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Estimation of Micronutrient Contents in Traditional Green Leafy Vegetables and Their Potential Contribution to Dietary Recommended Intakes

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Abstract: Micronutrient deficiency is a public health problem even though vegetable consumption could avert it. Vitamin C [Ascorbic acid (AA) and dehydroascorbate (DHAA)], β-carotene, lutein and vitamin B9 contents in seven traditional green leafy vegetables (raw and cooked) from Ghana were determined, to identify good sources and their potential contribution to Dietary Recommended Intakes. The micronutrients were quantified using spectrofluorimetric and HPLC/DAD analytical systems. Vitamin C content of samples ranged between 7.2 and 161 mg/100 g fresh weight. β-carotene content was within the ranges of 2.97 to 10.35 mg/100 g, Lutein 13.5 to 31.6 mg/100 g and total folate 18 to 146 µg /100 g. Lutein and β-carotene were in variable relative proportions (L/C from 1.6 to 6); *Solanum macrocarpon* and *Amaranthus hybridus* samples were particularly rich in lutein and β-carotene, respectively. Losses between 45 and 94% were observed for vitamin C, between 15 to 81% for β-carotene with the exception of an increase in *Solanum macrocarpon* and 17 to 80% for lutein under boiling. Similar drastic losses were recorded in microwaved samples; however, losses in lutein and folate were comparatively lower. The traditional green leafy vegetables studied were found to be very rich in the studied micronutrients, but cooking led to considerable losses. However, the cooked vegetables represent non-negligible sources of folate, good source of lutein and could provide up to 97% and 90% vitamin A and C Recommended Dietary Intakes respectively, when a 100 g is consumed. Improved cooking methods over the traditional methods of preparation are essential for retaining more micronutrients, for the benefit of consumers.

Keywords: Traditional Green Leafy Vegetables (TGLV), Ascorbic Acid, β-Carotene, Lutein, Folate, Cooking Methods

1. Introduction

Vegetables are well known to be good sources of micronutrients, such as ascorbic acid, β-carotene, lutein, vitamin B9 among others, which confer good health to consumers. While ascorbic acid enhances non-heme iron absorption and reduces the risk of many viral diseases, carotenoids (β-carotene and lutein) are needed for stronger immune systems and good eyesight while vitamin B9 (folic acid) plays a key role in cell division, differentiation and regulation. Micronutrient deficiencies have significant effect on health, and thus pose serious economic implications on a nation in terms of medical cost and reduced productivity [1].

Ghana has a rich diversity of green leafy vegetables (both cultivated and non-cultivated) which are utilized as integral food sources, especially as major ingredients in soups, sauces and salads. Most traditionally cultivated green leafy vegetables (TGLVs) commonly consumed in Ghana include leaves of *Xanthosoma saggitifolium*, *Corchorus olitorius*, *Solanum*

macrocapon, *Amaranthus hybridus* among others. However, only few of the known TGLVs are consumed routinely in specific areas of the country because they have been part of their food and cultural systems for a long time [2].

Other consumed green leafy vegetables which are not cultivated include *Launaea taraxacifolia, Nephrolepis undulate, Talinum triangulare*, *A. viridis* among others. Preference of vegetables especially in the urban areas and among the youth, tend to shift to more exotic and expensive ones such as cabbage, lettuce, spring onions, carrot and cucumber, to the disadvantage of traditional green leafy vegetables that are cultivated or grown in the wild. Knowledge of the nutritional contributions of TGLVs is not widespread. The trend has contributed to increased micronutrient deficiencies and diet related non-communicable diseases [4]. The per capita intake of fruits and vegetables in developing countries is still considered far below the 400 g per capita per day recommended by WHO and FAO [3]. The good news is that, there is increased research attention on traditional underutilized food crops due to their potential to contribute to feeding the ever increasing world population and attaining the Sustainable Development Goal number two (2), which seeks to "end hunger, achieve food security, improve nutrition and promote sustainable agriculture" by 2030. This goal is not merely to double production of agricultural commodities but also to ensure the already existing yet underutilized species are domesticated, preserved and utilized to the utmost degree.

Reports show that traditional green leafy vegetables have comparable good micronutrient contents and are more affordable than the exotic cultivated ones [5-6]. Until recently, most efforts in Ghana have focused on vitamin supplementation to combat micronutrient malnutrition, but this is donor dependent. Vegetables consumption in diverse forms have therefore been proposed as one of the important sources of micronutrients to advocate for, especially when the traditional diets made from unrefined ingredients, including vegetables, are shifting to more refined, processed and simple sugar food products. Smith and Eyzaguirre, [2], Schonfeldt and Pretoriuos, [10], Jaarsveld *et al*. [11] and others have reported on the nutritional composition of African vegetables, and given an indication of the huge potential of overcoming micronutrient malnutrition with its associated health challenges at a negligible cost with consumption of indigenous vegetables. Identification of traditional vegetables with potential to contribute to micronutrient status of Ghanaians will be a more sustainable means of tackling health related problems associated with their deficiencies.

