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Nutrient and Total Polyphenols Contents of Some Dark Green Leafy Vegetables, and Estimation of Their Iron Bioaccessibility Using the In Vitro Digestion/Caco-2 Cell Model

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Abstract: Dark green leafy vegetables (DGLV) are considered as important sources of iron and vitamin A. However, iron concentration may not indicate bioaccessibility. The objectives of this study were to compare the nutrient content and iron bioaccessibility of five sweetpotato cultivars including three orange-fleshed types with other DGLV commonly consumed DGLV in Ghana: cocoyam; corchorus; baobab; kenaf and moringa, using the in vitro digestion/Caco-2 cell model. Moringa had the highest amounts of iron absorption enhancers on an as-would-be-eaten basis, β-carotene (14,169 μg/100 g, $p < 0.05$) and ascorbic acid (46.30 mg/100 g, $p < 0.001$), and the best iron bioaccessibility (10.28 ng ferritin/mg protein). Baobab and an orange-fleshed sweetpotato with purplish young leaves had lower iron bioaccessibility (6.51 ng and 6.76 ng ferritin/mg protein, respectively) compared with that of moringa although these three greens contained similar ($p > 0.05$) iron (averaging 4.18 mg/100 g) and β-carotene levels. The ascorbic acid concentration of 25.50 mg/100 g in the cooked Baobab did not enhance iron bioaccessibility. Baobab and the orange-fleshed sweetpotato with purplish young leaves contained the highest level of total polyphenols (1646.75 and 506.95 mg Gallic Acid Equivalents/100 g, $p < 0.001$, respectively). This suggests iron bioaccessibility in greens could not be inferred based on the mineral concentration. Based on the similarity of the iron bioaccessibility of the sweetpotato leaves and cocoyam leaf (widely-promoted "nutritious" DGLV in Ghana), the former greens have an added advantage of increasing dietary intake of provitamin A.

Keywords: β-carotene; Caco-2 cell; iron bioaccessibility; leafy vegetable; polyphenols

1. Introduction

It is generally accepted that dark green leafy vegetables (DGLV) are important sources of micronutrients such as iron and vitamin A. For example, on the basis of compositional data, DGLV were reported to contribute about 19–39% of iron and 42–68% of vitamin A [1] in the diets of rural South Africans. However, iron and vitamin A deficiencies are perennial malnutrition problems in

developing countries where DGLV are important food ingredients [2,3]. One of the common food ingredients, possibly with high concentration of micronutrients such as iron and β-carotene (provitamin A), are the greens. However, Cercamondi and co-workers [4] reported that sauce prepared from Amaranth (*Amaranthus cruentus*) or Jew's mallow/corchorus (*Corchorus olitorius*), examples of DGLV, and eaten with a thick maize paste by young Burkinabe women did not increase the amount of iron absorbed. An inadequate dietary intake of bioavailable iron and vitamin A could be the primary cause of iron and vitamin A deficiencies. Therefore, bioaccessibility of minerals from food may not solely depend on their concentration, but other constituents in the food.

Polyphenols and phytates in cereal and leguminous foods have been shown to limit bioaccessibility and consequently bioavailability of essential micronutrients including iron, calcium and zinc [5,6]; and these staples are usually consumed with these DGLV that may also contain significant levels of these inhibitors. In a human feeding trial conducted by Garcia-Casal and co-workers [7], it was found that β-carotene enhances iron absorption when added to cereal-based diets. This finding was confirmed using Caco-2 cells as model for iron availability [8]. Thus, the consumption of these greens reported to be rich in micronutrients such as β-carotene [9,10], should have double impact as a provitamin A dietary source and also as an enhancer of iron absorption. However, this was contrary to the findings of Cercamondi and co-workers [4]. This calls for the need to investigate iron bioaccessibility from commonly consumed DGLV in Ghana as anaemia (not categorised) prevalence has consistently been stated to be above 73% for children under 5 years, and 35% among women in the reproductive age in northern Ghana [11–13] where the consumption of greens is high. Expectedly, vitamin A deficiency among Ghanaian children under 5 years was approximately 79% [14], as micronutrients deficiencies usually occur together. In Ghana, DGLV have been reported to be reliable sources of β-carotene for the majority of the population [10].

