100 g dry weight, the zinc levels from 11-22 mg/100 g dry weight, and the calcium levels from 25-4637 mg/100 g dry weight. The beta carotene levels were between 0 and 1772 µg/100 g dry weight and the protein levels between 6.6 and 26.4 g/100 g dry weight. The variation between species was often greater than the variation between sites, for a given species. However, large differences in nutrient content between collection sites were seen in many species for many nutrients. Across all species, calcium and protein tended to be higher in forested areas while zinc and iron tended to be lower and beta carotene was highly variable. We sought to better understand the impact of ecosystems services from forests on nutrient composition. Given our modest sample size and the high levels of variation in nutrient content it was difficult to draw conclusions from our results. Despite this, it is increasing clear that wild and traditional African leafy vegetables can play an important role in meeting the international recommendations for fruit and vegetable intake.

Keywords: African leafy vegetables; Traditional and under-utilized foods; Biodiversity; Nutrient composition; Ecosystem services

Introduction

The challenge of global malnutrition may be one of the most pressing issues facing humanity today [1]. Although the global rates of hunger and undernutrition (in terms of stunting, wasting and underweight in children) have decreased; rates of micronutrient deficiency have remained stubbornly high, affecting more than 2 billion people world-wide [1]. Micronutrient deficiencies now coexist in the same populations and even the same individuals that also suffer from overweight and obesity [1-4] estimated that 45% of childhood mortality in 2011 was associated with malnutrition.

While micronutrient deficiencies are exacerbated by infection, the underlying problems of poor dietary quality, low dietary diversity and inadequate consumption of micronutrient-rich foods must be addressed to improve global rates of malnutrition [5,6]. In the face of dietary transitions [7], increasingly globalized and homogenized food systems [8-12] and unfavorable food environments [5], the nutrition and public health community need to make use of every tool in the tool box to promote healthy diets and reduce rates of micronutrient deficiencies globally [6,13]. Many experts are calling for greater attention to biodiversity of traditional and under-utilized foods as an important tool in the fight against micronutrient malnutrition [14-19]. The push for more research on biodiverse foods for nutrition calls for interdisciplinary research that links species and biological information (through proper botanical, species and varietal identification of under-utilized foods) with the nutrient content of those foods. The belief is that better demonstration of the potential of traditional and underutilized for nutrition, will help support their incorporation into nutrition programming and education.

Close to three million deaths annually are attributed to insufficient vegetable consumption [20-22], and few populations globally meet the FAO/WHO recommended intake of 400 grams of fruit and vegetables per person per day [23,24], including in many Sub-Saharan African countries [25]. In this context, African Leafy Vegetables (ALVs) are an important type of traditional and under-utilized food. Because ALV are often wild, or easy to cultivate, they are readily available and affordable, as well as culturally appropriate [26]. ALVs have been used for generations; however, their use is reported to be declining in many settings, perhaps due to cultural stigmas associated with their consumption [26]. In some settings, there are signs of a reversal of this trend [27]. ALVs are incredibly diverse and represent a major portion of the vegetables consumed in many African settings [26,28-32]. ALVs are a key source of micronutrients for many populations and in many cases, contain more micronutrients than introduced vegetables such as spinach or cabbage [32]. For example, in South Africa, 20 traditional wild vegetables provide close to 10% of the recommended intake in minerals [33].

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The push to develop better nutrient composition information for a biodiverse range of foods will help overcome issues related to gaps in data and errors that have crept into international nutrient composition tables due to data recycling [18]. However, these efforts are complicated by the fact that is often a great variation in the nutrient content of foods from the same species. For example, different varieties of banana can have 150-fold differences in carotenoid content [34-36]. Similarly, Ref. [37] reported coefficients of variation frequently over 100% for content of iron, zinc, thiamine and niacin in grains (rice, sorghum, millet) from various regions of Mali. Ref. [38] reported 10 fold differences in beta-carotene, iron and zinc content in leafy greens from different regions of Tanzania and Stadlmayr et al. report large variations in nutrient content of indigenous fruits.