With the exception of a few vegetables used in salads, virtually all traditional green leafy vegetables in Ghana are cooked to increase palatability, reduce bitterness and remove itchiness. However, their nutrient concentrations are affected during cooking [7-9]. This study, therefore, aimed at estimating the micronutrient contents of traditional green leafy vegetables from Ghana and their potential contribution to dietary recommended intakes. The data obtained will be valuable for educating the public about micronutrient rich traditional green leafy vegetables in order to encourage their consumption, recommend appropriate preparation methods and prevent health consequences of micronutrient deficiencies.

2. Materials and Methods

2.1. Materials

2.1.1. Sample Collection and Preparation

Seven green leafy vegetables were sourced from a market in Ejisu, Kumasi (a forest zone between the North and the South of Ghana) and transported under cold chain to Avignon, France. Within 36 h, edible portions (leaves and stalk) of the raw vegetables were sorted, mixed evenly and weighed in three (3) sets of 150 g each. The first group was stabilized by grinding in liquid nitrogen (A11 analytical mill, IKA, Staufen, Germany) into fine powder. The second group was microwaved at full power with 20 ml of water for 10 min, allowed to cool and also ground in liquid nitrogen. The third set of vegetables was cooked in already boiling demineralized water for 5 min. Vegetable to water ratio was 1: 4 (g: ml). The remaining water after cooking was collected by draining the vegetable on a sieve. Both the cooked vegetables and the water remaining were each cooled, weighed, packaged in labelled plastic bags and rapidly frozen at -30 ˚C in a cold air active freezer (Usifroid, Elancourt, France). The frozen liquid was subsequently kept at -20°C while the frozen cooked vegetable was ground in liquid nitrogen. Both raw and cooked ground samples from the different treatments were each divided into three, packaged in labelled plastic containers and stored at -80°C until analysis. Table 1 shows details of leafy vegetables used. Spinach was bought from a local market in Avignon.

Table 1. *Selected Ghanaian Traditional Green Leafy Vegetables Studied.*

Scientific name	Family	English/Local name	Status of cultivation	Edible part and use
Amaranthus hybridus	Amaranthaceae	Efan	Cultivated	Leaves used in soups[4]
Corchorus olitorius	Malvaceae	Jute mallow/Ayoyo	Cultivated	Leaves used in soups[2]
Launaea taraxacifolia	Asteraceae	Dandelion/African lettuce	Uncultivated	The leaves-raw, cooked or dried are used in soup, sauces, salads and teas[3]
Nephrolepis undulate sp	Oleandraceae	Fern in swampy/Aya	Tree, uncultivated	Leaves used in soups for lactating mothers[3]
Nephrolepis undulate p	Oleandraceae	Fern on palm tree/Aya	Tree, uncultivated	Leaves used in soups for lactating mothers
Solanum macrocarpon	Solanaceae	Boma	Cultivated	Both fruit and leaves of parent plant are used in soups, sauces[3] Used in sauces and soups[1]
Xanthosoma sagittifolium	Araceae	Cocoyam leaves/Kontomire	Cultivated	Tubers and leaves are used. Leaves for soups, stews[3]

[1]Duru *et al*. [12, 13], [2]Osonwa *et al*. [14], [3]Personal communication, [4]Oke [15], [4]Mepha *et al*. [17]

2.1.2. Chemicals

Ascorbic acid analysis: Ascorbic acid (>99%), Trichloroacetic acid (>99%) (TCA), 2, 2-Dipyridyl (>99%), Orthophosphoric acid (85%) (H_3PO_4), Ferric chloride (>99%) ($FeCl_3$), Sodium phosphate monobasic monohydrate (>99%) (NaH_2PO_4, $1H_2O$), DL-Dithiothreitol (>99%) (DTT), Absolute Ethanol (100%), Sodium Dihydrogen Phosphate Dodecahydrate (>99%) (Na_2HPO_4, $12H_2O$), N-ethylmaleimide (>98%) (NEM) and Ultrapure water.

Carotenoid analysis: Sodium Chloride (NaCl), n-Hexane (analytical grade), Dicholoromethane, ethyl acetat, methanol, and Methyl Terbutyl ether (all HPLC grade), lycopene, ultra pure water and zirconium beads

Folates: 0.1 M phosphate buffer, ascorbic acid, chicken pancrease conjugase solution, 0.066 M Tris buffer, 2-octanol, sodium borohydride, 5 M acetic acid, formaldehyde, HCl, 5 M NaOH, 0.02 M DL-dithiothreitol (DTT), 0.02 M Trifluoroacetic (TFA) and ultra pure water

2.2. Analytical Methods

The homogenized samples stored at -80°C were analyzed for micronutrients.

