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Bioavailability of Iron and Related Components in Cooked Green Leafy Vegetables Consumed in Cameroon

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Abstract

Green leafy vegetables (GLVs) are a potential source of iron to combat iron deficiency in iron deficient population. The aim of this study was to determine the bioavailability of iron in seven species of leafy vegetables (*Solanum-scrabrun*, *Venonia amygdalina*, *Cucurbita maxima*, *Amarathus hybridus*, *Colococia esculenta*, *Solanum macrocarpon and Telfairia occidentalis*) consumed in Bamenda, Cameroon. A survey was carried out in 70 households in Bamenda, Cameroon to determine methods of preparation of these green leafy vegetables. Iron, antinutrients and vitamin C levels were determined using standard methods and the bioavailability of iron was determined using an *in vitro* dialys ability method. The vegetables used for the study were cooked with the addition of tomatoes, peanuts, melon seeds and soybean seeds. The loss of iron in GLVs was as a result of dilution caused by addition of the principal ingredients. The *V. amygdalina* cooked with soybean contained the highest level of iron (128.28 mg/100g). The *S. scrabrum* cooked with tomatoes had the highest Total phenolic coumponds of 0.91 g/100g; the *C. esculenta* recorded the highest with values ranging between 0.14 - 0.35 g/100g; the *C. maxima* cooked with soybean recorded the highest oxalate level (6.46 g/100g); and the vegetables cooked with melon seeds recording the highest in phytatelevels (70 - 1.63 g/100g). Vitamin C levels were highest in the *S. macrocarpon* cooked with tomatoes (199.96 mg/100g). Iron bioavailability was highest in *A. hybridus* cooked with tomatoes (28.09%). The iron bioavailability negatively correlated with phytates and positively with vitamin C. GLV consumed in Bamenda are good sources of iron whose bioavailability can be improved by using tomatoes in cooking.

Keywords

Green Leafy Vegetables, Iron Bioavailability, Cooking, Antinutrients, Vitamin C

1. Introduction

Iron deficiency is one of the most devastating and widespread micronutrient deficiencies, affecting about a third of the world's population [1]. It is the most prevalent cause of anaemia [2]. Its other consequences include impaired cognitive development, increased susceptibility to infection [3], increased risk of morbidity in children, unfavorable pregnancy outcomes and decreased work productivity in adults [4] (WHO, 2000).

Animal-based foods are better sources of iron, but due to their higher costs, people with low incomes get iron from plant-based foods [5]. Among these plant based foods, green leafy vegetables (GLVs) have been shown to be rich sources of iron [6]. Due to their low costs and wide availability, they can serve as an important source of iron for low income populations [5]. Unfortunately, they contain high levels of antinutritional factors such as phytates and polyphenols, which chelate iron and make it unavailable for absorption, thus decreasing its bioavailability [7]. However, heat treatment can reduce the levels of antinutritional factors in vegetables, and so increase the bioavailability of iron.

In Cameroon, a number of studies have been carried out to determine the nutrient composition of boiled GLVs [8] [9] [10].The bioavailability of carotenoids in some lesser-known vegetable recipes has also been studied [11] [12] [13]. However, there is limited information on the bioavailability of iron in Cameroonian vegetable recipes [14]. This limits their use in the fight against iron deficiency [15]. Given the above, this study was carried out to evaluate the bioavailability of iron in some GLVs consumed in Bamendain the North West Region (NWR) of Cameroon.

2. Materials and Methods

2.1. Study Design

In order to identify common indigenous GLVs consumed in Bamenda, a survey as carried out in three markets located within Bamenda (Bamenda Food market, Nkwen market and Bamenda main market). To describe the recipes and cooking methods used in the preparation of GLVs, a pretested questionnaire was administered to 70 randomly selected households in Bamenda in August 2017. Only those principally involved in cooking food for a the households provided information for the questionnaire.

2.2. Selection of Recipes, Preparation of the Vegetables

The ingredients included in the final recipes used for the study were ingredients used by at least 50% of surveyed households. Preparation and cooking methods employed for the study were also those used by at least 50% of the surveyed population. Earlyin the morning, about 4 kg of the GLVs were randomly obtained from some farmlands in mile 5 Nkwen, Bamenda, and were immediately transported in open bags to the Food Science laboratory at the University of Bamenda. All other ingredients used in the cooking of the vegetable (bouillon cubes-4 g, salt-6 g, tomato-100 g for tomato sauce, onion 50 - 60 g, peanut, melon seeds, soybean seeds and cooking oil) were purchased from the local market.

After harvesting each GLV, the leaves were destalked, rinsed under tap water and left for 20 min to drain, and then subjected to different processing conditions. The drained vegetable was divided into 6 lots of about 600 g each. One lot was left to dry at 50˚C in an electric oven (DGH, model 108,090) for 24 h while the other lots were mixed together, washed, sliced and boiled. *Solanium scrabrum* and *Colococia esculenta* were not sliced before boiling.