Amaranth and jute are widely consumed DGLV in northern Ghana, in addition to others such as baobab (*Adansonia digitata*), and moringa (*Moringa oleifera)* [15]. Sweetpotato (*Ipomoea batatas*) is available in northern Ghana [16], but is mainly cultivated for the roots. Sweetpotato leaf has been reported to contain appreciable levels of vitamin A, iron and other essential nutrients including water-soluble vitamins [17,18]; and the crop can be cultivated with low agricultural inputs [19]. Also, it has been reported that the sweetpotato leaves have higher caffeoylquinic acid derivatives (polyphenols) than commercial vegetables with physiological functions due to their enhanced antimutagenic and antioxidative properties [20]. Although the polyphenols have health benefits, they may compromise iron bioaccessibility from the DGLV. Different polyphenols exist, and have differing effects on iron bioaccessibility [21–24]. Based on the nutrient superiority of the sweetpotato leaf [17], it could serve as an alternative source of leafy vegetable to the populace in tropical regions of the world, particularly in Africa where vitamin A and iron deficiencies often co-exist and remain public health problems [2,3]. The compositional data suggest that sweetpotato and moringa leaves might be better sources of bioavailable iron, compared with other leafy green vegetables, as both have high levels of iron and also β-carotene—a dietary factor that has been reported to improve iron bioaccessibility. However, the use of the greens of sweetpotato as a leafy vegetable in Ghana is limited.

There is the need to do a comparative study of leaves commonly consumed and sweetpotato leaf before the latter could be suggested as an alternative green in Ghana as a source of bioavailable iron or β-carotene. The in vitro digestion/Caco-2 cell model has been suggested to be less expensive than human trials [25,26], a more physiological tool for screening iron availability in comparison with solubility and dialysability methods, and an effective approach for predicting iron bioaccessibility from food for humans [27,28]. Therefore, the in vitro digestion/Caco-2 cell model, with ferritin formation as a marker for iron absorption, was used to measure iron bioaccessibility of selected greens available in Ghana in comparison with sweetpotato leaves.

The objectives of this study were to compare the nutrient contents and iron bioaccessibility using the in vitro digestion/Caco-2 cell model of five different cultivars of sweetpotato with five other commonly consumed DGLV in Ghana: cocoyam *(Xanthosoma sagittifolium*), corchorus, baobab, kenaf (*Hibiscus cannabinus*) and moringa.

2. Materials and Methods

2.1. Sample Cultivation and Collection

Five cultivars of sweetpotato: three orange-fleshed (Coded OFSP1, OFSP2 and OFSP3); one purple-fleshed (PFSP); and one white-fleshed (WFSP) and three other DGLV, namely moringa; corchorus and kenaf were nursed in a screen house up to maturity (8 weeks). Each DGLV was cultivated in three replicates, and each replicate contained five pots of the particular green. Baobab and cocoyam were purposively sampled from three different geographical locations. Baobab leaves were collected from trees near settlements from Upper East, Upper West and Northern regions; while cocoyam leaves were harvested from farmlands from Ashanti, Eastern and Brong-Ahafo regions of Ghana. The baobab was not nursed due to relatively long time for initiation of vegetative growth. Cocoyam is normally cultivated in the rainforest regions in Ghana, and not in northern Ghana.

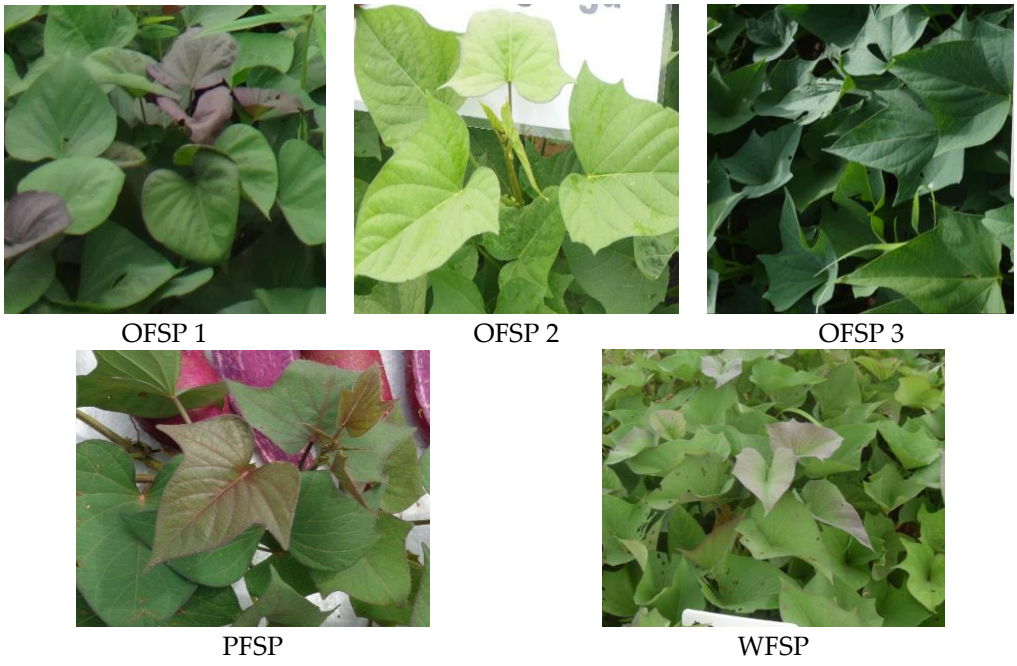

Plate 1. Cultivars of sweetpotato (*Ipomoea batatas*) leaves used in the study.