2.2.1. Determination of Dry Matter

About 3 g of samples was weighed into previously dried and weighed cans, dried at 70 ˚C until constant weight was achieved within 72 h. Moisture content was estimated by difference in weights before and after drying.

2.2.2. Determination of Ascorbic Acid

Ascorbic acid was determined colorimetrically using a micro titration method as described by Stevens *et al*. [12] with little modification. In summary: 0.3 g – 0.5 g of sample was added to eppendorf tube containing 600 µl of 6% cold TCA, and immediately vortexed for about a minute to aid the extraction by the TCA. The sample was quickly centrifuged for 15 min at 4°C and 1300 g. As described in the protocol used, DTT was used to reduce dehydroascorbic acid (DHAA) to ascorbic acid. The vitamin C was quantified at 525 nm on a spectrophotometer (Safas Xenius, Monaco). Duplicate analysis was done for each sample and quantification was determined by an external calibration against ascorbic acid (Sigma Aldrich, France). Results were expressed as the sum of ascorbic acid and dehydroascorbate, in mg/100 g of fresh weight.

2.2.3. Determination of β-carotene and Lutein

Carotenoids were extracted from the powders using the micro extraction technique described by Bureau *et al.* [9].

About 500 mg of sample was weighed into 2 ml Eppendorf tube already containing about 80 µg of 0.1 mm Zirconia beads and lycopene (10 – 70 µl of a 0.2 mg ml^{-1} solution of lycopene in ethyl acetate served as internal standard) was added to the sample in the tube. Extraction was achieved by sequentially adding 100 µl of saturated aqueous NaCl solution and 50 µl of n-hexane followed by 40 s agitation using a FastPrep® homogenizer (Thermo scientific, Waltham, USA) and 2 min centrifugation at high speed (10 000 *g* at 4°C). About 200 µl of dichloromethane was added following a similar agitation and centrifugation as done previously. Finally, 800 µl of ethyl acetate was added to the sample, agitated for 5 min and centrifuged. The supernatant containing the carotenoids obtained was filtered for assay using reverse phase HPLC.

Quantification was done by HPLC-DAD (SPD-M20A Shimadzu Inc., Kyoto, Japan) using a C30 column (250×4.6 mm, particle size 3µm; YMC Co, Kyoto, Japan) under the following condition: elution at 30°C, flow rate of 1.4 ml min^{-1} and injection volume 20 µl with a methanol–methyl tert-butyl ether (MTBE) gradients.

Lutein and β-carotene were quantified at 450 nm and lycopene (used as internal standard) at 503 nm. Quantification was performed relative to the peak area of the internal standard (lycopene).

2.2.4. Determination of Folate

Folate was measured according to the method of Delchier *et al.* [18] with slight modifications. The process was in four main steps: extraction, deconjugation, transformation and purification.

i. Extraction:

30 ml of 0.1 M phosphate buffer containing 1% ascorbic acid was added to 10 g of sample and boiled in a water bath at 100°C for 10 min to achieve extraction. After cooling for 15 min, the volume was adjusted to 50 ml with phosphate buffer (0.1 M containing 1% ascorbic acid) and centrifuged for 10 min at 5000 g.

ii. *Deconjugation*: 1 ml of chicken pancreas suspension at 10 g/l was added to 10 ml of extract and incubated at 37°C for 2 h. This was to deconjugate polyglutamate forms of folate into mono- and di-glutamate.

iii. Chemical transformation of folates 5-CH_3THF: 5 ml of phosphate buffer with 0.4% ascorbic acid, 15 ml of 0.066 M Tris buffer, 1 ml of 2-octanol and 10 ml of sodium borohydride at 120 g/l were gently added to the incubated extract to avoid effervescence. The content was stirred and allowed to stand for 10 min. The pH of sample solution was adjusted to 7.4 with 5 M acetic acid. 80 µl of 37% formaldehyde and 10 ml sodium borohydride were added slowly and the pH reduced to a value less than 1 with 37% HCl. The mixture was allowed to stand for 10 min and its pH adjusted back to 5 by gradually adding 5 M NaOH and sodium borohydride. The solution was left to stand for 20 min and later transferred into the 100 ml volumetric flasks. Tris buffer was added to the volumetric flask to make it to the mark. The flasks were covered with parafilm and mixed thoroughly by shaking. The solution was filtered with a 10 ml syringe attached to the 80 micron filters (with cellulose acetate membrane) into 15 ml falcon tubes and labelled.