The sliced vegetables were added to about 3 L of boiled water and left to boil for about 5 - 15 minutes. Before being boiled, *Vernonia amygdalina* sliced leaves were hand washed to reduce the level of bitterness as described by survey participants. In the case of *S. Scabrum*, the leaves were boiled with water for 15 minutes while *C. escuelenta* leaves were boiled for 45 min with the addition of about 0.5 L of water. The boiled vegetables were allowed to drain in a strainer for 20 min, and then shared into 5 equal lots. One of the lots was allowed to dry in the oven at 50˚C for 24 h, while the other lots were processed based on the survey findings on recipes, preparation and cooking methods. In the cooking of the vegetables with "egusi", peanut and soy bean (100 - 150 g in each case), 400 g of the boiled vegetable was used and for tomatoes 100 g was used. In all cases seasoning (6 g salt and 4 g Cube) was added as well as unrefined palmoil (40 g) and onions (40 - 60 g). The cooking process was as discribed in Chagomoka *et al.* [16].

After the vegetables were cooked, they were allowed to cool to room temperature before drying. The samples (raw, boiled and four preparations for each vegetable species) were spread thinly on aluminum foil trays and dried at 50˚C for 24 h. They were then milled using an electric blender and sieved using a 500 µm mesh pore size sieve (Fisher scientific, AFNOR NFX11804), packaged in polythene bags and transported to the Food and Nutrition Research Center at the Institute of Medical Research and Medicinal Plant studies (IMPM), Yaounde Cameroon, for analysis.

2.3. Chemical Analysis

2.3.1. Total iron Content Determination

Iron contents in all the samples were determined after wet digestion with a mixture of nitric, sulphuric and hydrochloric acid using Atomic Absorption Spectrophotometer (AAS aalae spectrophotometer S11) [17].

2.3.2. Bioavailability and Ionizable Iron Estimation

The *in vitro* bioavailability of iron from the leafy-vegetables samples were determined [18] and the estimation of ionizable iron in the filtrates estimated by adaptation of the α-dipyridyl method [19]. Duplicate aliquots (5 ml) of the filtrates were taken and the volume in one set was made up to 15 ml and served as the extract blank. The other aliquot was treated with 1 ml of 10% hydroxylamine hydrochloride, 5 ml of acetate buffer pH 4.2, 2 ml of 0.1% of α, α-dipyridyl reagent, and made up to 15 ml with water. The colour developed was measured at 510 nm in the Spectronic S 11 against a reagent blank. Iron standards (1 - 15 mg) were simultaneously run in the assay. The ionizable iron content in the sample was derived after subtracting the optical density of the extract blank from that of the sample.

The levels of phytates [20], oxalates [21], phenolic compounds [22], saponin [23] and tannins [24] were determined for the samples as described in the cited references. Vitamin C was also determined according to the method described by AOAC [21].

2.4. Statistical Analysis

All chemical analyses were done in triplicates. STATISTICA version 10.0 was used for statistical analysis. One-factor analysis of variance (ANOVA) was used to test for differences among means for the raw and cooked vegetables. Tukey's post hoc multiple test was applied to determine significant differences between specific means and Pearson's correlation analysis was used for associations between antinutrients and bioavailable iron in the raw and cooked green leafy vegetables. Values of $p < 0.05$ were considered statistically significant for all analyses.

3. Results and Discussion

3.1. Vegetable Consumption and Cooking Techniques

Results of survey in the markets as seen in **Table 1** show that there were seven species of GLVs mostly consumed in Bamenda (*Solanum scrabrum*, *Vernonia amygdalina*, *Colocasia esculenta*, *Cucurbita maxima*, *Telfairia occidentalis*, *Amaranthus hybridus* and *Solanum macrocarpon)* with two consumed by all the household that is *S. scrabrum and V. amygdalina.*

As shown in **Table 2**, *S. scrabrum* was prepared using only three principal ingredients (tomatoes, melon seeds and peanuts), while all the other leafy vegetables were prepared using4 major ingredients (tomatoes, pumpkinseeds, peanuts and soybeans). Tomato was the most used ingredient in this region for the preparation of vegetables with 82.9% of the housholds using it in the prepation of *A. hybridus.* This population used pumpkin seeds mostly in the prepation of *V.*

Table 1. Names and consumption frequency for the seven selected green leafy vegetables.

Scientific name	Common name	Local names	Households consuming (%)*	Households consuming at least once a week (%)*
S. scabrum	Huckleberry	*NjamaNjama*	100	71.4
V. amygdalina	Bitter herbs	*Bitter leaf*	100	40.0
C. esculenta	Taro leaves	*Coco leaf*	78.6	11.4
C. maxima	Pumpkin leaves	*Pumpkin*	74.3	15.7
T. occidentalis	Fluted pumpkin	*Okohobong leaf*	61.4	21.4
A. hybridus	Green amaranth	*Green*	82.9	41.4
S. macrocarpon	African egg plant	*Anchia leaf*	60	22.9

*Consumption in different recipes.