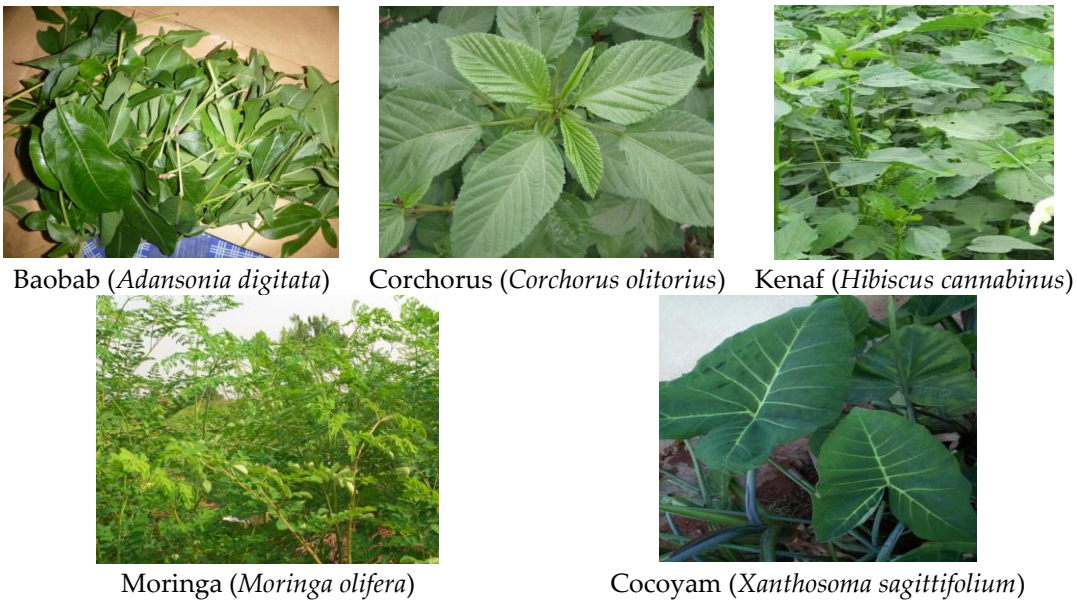

Plate 2. Commonly consumed DGLV used in the study.

2.2. Sample Preparation

The replicates of the DGLV were separately washed twice under running tap water and rinsed in distilled water; about two handfuls DGLV were put into a stainless-steel cup and 100 mL of distilled water added, covered with aluminium foil and boiled until soft, approximately between 15 min and 20 min. The cooked DGLV were allowed to cool, and all the contents of the cup were transferred into coded transparent low-density polyethylene zip lock bags, and stored in a freezer at −18 °C for two weeks. Prior to storage in the freezer, about 5 g aliquot portions were taken for moisture determination. The frozen samples were then freeze-dried (TK-118 Vacuum Freeze-Dryer, True Ten Industrial Company Limited, Taichung, Taiwan) for 72 h. The samples were then milled (Thomas Scientific, Dayton Electric Manufacturing Company Limited, Niles, IL 60714, USA) and sieved into fine powder using a 60-mm sieve.

Triplicate aliquots of three-letter coded powdered samples were couriered to University of Greenwich at Medway, UK, and Massey University, New Zealand, from Ghana. Moisture determination of fresh leaves was done in Ghana.

2.3. Compositional Analysis

2.3.1. Moisture and Protein

The moisture contents of freshly harvested leaves and cooked leaves were gravimetrically determined using forced air oven method (AOAC 925.10). For the milled freeze-dried samples, the vacuum oven protocol (AOAC 926.12), as published in the official methods of analysis of AOAC International [29] was used for moisture determination.

The concentration of nitrogen in the freeze-dried greens was done by the Dumas method (AOAC 968.06) and nitrogen to protein conversion factor of 6.25 were used to quantify the amount of protein in the leaves on a fee-for-service basis by Massey University Nutrition Laboratory.

2.3.2. Mineral Analysis: Calcium, Iron, and Zinc

Approximately 0.50 g of the freeze-dried DGLV samples was microwave-digested using an accelerated reaction system (CEM MARS 5H with XP-1500 vessels) for 20 min at 400 psi and 1200 W. Subsequently, calcium, iron and zinc were quantified using an Inductivity Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES, Perkin–Elmer Optima 4300 DV) using protocol as previously described [30]. A certified reference material (ERMCD281, Sigma) was included and run parallel with the DGLV samples. The data obtained for all the three minerals in the reference material were within 5% of the expected values.