The reasons for such high variations are complex. General knowledge in nutrition holds that selenium is the only micronutrient for which plant contents vary related to soil content; however, research from agriculture, soil and plant science indicates a number of other factors that can impact nutrient content of fruits and vegetables [39]. These include: soil characteristics (soil pH, soil structure, soil humidity), climate variables (temperature, rainfall and light intensity) and agricultural practices (ripeness, water content, pest exposure, pre- and post-harvest handling) [39,40]. The impacts of each of these differ from one nutrient to another. For example, light intensity has been found to have a positive impact on Vitamin C content and a negative impact on carotenoid content [41,42].

Ecosystem Services include the services that forests, and biodiversity provide to human well-being including: provisioning (such as food, water, fuel); regulating (such as weather, water and nutrient cycles, pollination and micro climates); and, cultural (recreation, religious, cultural uses of nature) services [43-45]. Many of the factors that impact nutrient content are related to regulating ecosystem services from forests and biodiversity: to our knowledge the impact of ecosystem services on food nutrient composition has not yet been examined.

The purpose of this study is to determine the nutrient content (protein, iron, zinc, calcium and beta-carotene) of ten wild vegetable species within and outside forested area in a semi-arid region of South-West Burkina Faso (in the Bontioli Forest Reserve and near the town of Dissine). The study pairs nutrient composition data with proper botanical identification, thereby contributing to the growing effort to develop an international data base on biodiverse foods for nutrition. Additionally, the study provides a first effort to examine the effect of forest cover on the amount of micronutrients in vegetables (iron, zinc, beta carotene, calcium).

Equipment and Methods

The research sites

The research was conducted in the South-West Region of Burkina Faso in Loba Province. The landscape of the region is a mosaic of savannahs, cultivated land, parklands and dry forest [46], including one of the largest protected forested areas in the country, the Bontioli Forest Reserve [47]. The local communities are comprised mainly of people from the Dagara ethic group, who practice hoe-based agriculture with Millet and Sorghum as their staple crops. As is the case in many African countries [48,49], the diet of communities in rural Burkina Faso is mainly based on carbohydrate rich staple crops, and is low in diversity, animal source foods, vegetables and fruit [27,28]. Across Burkina Faso, close to 50.5% of the households have a food diversity score of under 4 [50]. Vegetable collection for botanical identification and nutrient analysis took place in communities with greater forest cover living adjacent to the Bontioli Forest Reserve and communities with less forest cover living near the town of Dissine.

Selection of species

Species selection drew on interviews with local women, focus group discussions and feedback from a team collecting dietary intake data in the same region concurrently. The project team generated a list of wild, edible leafy vegetables. We selected the 10 species leafy vegetables that were reportedly most frequently consumed in local households.

Sample collection for nutrient composition analysis

For each of the ten leafy vegetable species, three samples were collected within and three samples outside of a forest area. The list of species collected, and the locations of collection are given in Table 1. The forest sites were located in the villages of Zambo and Bontioli, two villages located immediately adjacent to the Bontioli Wildlife Reserve. The non-forest (or lower forest cover) sites were located in the villages of Yô, Complan, and Benvar, all more than 30 km from the Bontioli reserve. The samples were collected between the months of May and July, the season when local communities consume fresh wild vegetables most frequently. The samples were composed of young leafy material, similar to what would be collected and consumed by local households. Material was harvested using a knife.

Sample preparation

After harvest, vegetable material was washed in potable water and rinsed in distilled water. The leafy vegetable was dried in the shade, in a ventilated enclosure and kept at a temperature of 25°C (77°F), then ground using a stainless steel (IKA) grinder. The fine grounded leaves were stored at 4°C (39°F) and analyzed to determine nutrient content.

Botanical identification

Botanical voucher specimens were collected by a different time who were in the field at the same time as the team collecting the material for nutrient composition. Botanical vouchers were collected by a highly ethnobotanist, Patrick Maundu, from the National Museum of Kenya. The local name, use, date and location of each specimen was recorded at the time of collection (see Table 1). Vouchers were pressed and dried and taken to the Herbarium at the University of Ouagadougou, where Siril SAWADOGO, an expert local Botanist who confirmed the identification and Latin name of each voucher.