Table 2. Frequency of use of major ingredients in the preparation of green leafy vegetables expressed as percentage.

Green leafy vegetables	Tomato	Pumpkin seeds	Peanuts	Soybean
A. hybridus	82.9	17.1	55.7	12.9
C. esculenta	31.4	18.6	12.9	7.1
S. scrabrum	67.1	58.6	32.9)	0
V. amygdalina	72.9	67.1	84.3	35.7
C. maxima	74.3	52.9	62.9	30
T. occidentalis	17.1	60	12.9	10
S. macrocarpon	12.9	30	11.4	8.6

amygdalina leaves (67.1%) followed by *T. occidentalis* (60%). Fewer persons used this ingredient in the prepation of the other vegetables. Peanut was principally used in Bamenda for the prepation of *V. amygdalina leaves* (84.3%). It was also used though to a lesser extent in the prepation of other vegetables with *A. hybridus* (55.7%) being the most perculiar. Soybean was used to substitute peanuts in *V. amygdalina* by 35.7% of those surveyed. Fewer people used it as an ingredient for the other vegetables. Leaks, onions, galic, ginger, crayfish, vegetable oil, and "kanwa" (natron) were used in the preparation of these vegetables as minor ingredients.

3.2. Influence of Boiling and Processing on Iron Levels

The effect of boiling and processing on the level of iron in each of the GLVs is presented in Table 3. The iron level in the raw GLVs ranged from 45.90 ± 1.73 in *C. esculentato* 180.03 ± 3.92 mg/100g in *S. macrocarpon* on DW basis. Similar values have been observed for GLVs. Ejoh *et al.* [25] reported 89.98 mg/100g DW for *C. maxima*, 164.58 mg/100 DW for *S. macrocarpon* and 87.24 mg/100g DW for *A. hybridus*. Oulai *et al.* [26] also reported similar values for *A. hybridus*.

Boiling of the vegetables significantly reduced the iron content in the vegetables with losses ranging from 12.42% in *S. scrabrum* to 65.43% in *S. macrocarpon*. The results are comparable to that of Tsado *et al.* [27] for *V. amygdalina*. For the vegetables cooked with tomatoes, the iron content is significantly lower than that of the boiled samples except for those of *A. hybridus C. esculenta* and *T. occidentalis*. A dilution effect is therefore expected in the recipes cooked with tomatoes because it contains relatively lower amounts of iron (0.9 mg/100g FW), when compared with the green leafy vegetables [28]. Similar results were also observed for the vegetables that were cooked with groundnut, except in *C. esculenta* and *C. maxima*, which recorded higher values. All vegetables that were cooked with melon seeds also had significantly lower iron levels except for *C. esculenta*, which recorded an increase. The dilution effect observed after cooking with peanuts and melon seeds were expected as these seeds contain relatively lower iron content than the raw GLVs [29]. Djuikwo *et al.* [11], demonstrated loweriron levels after cooking green leafy vegetables with peanuts.

Table 3. Iron content in the raw, boiled and cooked green leafy vegetables (mg/100g DW).

GLVs	Raw	Boiled	A	B	C	D
A.hybridus	81.96 ± 0.36^{c}	50.09 ± 1.86^{b}	78.15 ± 0.47^{c}	35.38 ± 6.11^{a}	31.70 ± 3.97^{a}	77.29 ± 4.46^{c}
C. esculenta	45.90 ± 1.73^{b}	32.47 ± 3.04^{a*}	46.09 ± 4.26^{bc}	53.34 ± 2.10^{c}	46.47 ± 0.46^{bc}	66.85 ± 2.91^{d}
S.scrabrum	96.14 ± 6.46^{e}	84.20 ± 2.54^{d}	63.13 ± 1.85^{c}	46.77 ± 3.27^{b}	45.26 ± 3.28^{b}	81.18 ± 1.51^{a**}
V.amygdalina	93.22 ± 3.92^{d}	71.68 ± 1.73^{c}	63.97 ± 3.73b^{c}	59.75 ± 6.65^{b}	35.71 ± 4.03^{a}	128.28 ± 4.48^{e}
C. maxima	82.60 ± 0.80^{e}	31.57 ± 3.30^{c}	27.10 ± 1.92^{b}	42.59 ± 1.79^{d}	12.10 ± 0.57^{a}	42.20 ± 0.35^{d}
T.occidentalis	138.37 ± 1.93^{d}	71.49 ± 1.87^{c}	134.65 ± 1.88^{d}	50.46 ± 5.39^{b}	34.61 ± 3.09^{a}	52.64 ± 6.30^{b}
S.macrocarpon	180.03 ± 3.92^{e}	62.23 ± 1.69^{c}	52.66 ± 2.52^{b}	46.05 ± 3.22^{b}	31.51 ± 1.51^{a}	80.50 ± 4.43^{d}

Values with different superscript in the same row are significantly different ($p < 0.05$); *no tomato was added in the preparation of the sauce; **the vegetable was cooked only with red palm oil and seasoning cube; A: with tomato; B: with pumpkin seeds; C: with peanut seeds; D: with soybean seeds

All the sauces cooked with soybean recorded significant higher iron content than in the boiled vegetables, with levels in *V. amygdalina* and *C. esculenta* being significantly higher than even the raw samples. This is as a result of high iron levels (600 mg/100g) in the soybean [30].