2.3.3. β-Carotene

Other researchers have described the extraction and quantification methods used in this study [31]. Averagely, 0.50 g of the freeze-dried samples of the leaves was used for the extraction. A certified reference material (BCR®—485, European Commission Research Centre, Institute for Reference Materials and Measurements, Belgium) was included in three out of the five batches of extraction carried out on DGLV samples. A mean recovery of 128% was obtained for β-carotene level for the reference material. Therefore, the values obtained for DGLV were adjusted for a systematic error of 28%.

2.3.4. Ascorbic Acid

The method for vitamin C determination as published by Lee and Coates [32] was carried out by the Massey University Nutrition Laboratory on a fee-for-service basis.

2.3.5. Polyphenols

The Folin–Ciocalteu method described by Isabelle and co-workers [33] was used to quantify the total polyphenols in the samples, as gallic acid equivalents. The Nutrition Laboratory, Massey University, New Zealand carried out the analysis on a fee-for-service basis.

2.4. In Vitro Digestion/Caco-2 Cell Model for Iron Availability

Iron availability from the freeze-dried DGLV "as received" from Ghana was assessed using the TC7 Caco-2 cell clone (INSERM U505) from cell passages 42–45 in the in vitro digestion/Caco-2 cell model as previously described [34] with slight modification. Averagely, 0.5 g instead of 1 g of the sample was weighed for the assessment as the 1 g of starting material lead to a matrix that was too viscous for the multiple mixing and pH adjustments required in this method. Cells were grown in six-well tissue culture plates for experiments and maintained in DMEM supplemented with 10% *v*/*v* foetal bovine serum (FBS). On days 12–13, cell media was changed to MEM without FBS in the method developed by Glahn [35,36], to ensure a low iron media but optimal expression if Caco iron transport proteins [37]. On day 14, foods were subjected to in vitro digestion with sequential addition of digestive enzymes to mimic exposure to the stomach and small intestine (pepsin at pH 2, followed by bile/pancreatin at pH 7). Digested foods (digestates), and controls including a blank "No food/added iron" digestate, were then applied to Caco-2 cells through an upper chamber suspended over the plate wells created using a 15-kD dialysis membrane fitted over a Transwell insert and held in place with a silicon ring. The membrane protects the cells from the digestive enzymes, and also mimics the gut mucous layer in only allowing soluble iron a selected size to be available for enterocyte absorption. Cells were treated for two hours, digestates removed, and the cells returned to the incubator. The cells were harvested for ferritin 24 h after the initiation of the digestive process. Ferritin was measured using a commercial enzyme-linked immunosorbent assay (Spectro ferritin, RAMCO Laboratories Inc., Stafford, TX, USA), and corrected for differing cell numbers/well by measurement of cell protein; cell protein was measured using the Pierce protein bicinchoninic acid assay. Ferritin values are expressed as ng ferritin/mg cell protein.

2.5 Statistical Analysis

The compositional data were converted to "as-would-be-eaten" basis prior to statistical analysis using the dry matter content obtained for the cooked samples prior to storage in the freezer. The univariate analysis, followed by Tukey's studentised range test with significance set at $p < 0.05$ was used for the compositional data. For the in vitro digestion/Caco-2 cell model for iron availability, the data generated were normalised prior to using the general linear model procedure for one-factor analysis, and the results presented as interval plots of mean with 95% confidence interval. Minitab® 16.2.2 (Minitab Inc., State College, PA, USA) statistical package was employed for the data analysis.

3. Results

3.1. Compositional Profile

The data in Table 1 is expressed on "as-would-be-eaten" basis, with the exception of moisture value that was on the freshly harvested leaves. The moisture content of the sweetpotato cultivars ranged from 83 to 87 g/100 g, and it was similar to other cultivars cultivated in China [38]. The greens of the sweetpotato cultivars were generally not significantly different ($p > 0.05$) from each other for all the components analysed with the exception of total polyphenols.

Table 1. Moisture, micronutrient and total polyphenols levels per 100 g in some DGLV on as-would-be-eaten basis [#].