Nutrient composition analyses

Protein: Total protein in various samples was measured using the Kjeldahl AOAC 979.09 method [51]. In a Kjeldahl flask, 0.2 g of ground leaves were mixed with a catalyser tablet, 10 ml of 96% sulfuric acid and a few drops of oxygenated water. The mixture was then heated until mineralized, the residue diluted in distilled water and phenolphthalein. The mixture was then placed in the Kjeldahl distillation apparatus with 60 ml of a 10 N sodium hydroxide (NaOH) solution. The distillate was collected into a 250 ml Erlenmeyer flask containing 20 ml of a boric acid, phenolphthalein, an indicator dye solution. The distillate was titrated with 0.1 N sulfuric acid and A conversion factor of 6.25 was used in the calculations.

Mineral content: Levels of iron, zinc and calcium contents were determined using the spectrophotometric method (Fast Sequential Atomic Absorption Spectrometer AA240 FS). Sample residue weighing 0.2 g was ground in a glass jar and 10 ml of concentrated nitric acid

Scientific name (Latin)	Name in Dagara	Botanical Identification	Material Collection	
		Voucher number, Date of Collection, Site of Collection	Forest site (village name)	Non-forest site
Adansonia digitata L.	Tokoura	PM#8, 6/12/2014, Nakar PM#61, 6/13/2014, Nakar PM#161, 6/16/2014, Zambo PM#210, 6/17/2014, Complan PM#278, 6/17/2014, Complan	Zambo	Complan
Balanites aegyptiaca (L.) Del.	Sansan	PM#46, 6/13/2014, Nakar PM#85, 6/13/2014, Nakar PM#158, 6/16/2014, Zambo PM#185, 6/17/2014, Complan PM#266, 6/17/2014, Complan	Zambo	Complan
Boerhavia diffusa L.	Tchonpegué	PM#2, 6/12/2014, Nakar PM#30, 6/12/2014, Nakar PM#56, 6/13/2014, Nakar PM#129, 6/16/2014, Zambo PM#204, 6/17/2014, Complan PM#214, 6/17/2014, Complan	Zambo	Benvar
Ceiba pentandra (L.) Gaertn.	Gonkoura	PM#190, 6/17/2014, Complan PM#209, 6/17/2014, Complan PM#280, 6/17/2014, Complan	Bontioli	Complan
Cerathoteca sesamoides Endl.	Saapla	PM#34, 6/12/2014, Nakar PM#41, 6/13/2014, Nakar PM#237, 6/17/2014, Complan	Zambo	Complan
Crataeva religiosa Sieber	Dokoum	PM#11, 6/12/2014, Nakar PM#40, 6/13/2014, Nakar PM#137, 6/16/2014, Zambo PM#195, 6/16/2014, Complan PM#264, 6/16/2014, Complan PM#281, 6/16/2014, Complan	Zambo	Complan
Ficus ovata Vahl	Kankantchita	PM#187, 6/17/2014, Complan	Bontioli	Yô
Moringa oleifera Lam.	Wobgnoukouon	PM#33, 6/12/2014, Nakar PM#72, 6/13/2014, Nakar PM#112, 6/16/2014, Zambo PM#179, 6/17/2014, Complan PM#262, 6/17/2014, Complan PM#279, 6/17/2014, Complan	Bontioli	Benvar
Strycnos spinosa Lam.	Gbeme / Poupoulourakolé	PM#7, 6/12/2014, Nakar PM#42, 6/13/2014, Nakar PM#102, 6/14/2014, Nakar PM#144, 6/16/2014, Zambo	Zambo	Complan
Vitex doniana Sweet	Baonigbe	PM#6, 6/12/2014, Nakar PM#39, 6/13/2014, Nakar PM#173, 6/17/2014, Complan PM#243, 6/17/2014, Complan	Zambo	Complan

Table 1: Vegetable species collection information.

solution (69%) was added. The mixture was then incubated at room temperature for 24 hours. A calibration solution was prepared with 19.71 ml of concentrated nitric acid (69%) in a 1000 ml graduated flask to obtain a solution of 0.5 N normality. Iron and zinc were measured by atomic absorption at 248.3 nm and 213.9 nm wavelengths respectively, and calcium by atomic emission at 422.7 nm wavelength, detected using Spectra AA software.