3.3. Influence of Cooking of Different Vegetables on the Anti-Nutrient Levels

The antinutrient content of the raw and cooked green leafy vegetables are shown in Table 4. The total phenolics contents in the raw samples ranged from 0.65 ± 0.02 g/100g DW in *C. maxima* to 1.95 ± 0.32 g/100g DW in *V. amygdalina* on dry weight (DW) basis. Boiling GLVs resulted in significant reductions in the total phenolic content of the vegetables except in *C. esculenta* leaves. The least loss was observed in *C. esculenta*, which may be because this vegetable was not drained after boiling. Oulai *et al.* [26] recorded losses in total phenolics content after boiling green leafy vegetables for 15 min. Higher losses recorded in *V. amygdalina* leaves can be explained by the physical crushing (Squeeze-washing), which certainly caused more destruction of the plant cell wall, and therefore enhancing the leaching of the compounds. These results indicate that the reduction in total phenolics observed as a result of boiling the vegetables could be attributed to the leaching effect.

Vegetables cooked with tomatoes had no change in the total phenolic content though in *S. scrabrum* there was an increase. Tomatoes contain relatively lower quantities of total phenolics (312.2 - 557.8 mg GAE/Kg fresh weight [31]. For *C. maxima*, only those cooked with peanut recorded an increase in total phenolics when compared with the boiled vegetables. The increase in total phenolics can be explained by the high content of total phenolic content in peanuts (8.2 g GAE/100g DW) [32]. The lower values observed for other vegetables could be due to of leaching of the compounds from the peanuts during the soaking process as peanuts were soaked before use. Cooking the boiled vegetables with melon seeds ("egusi") resulted in the highest percentage reductions of total phenolics content when compared with the boiled respective vegetables. The

Table 4. Antinutrient contents of the raw and cooked green leafy vegetables (g/100g) DW.