DGLV	Moisture (g) [¥]	Calcium (mg) [§]	Iron (mg)	Zinc (mg)	β-Carotene (μg)	Ascorbic Acid (mg)	Total Polyphenols (mg GAE) [†]
OFSP1	84.09 ± 0.34 ^{c,d}	95.61 ± 8.01 ^{c,d}	3.41 ± 0.36 ^{a,b}	0.44 ± 0.01 ^{b}	10,533 ^{a,b}	0.74 ± 0.16 ^{c}	506.93 ± 86.76 ^{b}
OFSP2	84.76 ± 0.75 ^{b,c,d}	81.04 ± 3.24 ^{d}	1.89 ± 0.29 ^{b}	0.42 ± 0.02 ^{b}	8280 ^{a,b,c}	0.50 ± 0.15 ^{c}	356.69 ± 79.60 ^{c}
OFSP3	87.24 ± 0.13 ^{a}	103.25 ± 2.59 ^{c,d}	2.58 ± 0.21 ^{a,b}	0.36 ± 0.03 ^{b}	7053 ^{b,c}	0.45 ± 0.07 ^{c}	336.38 ± 63.15 ^{c,d,e}
PFSP	84.30 ± 0.26 ^{c,d}	84.75 ± 8.83 ^{c,d}	2.04 ± 0.36 ^{b}	0.44 ± 0.04 ^{b}	4472 ^{b,c}	0.48 ± 0.03 ^{c}	231.44 ± 49.77 ^{c,d,e}
WFSP	83.91 ± 0.26 ^{d}	87.02 ± 6.80 ^{c,d}	3.27 ± 0.34 ^{a,b}	0.40 ± 0.03 ^{b}	9501 ^{a,b,c}	0.34 ± 0.10 ^{c}	234.86 ± 0.16 ^{c,d,e}
Baobab	85.97 ± 0.53 ^{b}	535.63 ± 22.93 ^{a}	4.59 ± 1.28 ^{a}	0.65 ± 0.03 ^{b}	7166 ^{b,c}	25.50 ± 0.01 ^{b}	1646.75 ± 69.44 ^{a}
Cocoyam	85.23 ± 0.64 ^{b,c}	166.39 ± 15.13 ^{b}	2.64 ± 0.16 ^{a,b}	1.49 ± 0.47 ^{a}	3911 ^{c}	1.14 ± 0.01 ^{c}	196.05 ± 10.96 ^{e}
Corchorus	78.99 ± 0.38 ^{f}	121.41 ± 3.61 ^{c}	2.48 ± 0.23 ^{a,b}	0.45 ± 0.02 ^{b}	9298 ^{a,b,c}	3.53 ± 0.58 ^{c}	337.94 ± 16.44 ^{c,d,e}
Kenaf	80.68 ± 0.18 ^{e}	90.24 ± 17.76 ^{c,d}	2.94 ± 0.25 ^{a,b}	0.35 ± 0.05 ^{b}	8959 ^{a,b,c}	21.79 ± 1.54 ^{b}	202.42 ± 9.29 ^{d,e}
Moringa	78.81 ± 0.42 ^{f}	186.22 ± 23.81 ^{b}	4.55 ± 1.88 ^{a}	0.77 ± 0.06 ^{b}	14,169 ^{a}	46.30 ± 4.78 ^{a}	347.38 ± 14.59 ^{c,d}
p-Value	*<0.001*	*<0.001*	*0.002*	*<0.001*	*<0.001*	*<0.001*	*<0.001*

[#] Value (mean ± standard deviation, $n = 3$) except for β-carotene-value is mean only; value with different letter was significantly different ($p < 0.0001$); DGLV-dark green leafy vegetable; OFSP-orange-fleshed sweetpotato; PFSP-purple-fleshed sweetpotato; WFSP-white-fleshed sweetpotato; [¥] Moisture determined on freshly harvested leaves; [†] GAE (Gallic Acid Equivalents).

OFSP 1, Apomuden, a variety being promoted in Ghana because of the β-carotene content in the storage root [39], had approximately 1.7 times more of total polyphenols than the other sweetpotato cultivars. The leaves of the sweetpotato cultivars were not distinctively superior in the levels of the micronutrients analysed compared with the other DGLV. However, OFSP1 contained appreciable higher levels of β-carotene (10,533 μg/100 g) and total polyphenols than the other greens apart from the β-carotene in moringa (1.3 times more) and total polyphenols in baobab, which was about thrice higher. Although the roots of the OFSP cultivars are promoted as a dietary source of vitamin A, moringa leaves actually had the highest β-carotene concentration among the DGLV investigated. Although the WFSP root is devoid of β-carotene [40], the amount of this provitamin A in the leaf was more than that in the greens of OFSP2 and OFSP3.

In contrast, among only the commonly consumed DGLV only, baobab leaves contained the highest amount of calcium ($p < 0.001$), on the average, about four times more. There was no significant difference in iron concentration ($p > 0.05$), but the data show that the iron level in baobab and moringa (4.59 ± 1.28 and 4.55 ± 1.88 mg/100 g, respectively) was higher. Previous data indicated that moringa contained 28.29 ± 0.05 mg/100 g of compositional iron [17], the highest compared with the seven sweetpotato varieties in Ghana; the data in this study follows a similar trend.

Three of the DGLV with notable amount of ascorbic acid were moringa, baobab, and kenaf. The total polyphenols in baobab were the highest (1646.75 ± 69.44 mg GAE, $p < 0.001$) among all the DGLV including the sweetpotato cultivars considered in this study. Moringa had moderate content of total polyphenols, about a fifth of that in Baobab ($p < 0.05$).