Beta-carotene content: The beta-carotene content was determined by High Performance Liquid Chromatography (HPLC) (Craft Method). First a beta-carotene for the calibration curve was prepared using a standard beta-carotene solution in HPLC grade hexane, at a concentration of 60 pico-moles/20 microliters. Sample residue weighing 0.5 g was used for extraction with 2 ml of n-hexane, 1 ml distilled water and l ml ethanol. The mixture was vortexed for two minutes, left too steep for 24 hours at a temperature of 4°C, then centrifuged at 3000 rpm for 10 min at-5°C. The n-hexane upper layer was removed in a 5-ml test tube and 2 ml of n-hexane were added to the base to prepare a second extraction, as per the preceding procedure. The chlorophyll was precipitated using 1 ml of dimethylformamide (DMF), vortex and centrifuge at 3000 rpm for 10 min at-5°. The supernatant was then evaporated under nitrogen flow. The residue, after evaporation, was put in 1 ml of acetonitrile. After agitation in the vortex, 20 µl of the solution was injected into the HPLC, with the analytical wavelength set at 450 nm. The concentration of the sample was calculated from the value of the peak area of the standard, its concentration and the area of the sample. The beta-carotene content was then converted into micrograms/100 grams (µg/100 g).

Statistical analyses

The mean and standard deviation of nutrient content of each of the three samples per species in forest and three samples per species in non-forest sites were calculated, expressed in relation to the dry matter. In order to be able to compare differences between forest and non-forest nutrient content across species with varying average nutrient contents (due to genetic, species level factors), we calculated a ratio for the amount of each nutrient found in a given species in forested relative to non-forested collection sites (when the nutrient content was greater in forested areas) and non-forested relative to forested collection sites (when the nutrient content was greater in the non-forested sites). The ratios where the content was greater in the non-forest site were given a negative value. Finally, the mean of the ratios was used to examine the general trend in nutrient content between forest and non-forest sites across species (where a positive mean would indicate greater nutrient content in forest areas, a negative mean suggests greater nutrient content in non-forest areas and a mean of zero would indicate no difference).

Results

The levels of protein, iron, zinc, calcium and beta carotene in the ten-species collected in forested and non-forested areas are reported in Table 2. There was great variation between species in terms of nutrient content, as well as in terms of which species had highest content for each nutrient. For Beta-carotene, *Boerhavia diffusa*, *Moringa oleifera*, *Ceiba pentandra* and *Cerathoteca sesamoides* had the highest content (Figure 1). For iron, *Cerathoteca sesamoides*, *Boerhavia diffusa* and *Strycnos spinose* had the highest content (Figure 2). The zinc content varied little between species, with *Boerhavia diffusa* having the highest content (Figure 3). *Balanites aegyptiaca* and *Boerhavia diffusa* had higher calcium content (Figure 4). Protein content was high in *Boerhavia diffusa*, *Crataeva religiosa*, *Ficus ovata*, and *Moringa oleifera* (Figure 5).

The variation between species was often greater than the variation between forested or a non-forested site, for a given species. However, large differences in nutrient content between forest and non-forest collection sites were seen in many species for many nutrients. For each nutrient there were some species that had higher content in forest sites and others with higher content in non-forest sites (Figures 1-5). For example, *Ceiba pentandra* had 3 times more calcium in non-forested sites compared to forest sites, while *Strycnos spinosa* had 20 time more calcium in forested sites. Similarly, *Boerhavia diffusa* had 3.22 times more beta-carotene in non-forest sites than forest sites, and *Adansonia digitate* had 4.13 times more beta-carotene in forest sites than non-forest sites. For iron, *Strycnos spinosa* had 5.88 times greater content in non-forested sites compared to forest sites, *Crataeva religiosa* had 1.53 times greater content in forested sites compared to non-forest sites. Using the average ratio across all ten species, the greatest variation between forest and non-forest sites were seen in calcium (average ratio=2.809) and protein (average ratio=0.855). The average ratios for both zinc and iron were negative (-0.162 and-0.681, respectively) and beta carotene had an average ratio close to zero (0.198) (Table 2).