GLVs	Form	Phytates	Tannins	Oxalates	Phenolic C
A. hybridus	Raw	0.13 ± 0.01^{a}	0.38 ± 0.02^{d}	5.80 ± 0.33^{e}	1.41 ± 0.11^{c}
	Boiled	0.07 ± 0.01^{a}	0.21 ± 0.00^{b}	4.67 ± 0.18^{d}	0.40 ± 0.07^{ab}
	A	0.17 ± 0.04^{ab}	0.26 ± 0.01^{c}	4.76 ± 0.17^{d}	0.46 ± 0.04^{b}
	B	0.28 ± 0.06^{b}	0.12 ± 0.01^{a}	2.78 ± 0.17^{b}	0.41 ± 0.04^{ab}
	C	0.89 ± 0.07^{c}	0.11 ± 0.00^{a}	1.95 ± 0.00^{a}	0.29 ± 0.03^{a}
	D	0.09 ± 0.00^{a}	0.14 ± 0.00^{a}	3.64 ± 0.18^{c}	0.41 ± 0.02^{ab}
C. esculenta	Raw	0.12 ± 0.02^{b}	0.54 ± 0.02^{c}	4.51 ± 0.00^{d}	1.09 ± 0.11^{b}
	Boiled	0.07 ± 0.01^{ab}	0.49 ± 0.01^{c}	3.95 ± 0.00^{c}	0.98 ± 0.05^{b}
	A	ND*	0.35 ± 0.01^{b*}	4.67 ± 0.00^{d*}	1.03 ± 0.10^{b*}
	B	0.27 ± 0.02^{c}	0.19 ± 0.01^{a}	2.16 ± 0.18^{a}	0.62 ± 0.16^{a}
	C	0.70 ± 0.06^{d}	0.14 ± 0.00^{a}	2.72 ± 0.00^{b}	0.38 ± 0.02^{a}
	D	0.10 ± 0.02^{b}	0.18 ± 0.01^{a}	4.05 ± 0.00^{c}	0.61 ± 0.06^{a}
S. scrabrum	Raw	0.08 ± 0.01^{a}	0.49 ± 0.02^{c}	11.92 ± 0.00^{d}	1.92 ± 0.11^{d}
	Boiled	0.05 ± 0.00^{a}	0.24 ± 0.02^{b}	2.42 ± 0.18^{a}	0.33 ± 0.05^{ab}
	A	ND	0.29 ± 0.02^{b}	2.32 ± 0.17^{a}	0.91 ± 0.08^{c}
	B	0.08 ± 0.00^{a}	0.12 ± 0.00^{a}	6.16 ± 0.00^{c}	0.48 ± 0.04^{b}
	C	0.65 ± 0.07^{c}	0.10 ± 0.00^{a}	4.60 ± 0.18^{b}	0.25 ± 0.02^{a}
	D	0.02 ± 0.00^{a**}	0.45 ± 0.01^{c**}	2.26 ± 0.00^{a**}	0.98 ± 0.10^{c**}
V. amygdalina	Raw	0.32 ± 0.06^{b}	0.36 ± 0.01^{c}	7.32 ± 0.55^{e}	1.95 ± 0.32^{b}
	Boiled	ND	0.09 ± 0.01^{a}	1.29 ± 0.00^{a}	0.17 ± 0.02^{a}
	A	0.11 ± 0.00^{ab}	0.17 ± 0.01^{b}	5.20 ± 0.00^{d}	0.44 ± 0.06^{a}
	B	0.36 ± 0.03^{b}	0.05 ± 0.00^{a}	4.89 ± 0.18^{d}	0.50 ± 0.13^{a}
	C	1.50 ± 0.10^{c}	0.06 ± 0.01^{a}	2.42 ± 0.00^{b}	0.18 ± 0.01^{a}
	D	0.33 ± 0.08^{b}	0.10 ± 0.01^{a}	3.74 ± 0.18^{c}	0.51 ± 0.13^{a}
C. maxima	Raw	0.15 ± 0.03^{a}	0.18 ± 0.01^{b}	5.09 ± 0.00^{e}	0.65 ± 0.02^{c}
	Boiled	0.15 ± 0.02^{a}	0.09 ± 0.00^{ab}	1.70 ± 0.00^{b}	0.13 ± 0.01^{a}
	A	ND	0.08 ± 0.00^{a}	3.18 ± 0.00^{c}	0.23 ± 0.01^{ab}
	B	0.16 ± 0.06^{a}	0.17 ± 0.00^{b}	1.22 ± 0.00^{a}	0.37 ± 0.04^{b}
	C	0.64 ± 0.05^{c}	0.15 ± 0.00^{ab}	3.67 ± 0.00^{d}	0.20 ± 0.03^{a}
	D	0.29 ± 0.01^{cb}	0.15 ± 0.00^{ab}	6.46 ± 0.00^{f}	0.37 ± 0.05^{b}
T. occidentalis	Raw	0.08 ± 0.01^{a}	0.18 ± 0.02^{c}	6.33 ± 0.17^{d}	1.34 ± 0.05^{d}
	Boiled	ND	0.10 ± 0.01^{ab}	0.75 ± 0.00^{a}	0.49 ± 0.09^{ab}
	A	ND	0.14 ± 0.01^{bc}	2.45 ± 0.00^{b}	0.52 ± 0.05^{b}
	B	0.20 ± 0.06^{b}	0.06 ± 0.01^{a}	3.70 ± 0.00^{c}	0.45 ± 0.04^{ab}
	C	1.21 ± 0.01^{c}	0.12 ± 0.00^{b}	2.42 ± 0.00^{b}	0.65 ± 0.02^{a}
	D	0.26 ± 0.06^{b}	0.09 ± 0.01^{ab}	3.74 ± 0.00^{c}	0.72 ± 0.06^{c}
S. macrocarpon	Raw	0.13 ± 0.01^{b}	0.26 ± 0.03^{c}	2.49 ± 0.18^{b}	1.90 ± 0.13^{b}
	Boiled	0.08 ± 0.01^{ab}	0.15 ± 0.01^{a}	1.72 ± 0.00^{a}	0.44 ± 0.02^{a}
	A	ND	0.24 ± 0.01^{bc}	2.68 ± 0.18^{b}	0.44 ± 0.02^{a}
	B	0.40 ± 0.03^{d}	0.07 ± 0.00^{a}	3.24 ± 0.00^{c}	0.41 ± 0.01^{a}
	C	1.63 ± 0.07^{e}	0.08 ± 0.02^{a}	1.74 ± 0.00^{a}	0.34 ± 0.07^{a}
	D	0.28 ± 0.02^{c}	0.16 ± 0.02^{ab}	2.35 ± 0.17^{b}	0.48 ± 0.03^{a}

Values with different superscript in the same column are significantly different ($p < 0.05$). *indicates that no tomato was added in the preparation of the meal **indicates that the vegetable was cooked only with red palm oil and maggi seasoning cube, ND = non-detectable.

findings observed can be attributed to the relatively lower total phenolic levels of melon seeds (2.49 g GAE/100g DW) [33]. In the vegetables cooked with soybean, the total phenolic content was not significantly different except for sauces cooked with *C. maxima* and *T. occidentalis* which recorded significant higher values than the boiled samples. Soybean can contain as high as 3.6 g/100g total phenolics [34].