The concentration of zinc in the cocoyam leaf was 1.49 mg/100 g, about thrice more than the average of all the other DGLV ($p < 0.001$). A similar trend of the zinc data between moringa and the sweetpotato cultivars in this study was observed in a previous study in Ghana [17].

Figure 1 shows the crude protein content of all the DGLV, ranging from 3.62–6.54 g/100 g on "as-would-be-eaten" basis. Moringa contained the highest protein (6.54 ± 0.36 g/100 g), and was significantly different ($p < 0.05$) from the next DGLV, Baobab (5.67 ± 0.05 g/100 g), followed by two cultivars of sweetpotato: OFSP1 (5.37 ± 0.04 g/100 g) and OFSP3 (4.99 ± 0.17 g/100 g). The two DGLV with the lowest protein level were WFSP (3.87 ± 0.05 g/100 g) and Cocoyam (3.62 ± 0.17 g/100 g). A trend between the protein data for moringa and the sweetpotato cultivars was similar to a previous study in Ghana [17].

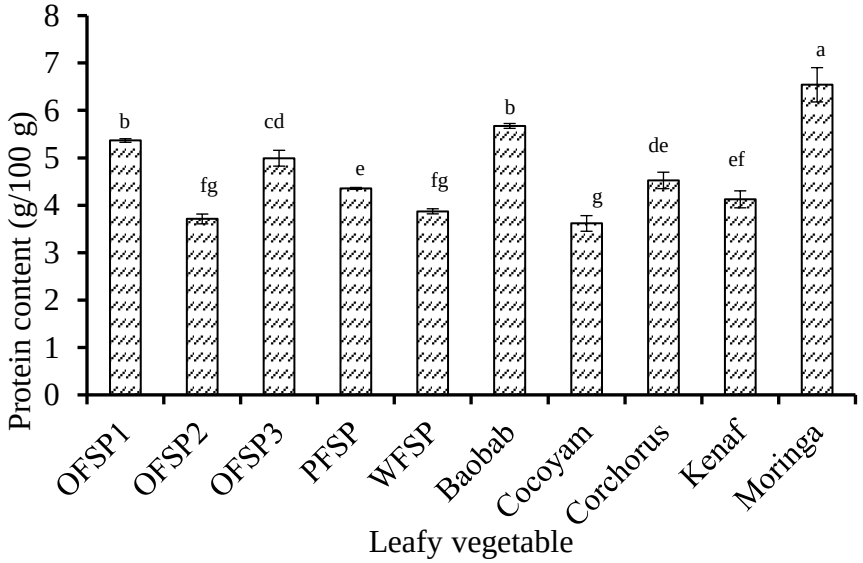

Figure 1. Protein content in "as-would-be-eaten" leafy vegetable. Bar value (mean ± standard deviation, $n = 3$); and Bar with different letter was significantly different ($p < 0.0001$). OFSP—Orange-fleshed sweetpotato (1, 2 & 3); PFSP—Purple-fleshed sweetpotato; WFSP—White-fleshed sweetpotato.

3.2. In Vitro Iron Bioaccessibility Using Caco-2 Cells as a Model

The data for representing in vitro iron bioaccessibility are in Figure 2. The overall mean of iron bioaccessibility was 7.71 ng ferritin/mg protein. Moringa markedly had the best iron bioaccessibility, 10.28 ± 2.73 ng ferritin/mg protein, and was significantly different ($p < 0.0001$) from all the DGLV investigated.

The two greens (Baobab and OFSP1) that can be ranked as first and second in terms of the concentration of total polyphenols had the lowest iron bioaccessibility using the Caco-2 cell model, and their bioaccessibility was below the group mean. Conversely, cocoyam had iron bioaccessibility as the overall mean although it contained the lowest concentration of polyphenols. Apart from baobab, moringa and OFSP1, all the other DGLV had bioaccessibility similar to that of the overall mean.