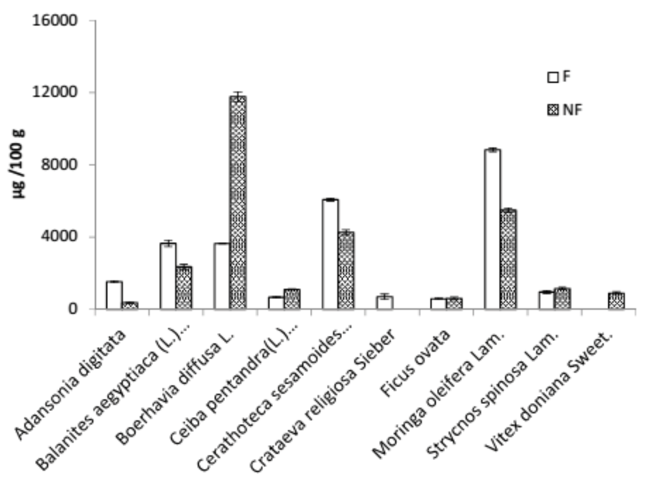

Figure 1: Beta-carotene content of vegetable plants inside (F) and outside (NF) the forest zone.

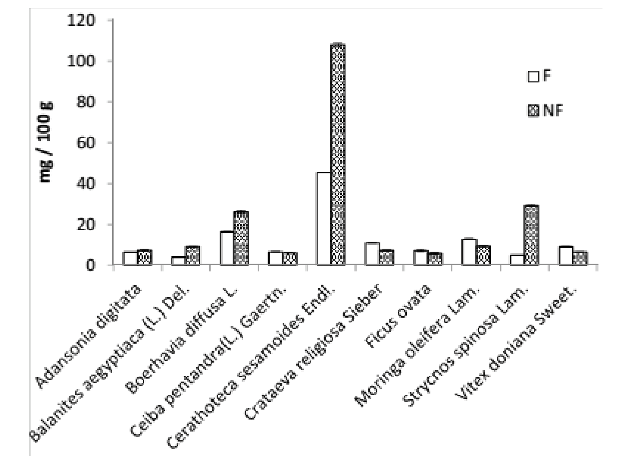

Figure 2: Iron content of vegetable plants inside (F) and outside (NF) the forest zone.

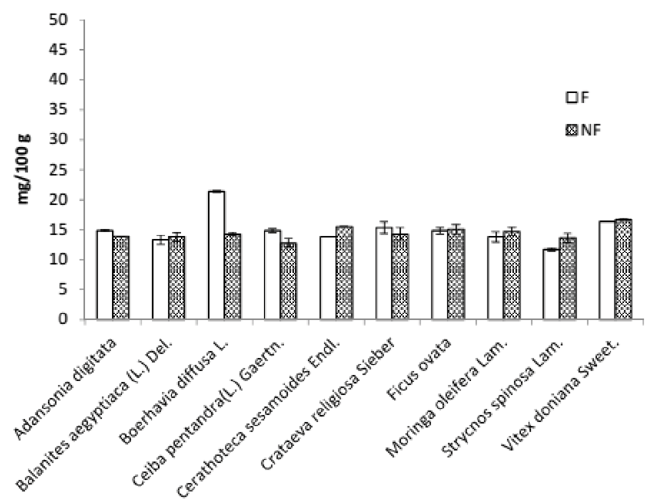

Figure 3: Zinc content of vegetable plants inside (F) and outside (NF) the forest zone.

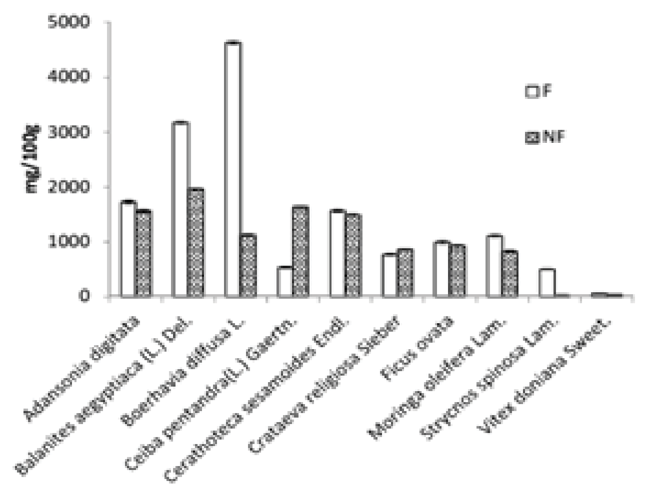

Figure 4: Calcium content of vegetable plants inside (F) and outside (NF) the forest zone.