The tannin levels in the raw samples ranged from 0.18 ± 0.01 g/100g DW in *C. maxima* to 0.54 ± 0.02 g/100g DW in *C. esculenta* leaves. Boiling the raw GLVs resulted in a significant reduction in tannin levels except for *C. esculenta*. Cooking the boiled vegetables with tomatoes significantly increased their tannin levels except in *C. maxima* and *S. scrabum* where levels were not affected by the addition of tomatoes. The increase in the tannin levels can be explained by the high tannins content in tomatoes (3.5 mg/g DW) as reported by Shen *et al.* [35]. Cooking the boiled vegetables with peanuts did not have an impact on the tannin content of the boiled vegetables except for *A. hybridus*, *C. esculenta* and *S. scrabrum* where significant reductions in the tannin levels were observed. One of the reasons for the reduction may be related to the tannin content of groundnut (1.6%) [36] as well as the effect of soaking of the peanuts. The vegetables cooked with melon seeds, *A. hybridus*, *C. esculenta and S. scrabrum* had significantly lower values than the boiled samples, while the others remained the same. The trend can be explained by the tannin levels in "egusi" melon seeds (49.67 ± 3.91 mg/100g) which are lower than the tannin levels in the boiled vegetables [37]. The sauces cooked with soybean had varied tannin levels with those of *A. hybridus and C. esculenta* recording lower levels than the boiled samples while the others remained statistically the same. Soybeans contain relatively lower tannins levels of (25.23 mg/100g) [38] compared to the green leafy vegetables. Sharma *et al.* [34], reported higher levels. These variations in the tannin levels could also account for its variations when cooked with the boiled vegetables.

The oxalate levels in the raw GLVs ranged between 2.49 ± 0.18 g/100g DW in *S. macrocarpon* and 11.92 ± 0.00 g/100g DW in *S. scrabrum*. Boiling caused a significant decrease in oxalate levels in all the samples with percentage decrease ranging between 12.38% in *C. esculenta* and 88.11% in *T. occidentalis*. The losses observed show that leaching of oxalates is more responsible for reduction in the oxalate levels than is the effect of heat as can be seen with minimal reduction in *C. esculenta*. Akhtar *et al.* [39] showed that the least oxalate loss observed in the *C. esculenta* leaves was because, the water used to boil leaves was retained and therefore the leached out oxalates remained with the boiled vegetable. Furthermore, *C. esculenta* leaves had the least lost in oxalate levels despite the fact that its boiling time (45 min) was about three times more than that of the other vegetables. Paul *et al.* [40] and Judprasong *et al.* [41] showed similar results with up to 23% - 59% loses after blanching and up to 21% - 76% loses in the oxalate content of green leafy vegetables after blanching for 10 min in boiled water respectively. The addition of tomatoes to the boiled vegetables caused an increase in their oxalate levels compared to the boiled samples except for the vegetable prepared from *A. hybridus* and *S. scrabrum* whose oxalate levels remained unchanged. Judprasong *et al*. [41] reported 11 mg/100g of oxalates in tomatoes. Cooking the boiled vegetables with groundnuts led to an increase in oxalate levels except in *A. hybridus*, *C. esculenta and C. maxima*. Boiled vegetables cooked with melon seeds also resulted in an increase in their oxalate levels except in *A. hybridus*, *C. esculenta* and *S. macrocarpon*. Cooking the boiled vegetables with soybean also increased the oxalate levels except in *A. hybridus* and *C. esculenta*. Most of the cooked vegetables recorded an increase in oxalate content compared with the boiled vegetables. The increases observed are likely from other minor ingredients added given that the added seeds contain relatively lower levels of oxalates compared to the boiled vegetables. Furthermore, Haron and Raob [42], showed that significant oxalate loses can occur after soaking in water. Cooking the boiled vegetables with tomato, peanuts, melon seeds and soybean all led to an increase in oxalate content of many of the samples even though literature reveals that their oxalate contents are lower than that of the raw vegetables. However, the added ingredients used in cooking process could account for the varying results observed [43].

The phytate levels were determined in the raw, boiled and cooked green leafy vegetables under study. The phytate levels in the raw GLVs ranged between 0.08 ± 0.01 g/100g DW in *S. scrabrum* and 0.32 ± 0.06 g/100g DW in *V. amygdalina*. Boiling resulted in lossesof phytates in the green leafy vegetables. These results corroborate with earlier studies [26]. The findings of this study show that the loss of phytate is not only related to its leaching in water [44], but it is also due to its heat lability as seen with *C. esculenta* (40.36% loss) after boiling for 45min.Further cooking of the boiled vegetables with tomatoes showed levels that were not significantly different from those of the boiled vegetables. This was most probably because of the quantity of tomatoes used which did not contribute significant quantities of phytate and the cooking method could also have led to losses of phytate. Cooking the boiled vegetables with the added seeds all led to increased levels of phytates in the vegetables. The vegetables cooked with "egusi" melon seeds recorded the highest phytate levels ranging from 0.64 g/100g in *C. maxima* to 1.63 g/100g in *S. marcrocarpon*. The significant increase in the phytate levels can be accounted for by the high phytate content of the added seeds [34] [45] [46].