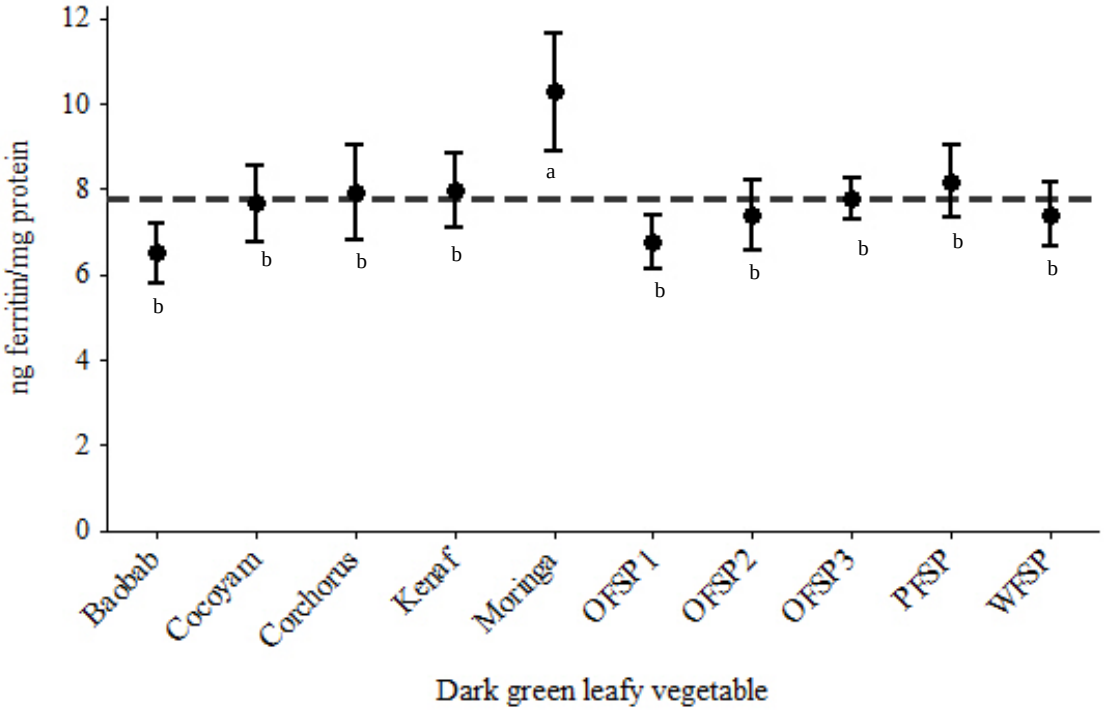

Figure 2. Ferritin formation per half a gram of freeze-dried green leafy vegetable. Vertical lines are means with 95% confidence interval of ng ferritin/mg protein from the various greens (n = 12 for Corchorus; n = 18 for OFSP1, PFSP, Baobab, Kenaf and Moringa; n = 21 for OFSP2, OFSP3, WFSP and Cocoyam) normalised to the blank digest ferritin level; Horizontal line indicates the overall mean of ng ferritin/mg protein; Mean with 95% confidence interval with a different letter is significantly different ($p < 0.0001$). OFSP—Orange-fleshed sweetpotato (1, 2 & 3); PFSP—Purple-fleshed sweetpotato; WFSP—White-fleshed sweetpotato.

To test the relationship between iron bioaccesibility and some of the components (on in the DGLV investigated, a multiple linear regression was conducted (Table 2).

Table 2. Effect of selected components (on as-would-be-eaten basis) in DGLV on iron bioaccesibility.

Variable [#]	Estimate (Standard Error)	*p*-Value
Intercept	10.26 (3.24)	0.09
Calcium (mg/100 g)	−0.00 (0.03)	0.99
Iron (mg/100 g)	0.13 (1.18)	0.92
Zinc (mg/100 g)	−0.87 (1.81)	0.68
β-carotene (μg/100 g)	−0.00 (0.00)	0.35
Ascorbic acid (mg/100 g)	0.01 (0.08)	0.93

Total polyphenols (mg GAE/100 g)	−0.00 (0.01)	0.91
Protein (g/100 g)	0.29 (0.78)	0.74

[#] Coefficient of determination (R^2 = 74.62).

Although the model explained about 75% of the variation in iron bioaccesibility, it was protein and iron levels that suggested that a unit increase in concentration could respectively lead to 0.29 and 0.13 ng ferritin/mg protein formation in Caco-2 cells. However, with regards to zinc, an increase in its concentration could result in reduction of ferritin formation by a 0.87. Ascorbic acid and β-carotene (known enhancers of iron absorption) as well as total polyphenols (inhibitor of iron) had almost no effect on iron bioaccessibility using the in vitro digestion/Caco-2 cell model.

Thus, the data from this study may suggest a complex interaction between the components investigated in the leaves and iron bioaccessibility. Moringa with the highest levels of protein, ascorbic acid, β-carotene and moderate concentration of total polyphenols, had the best iron bioaccessibility. Also, cocoyam with lowest levels of β-carotene and total polyphenols had average iron bioaccessibility. Among the DGLV investigated, iron content does not explicitly indicate its bioaccessibility. For example, the two DGLV with the highest amount of iron, had one of them, moringa, having the best iron bioaccessibility, and the other, baobab, with the least bioaccessibility compared to the other greens, although not significant ($p > 0.05$). However, PFSP, a sweetpotato cultivar, with the lowest level of iron, had iron bioaccessibility better than baobab, although not significant ($p > 0.05$). In addition, the level of total polyphenols irrespective of the concentrations of ascorbic acid, β-carotene and iron appeared to be the major factor limiting iron bioaccessibility of OFSP1 and baobab.