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Species	β carotene			Iron			Zinc			Calcium			Protein		
	F (µg)	NF (µg)	Ratio F/NF	F (mg)	NF (mg)	Ratio F/NF	F (mg)	NF (mg)	Ratio F/NF	F (mg)	NF (mg)	Ratio F/NF	F (g)	NF (g)	Ratio F/NF
Adansonia digitate L.	1528.85	370.01	4.13	6.24	7.27	0.86	14.82	13.86	1.07	1730.26	1568.34	1.10	9.56	9.00	1.06
Balanites aegyptiaca (L.) Del.	3652.94	2354.96	1.55	3.93	9.26	0.42	13.31	13.81	0.96	3175.60	1964.05	1.62	12.00	9.56	1.25
Boerhavia diffusa L.	3646.13	11772.14	0.31	16.31	26.17	0.62	21.38	14.27	1.50	4636.85	1127.69	4.11	18.56	21.00	0.88
Ceiba pentandra (L.) Gaertn.	668.19	1106.11	0.60	6.42	5.99	1.07	14.82	12.76	1.16	535.45	1647.08	0.33	10.19	6.56	1.55
Cerathotea sesamoides Endl.	6069.77	4261.11	1.42	45.40	107.90	0.42	13.76	15.51	0.89	1572.76	1495.35	1.05	13.81	12.56	1.10
Crataeva religiosa Sieber	701.38	1.00	x	10.99	7.18	1.53	15.34	14.26	1.08	772.43	865.34	0.89	26.38	16.19	1.63
Ficus ovata Vahl	584.92	602.07	0.97	6.98	5.68	1.23	14.83	15.06	0.98	996.94	936.55	1.06	21.56	18.00	1.20
Moringa oleifera Lam.	8830.24	5490.38	1.61	12.68	9.39	1.35	13.78	14.69	0.94	1113.21	824.23	1.35	24.56	16.19	1.52
Strycnos spinosa Lam.	954.10	1152.60	0.83	4.83	29.25	0.17	11.69	13.64	0.86	503.04	24.63	20.42	14.38	16.19	0.89
Vitex doniana Sweet.	0.00	901.49	x	9.17	6.39	1.43	16.37	16.64	0.98	60.78	39.67	1.53	16.19	10.81	1.50

Table 2: Mean Nutrient Content of ten wild vegetable species harvested in forested and non-forested sites (per 100 g dry weight). *F: Forest; *NF: Non-forest.

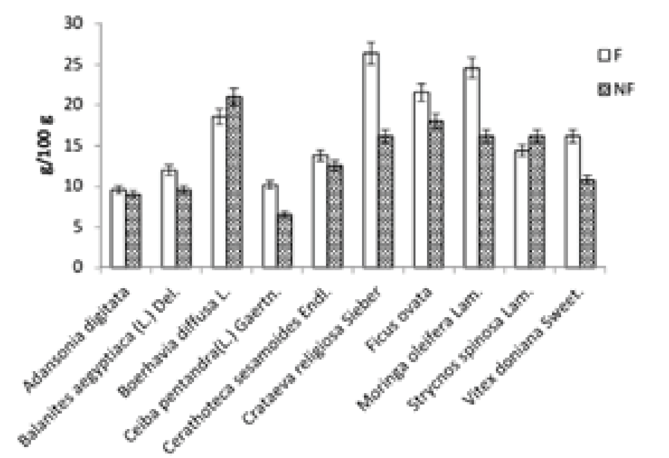

Figure 5: Protein content of vegetable plants inside (F) and outside (NF) the forest zone.