3.4. Influence of Processing of Different Sauces on the Vitamin C Levels of GLVs

The vitamin C levels in the raw GLVs ranged between 89.60 ± 6.34 mg/100g in *V. amygdalina to* 157.07 ± 5.55 mg/100g in *S. macrocarpon* DW (**Table 5**). Boiling the vegetables led to significant reduction in the levels of vitamin C ($P < 0.05$). These values confirm the results obtained by Oulai *et al*., [26] and Babalola *et al*. [47]. Most reduction in the vitamin C level in the GLVs can be accounted for by its leaching into the boiling water which is later on discarded. Cooking with tomatoes increases the vitamin C levels in the cooked vegetables compared

Table 5. Vitamin C content of raw and cooked green leafy vegetables (mg/100g Dry weight).

Green leafy vegetable/treatments	Raw	Boiled	Sauce cooked with tomato	Sauce cooked with peanut seeds	Sauce cooked with Melon seeds	Sauce cooked with soybean seeds
A. hybridus	129.50 ± 3.10[c]	70.95 ± 6.27[b]	86.55 ± 6.12[b]	43.26 ± 6.12[a]	43.62 ± 6.17[a]	44.17 ± 6.25[a]
C. esculenta	108.20 ± 6.38[c]	66.21 ± 6.69[b]	70.16 ± 8.27[b]*	35.71 ± 6.31[a]	40.06 ± 0.00[a]	49.94 ± 3.36[ab]
S. scrabrum	145.04 ± 6.41[c]	80.60 ± 8.14[b]	86.33 ± 6.10[b]	26.46 ± 3.12[a]	44.17 ± 7.81[a]	74.09 ± 3.88[b]**
V. amygdalina	89.60 ± 6.34[b]	26.87 ± 6.33[a]	71.74 ± 6.34[b]	17.38 ± 6.14[a]	8.74 ± 6.18[a]	9.08 ± 6.42[a]
C. maxima	136.07 ± 6.41[c]	87.58 ± 6.19[b]	127.59 ± 6.01[c]	31.44 ± 2.96[a]	33.92 ± 6.00[a]	43.46 ± 6.15[b]
T. occidentalis	105.83 ± 6.24[c]	52.44 ± 6.18[b]	113.21 ± 6.16[c]	17.20 ± 6.08	17.02 ± 6.02[a]	8.68 ± 6.14[a]
S. macrocarpon	157.07 ± 5.55[c]	91.44 ± 8.08[b]	199.96 ± 3.98	26.21 ± 6.18[a]	29.81 ± 3.83[a]	44.33 ± 6.27[a]

Values with different superscript in the same row are significantly different ($p < 0.05$). *indicates that no tomato was added in the preparation of the sauce; **indicates that the vegetable was cooked only with red palm oil and maggi seasoning cube.

to the boiled vegetables except in vegetable cooked with *A. hybridus* and *S. scrabrum* which remained unchanged. The increase in vitamin C levels can be accounted for by addition of high vitamin C tomatoes to the vegetable during preparation [48]. The addition of the boiled vegetables to groundnut, "egusi" and soybean, resulted in the highest reduction in vitamin C. This reduction is due to dilution of the vitamin C by the added paste due to their very low vitamin C content [49]. Vitamin C in iron absorption has been explained by its ability to prevent the formation of insoluble and unabsorbable iron compounds and to maintain iron in its reduced form (ferrous) which is absorbed from the intestine, hence improving on the bioavailability of iron [50]. Vegetables cooked with tomatoes provide vitamin C at levels that contribute significantly in meeting the daily requirements of the population. It has been shown that meals with high vitamin C levels are able to counteract the effect of anti-nutrients such as tannin and phytate [51].

3.5. Influence of Cooking of the Different Vegetables on Their Iron Bioavailability

The bioavailability of iron in the vegetables were determined in the raw, boiled and four cooked green leafy vegetables and the results are given in **Table 6**. The bioavailability of iron in the raw GLVs ranged between 1.90% ± 0.36% in *S. macrocarpon* and 20.61% ± 0.53% in *S. scrabrum.* Gupta *et al.* [5] obtained similar results. Boiling of the vegetables caused a significant increase in percent bioavailable iron for the vegetables except in *S. scrabrum* and *C. maxima* which had lower values. Djuikwo *et al.* [11] recorded lower values for bioavailability of boiled vegetables.

All the vegetables cooked with tomatoes had a significantly higher bioavailable iron than in the raw and boiled samples except for the boiled *A. hybridus* that remained unchanged. The increase in bioavailability can be accounted for by the increase in vitamin C brought about by the added tomato [48]. This enhancement in the bioavailability of iron by the addition of tomato is expected because of the enhancing effect of vitamin C on iron absorption. Furthermore, it has

Table 6. Iron bioavailability in the GLV samples (%).