4. Discussion

OFSP1 was the only sweetpotato cultivar with purplish young leaves [41] among the five sweetpotato genotypes evaluated in this study. This may have accounted for the highest total polyphenol contents of OFSP1 compared to the other sweetpotato cultivars. The difference in the iron data for moringa in this study and compared to previous work [17], was due to how the data were reported. In the previous study, the result was reported on powdered-samples, while in our study, it is on as-would-be-eaten basis. Nonetheless, the trend of iron being the highest in moringa was also confirmed in this study.

Although cocoyam leaf is widely consumed and promoted in Ghana as a "nutritious" green to improve iron status (anecdotally), on the basis of its composition data, it was only highest in zinc and lowest in β-carotene and total polyphenols compared with the OFSP cultivars. Because both the sweetpotato leaves and cocoyam had similar iron bioaccessibility, the sweetpotato leaves could be used in culinary preparations, and have an added advantage of increasing dietary intake of β-carotene compared to that of cocoyam.

Generally, the level of iron bioaccessibility from the DGLV was relatively low (6–10 ng ferritin/mg protein) compared with our previous work from the same laboratory on complementary food (12–34 ng ferritin/mg protein) [34]. However, a strong comparison cannot be made between the data from the two studies as different sample weights were used, 1 g in previous work, and 0.5 g in present study. Previous community-based feeding trial using Weanimix, which had iron bioaccessibility of 17.32 ± 2.84 ng ferritin/mg protein [34] resulted in poor iron status among older infants in Ghana [42,43]. The lower availability of iron of the greens in this study lends support to the finding on the work among young Burkinabe women that resulted in no increase in iron absorption after eating Jew's Mallow with a thick maize paste [4].

As mentioned earlier, moringa contained the highest amounts of enhancers of iron absorption: β-carotene [7,8]; and ascorbic acid [44]. Additionally, the concentration of total polyphenols in this DGLV was moderate. The composition of nutrients in moringa compared with the other DGLV contributed to the highest bioaccessibility of iron as obtained from the in vitro Caco-2 cells model study. Although OFSP1 had significantly similar levels of β-carotene and iron to moringa, and a third of the total polyphenols of baobab, its iron bioaccessibility was lower than moringa, indicating that

the reported caffeoylquinic acid derivatives in sweetpotato leaves [20] may have limited the bioaccessibility of iron. Baobab had the lowest iron bioaccessibility in spite of being one of the greens that contained the highest amount of iron and ascorbic acid. This may be attributed to high concentrations of calcium that is known to inhibit iron absorption [25,45] and total polyphenols [20] relative to the other DGLV suggesting that the polyphenols in Baobab may be very inhibitory even in the presence of endogenous ascorbic acid. However, the amount of calcium in the greens explicitly does not suggest inhibitory effect on iron as moringa contained the second highest of this mineral among all the DGLV investigated, but had markedly better iron availability. Therefore, predicting iron bioaccessibility based on only compositional data could lead to false conclusions.

The effect of concentration of plant protein on iron bioaccessibility cannot be explicitly substantiated in this study. Moringa, having the highest as-would-be-eaten protein was the green with the highest bioaccessibility. Both baobab and OFSP 1 that contained relatively high concentration of protein than the rest of the DGLV, were those that recorded the lowest bioaccessibility of iron, although not significant. Thus, from the data in this study, it is difficult to use the protein concentration to predict iron bioaccessibility. The inverse association between zinc concentration and the index of iron bioaccessibility, can be attributed to cocoyam leaf that had the highest zinc concentration and the lowest ferritin formation in the Caco-2 cells.

The major limitations in this study were that phytate and the constituent of the different classes of polyphenol were not quantified. The assay method previously used for phytate determination [46,47] gave very inconsistent results within replicates in this study; possibly the colour of DGLV intefers with the spectrophotometer readings.

5. Conclusions

The studied greens varied in terms of calcium, iron and zinc. In addition, moringa had the highest levels of β-carotene and ascorbic acid. Baobab had the highest level of calcium and total polyphenols. Within the limits of this study, iron bioaccessibility is influenced by a complex interplay of several components in DGLV including protein, ascorbic acid, β-carotene and total polyphenols. Moringa had the best iron bioaccessibility, and the lowest was found in baobab and one of the orange-fleshed sweetpotato with purplish young leaves. Estimating iron bioaccessibility in greens based on the mineral concentration may lead to incorrect conclusions. Based on the similarity of the iron bioaccessibility of the sweetpotato leaves and cocoyam leaf, the widely promoted "nutritious" DGLV in Ghana, the former greens have an added advantage of increasing dietary intake of provitamin A.

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