Discussion

Growing body of evidence on traditional and wild African vegetables

These results contribute to the efforts to improve global data on nutrient content of biodiverse foods [14,17]. Our results showed similar nutrient content as has been reported in past studies. [52] reported zinc levels between 3 and 109 mg/100 g dry weight for 20 wild leafy vegetables KwaZulu Natal in South Africa, and in another study from South Africa, [53] reported 4.2-5.5 mg of zinc/100 g dry weight in four wild leafy vegetables. In Cameroon, the zinc content of 21 traditional vegetables was reported to be between 0.45-3.8 mg/100 g dry weight [54]. The zinc levels in the vegetables we studied (between 11-22 mg/100 g dry weight) were comparable, possibly higher. [52] reported iron levels between 11 and 85 mg/100 g dry weight, while [53] reported levels between 25.5 and 88.3 mg/100 g dry weight for wild leafy vegetables in South Africa, and [54] reported levels between 15-167 mg/100 g dry weight in Cameroon. The iron levels in the vegetables we studied (between 3.9-107.9 mg/100 g dry weight) are comparable with even greater differences between the highest and lowest values. For calcium, our values ranged from 25 to 4637 mg/100 g dry weight, while values from the South African studies 100 to 12,262 mg/100 g dry weight [52,53]. Many other studies report similar values for nutrient content of beta carotene and protein in traditional and wild vegetables across Africa, as seen in our results, however reporting units are not always consistent.

While the drying, processing, storage and cooking of vegetables may affect their nutrient content [32], there remains significant potential for vegetables with the above listed nutrient values to make important contributions to meeting the recommended intake (RDA) for nutrients such as iron (maximum RDA for non-pregnant individuals of 18 mg), calcium (maximum RDA for non-pregnant individuals of 1300 mg), and zinc (maximum RDA for non-pregnant individuals of 11 mg). For example, 100 g of any of the species we tested would provide enough zinc to meet the daily recommendation (RDA). While cooking has been shown to cause the loss of some nutrients (less minerals than vitamins), it also reduces anti-nutrient content thereby increasing bioavailability [54].

High with-in species variation

As has been found in past studies, our results showed high variation in nutrient content within the same species harvested from different sites. Past studies have similarly reported large variation in nutrient content of micronutrients in African vegetables [37], and other foods in Africa [36]. Such variation may be associated with soil characteristics (soil pH, soil structure, soil humidity), climate variables (temperature, rainfall and light intensity) and agricultural practices (ripeness, water content, pest exposure, pre-and post-harvest handling), as well as genetic differences between plant populations [32,39,55].

Forest ecosystem services and nutrient content

Regulating Ecosystem services provided by forests were hypothesized to have an impact on nutrient content of leafy vegetables. The nutrient that showed the most consistent difference between forest and non-forest sites across species was calcium (which had an average ratio between forest and non-forest levels of=2.809). Differences between forest and non-forest sites for protein, zinc and iron were too highly variable to draw conclusions from about an impact of forest cover (or lack thereof) on nutrient content. Past research found that plants grown in more intense light had lower carotenoid content [41], suggesting that vegetables grown in forest or agroforestry settings might have higher carotenoid content. Our findings did not support this across all species; however, given the dry forest setting of our research (where the majority of trees are seasonally deciduous), it is difficult to be sure that the "forest" sites would have had different light intensities from the "non-forest" sites where we collected the vegetables. The impact of micro-climate variation on nutrient content should be tested in other settings, with large sample sizes and more controlled research designs.

Conclusion

There is still a long road ahead in developing a compressive data on the nutrient content of wild foods and underutilized crop species in Africa and globally. The high variations in nutrient content of all foods and the lack existing data for entire species of locally important foods has impaired their incorporation into nutrition and dietary recommendations. While nutrition community builds better data on nutrient content, traditional and wild African leafy vegetables should already be promoted as an important part of healthy diets: there is mounting evidence that they often have excellent nutrient levels for many important nutrients. Moreover, the evidence linking fruit and vegetable consumption with health outcomes is already very strong [20-24,55,56] and time to take actions to mitigate the impending dietary and nutrition transitions in countries like Burkina Faso is running out [1].

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