Green leafy vegetable/treatments	Raw	Boiled	Sauce cooked with tomato	Sauce cooked with peanut seeds	Sauce cooked with Melon seeds	Sauce cooked with soybean seeds
A. hybridus	16.44 ± 3.42^{a}	27.77 ± 6.08^{c}	28.09 ± 0.71^{c}	26.15 ± 0.29^{bc}	13.28 ± 2.04^{a}	19.48 ± 0.94^{ab}
C. esculenta	12.18 ± 1.15^{bc}	15.78 ± 2.47^{c}	9.29 ± 0.84^{ab*}	6.96 ± 0.91^{a}	7.51 ± 3.26^{ab}	8.55 ± 0.28^{ab}
S. scrabrum	20.61 ± 0.53^{de}	13.28 ± 0.38^{bc}	16.74 ± 1.84^{cd}	24.52 ± 0.77^{e}	7.03 ± 1.20^{a}	9.06 ± 3.34^{ab**}
V. amygdalina	12.91 ± 0.69^{c}	13.21 ± 0.86^{c}	18.94 ± 1.75^{d}	1.95 ± 0.74^{a}	5.98 ± 1.33^{b}	7.38 ± 0.14^{b}
C. maxima	14.69 ± 0.80^{c}	8.47 ± 0.87^{b}	43.20 ± 1.31^{d}	3.32 ± 0.00^{ab}	2.49 ± 4.69^{a}	3.33 ± 0.68^{ab}
T. occidentalis	7.76 ± 0.76^{b}	12.51 ± 0.32^{c}	17.40 ± 0.41^{d}	7.29 ± 0.82^{b}	4.69 ± 1.75^{a}	22.92 ± 0.52^{e}
S. macrocarpon	1.90 ± 0.36^{a}	12.04 ± 1.64^{b}	31.61 ± 1.60^{d}	8.07 ± 3.10^{b}	2.92 ± 0.55^{a}	25.46 ± 0.37^{c}

Values with different superscript letter in the same row are significantly different ($p < 0.05$). *indicates that no tomato was added in the preparation of the sauce, **indicates that the vegetable was cooked only with red palm oil and maggi seasoning cube.

been shown that meals with high vitamin C levels are able to counteract the effect of anti-nutrients such as tannin and phytate [50], hence improvement in iron bioavailability. Singh *et al.*, [52] reported an increase in iron bioavailability with the addition of tomatoes to leguminous seeds.

All the vegetables cooked with peanuts and melon seeds had significantly lower bioavailable iron than in the boiled samples. In vegetables cooked with soybean, the bioavailable iron was significantly lower than in the boiled samples except for those cooked of *T. occidentalis* and *S. macrocarpon* whose bioavailable iron percentages are higher than in the raw and boiled vegetable. The lower bioavailability is expected as these vegetables contain lower quantities of iron absorption enhancer (vitamin C) and an increased amount of phytate. However, the effect of ascorbic acid and phytate are not the only factors to be considered in bioavailability of iron.

3.6. Correlation between Anti-Nutrient Levels, Vitamin C Content and Iron Bioavailability

The bioavailability of iron was negatively related to the phytate and positively related to the vitamin C content of the green leafy vegetables and the vegetables prepared from them had correlation coefficients of $r = -0.43$, $P = 0.0046$ and $r = 0.44$, $P = 0.003$ respectively. Phenolic compounds had no significant impact on the percent bioavailability ($P = 0.94$) and corroborate the studies by Brune *et al.* [53] suggesting that condensed tannins do not necessarily interfere with iron absorption. There was also no significant correlation between the oxalic acid levels in the vegetable samples and iron bioavailability ($P = 0.36$) as had been shown earlier [54].

4. Conclusions

Seven principal vegetables are consumed in Bamenda in the North West region of Cameroon. These are *A. hybridus*, *C. esculenta*, *S. scrabrum*, *V. amygdalina*, *C. maxima*, *T. occidentalis* and *S. macrocarpon*. Apart for *S. scrabrum*, which is prepared using only three principal ingredients (tomatoes, melon seeds and peanuts), all the other leafy vegetables are prepared using 4 major ingredients (tomatoes, melon seeds, peanuts and soybeans). Onion and unrefined palm oil are also used in the preparations as minor ingredients.

Preparation and cooking of the GLVs with the addition of the principal ingredients all led to a decrease in the iron content except for the vegetables cooked with soybean as principal ingredient. Total phenolic contents were reduced in all the four cooked vegetables. Most of the vegetables were lower in tannin content compared to the raw and boiled GLV. The oxalate content of most of the vegetables had higher values when compared to the boiled vegetables; and for phytate levels, the addition of egusi melon seeds led to an increase in phytate levels. All vegetables cooked without tomatoes had lower vitamin C levels than those containing tomatoes.

The addition of tomatoes in the preparation of GLV improves the bioavailability of iron than any other major ingredient. Only phytate and vitamin C correlate with iron bioavailability; with phytates negatively correlating with iron bioavailability while vitamin C positively correlating with iron bioavailability. Therefore, addition of tomatoes to GLV can be useful in food-based strategies to fight iron deficiencies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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