The series of chemical reactions were to convert all folate present in the deconjugated sample extracts to 5-

methyltetrahydrofolic acid (THF-5CH_3) monosodium glutamate and / or diglutamate.

iv. *Purification of folates* - Transformed folate was purified by affinity chromatography with Folate Binding Protein, followed by columns condition using 5 ml phosphate buffer (0.1 M, pH 7). 10 ml of sample solution was filtered through the column gel for elution of folates with 8 ml eluent solution consisting of 0.02 M DL-dithiothreitol (DTT) and 0.02 M trifluoroacetic acid (TFA) into a beaker containing 40 µl of 60% NaOH and 200 µl of 25% ascorbic acid. The volume was adjusted to 10 ml with eluent solution. About 1 ml of eluted folate solution was filtered with 0.45 µm filters into HPLC chamber vials for quantification.

v. *Folates quantification*: With the use of HPLC equipped with fluorimetric detection (RF-10AXL, Shimadzu Inc., Kyoto, Japan), Column Licrospher ® RP-18 Column 250 x 4.6 mm, 5 µm; Precolumn Guard 7.5 x 4.6 mm Licrospher ® RP-18 5 µm; column oven temperature: 30°C; Autosampler Temperature: 4°C and a flow rate of 0.8 ml per minute, 25 µl of the eluted solution was injected. The mobile phases were: ultrapure Water + 0.1% formic acid (v/v) and acetonitrile.

2.3. Statistics

At least two replicate analyses of two or three separate aliquots of each vegetable sample were carried out for ascorbic acid, β-carotene and lutein. Folate samples were in duplicate. Data was summarized using means and standard deviations in MS Excel software (2010).

3. Results and Discussion

The mean micronutrients levels in the vegetables, both raw and boiled, have been presented in Tables 2 and 3, respectively. Table 4 has the mean micronutrient levels of microwaved vegetables.

From Tables 2 and 3, all the vegetables had increased moisture content after boiling as a result of water uptake from the surrounding cooking media. Water and water-soluble substances are exchanged through leaching and water absorption/uptake during boiling. This was shown in the higher moisture content of the boiled vegetables (Table 3) and the colour change in the water used to boil the vegetable (data not presented). Moisture contents of the raw vegetables studied were high (75% for *Nephrolepis undulata species* found at swampy areas; 92% for spinach) in accordance with the knowledge that vegetables generally have high moisture. Vegetable consumption thus puts less stress on the digestive system because of the high moisture content, which will require less energy and water for digestion and nutrients assimilation.

Table 2. *Ascorbic acid, beta-carotene, lutein and folate contents of raw green leafy vegetables (fresh weight).*

Vegetable name	Moisture (g)	Vit C mg/100 g	β-carotene mg/100 g	Lutein mg/100g	Total folate µg/100g
Amaranthus hybridus	87.3±1.5	24±1.7	10.4±0.85	16±1.4	73±2.3
Corchorus olitorius	82.0±0.1	48±4.1	6.4±0.18	22±0.3	89±5.8
Launaea taraxacifolia	90.8±0.9	11±0.7	7.5±0.19	14±1.1	60±6.5
Nephrolepis undulate (found on palm trees)	75.4±0.2	161±3.2	3.0±0.13	21±1.0	18±2.8
Nephrolepis undulata species (found at swampy areas)	74.8±0.6	97±4.8	4.9±0.10	25±0.7	74±5.6
Solanum macrocarpon	86.2±0.3	21±2.1	6.4±0.58	32±2.4	35±1.4
Spinach	92.1±0.1	7±0.8	4.1±0.04	19±0.1	146±5.2
Xanthosoma sagittifolium	84.0±0.7	60±5.3	4.7±0.79	20±4.8	99±3.4

Spinach* this spinach was not a traditional vegetable from Ghana, it was obtained from an open market in Avignon to compare
All samples were analyzed in triplicates for ascorbic acid, duplicate for carotenoids and folate

3.1. Vitamin C Content of Raw Vegetables

The vitamin C content in the vegetables as reported in this study is a measure of reduced ascorbic acid and dehydroascorbic acid (DHA). Among the seven (7) TGLVs analysed (Table 2), vitamin C content was highest in the *Nephrolepis undulata* leaves that were harvested from the wild on palm tree followed by the same species harvested from swampy area. *Corchorus olitorius* and *Xanthosoma saggitifolium* leaves were the second group of vegetables with relatively high vitamin C. The spinach from France had the least of vitamin C content as compared to the TGLVs from Ghana. This could be due to its high moisture content compared to the Ghanaian vegetables studied (Table 2). On the contrary, higher vitamin C contents (36, 39 and 89 mg/100 g) were obtained for spinach and 50-60 mg/100 g for *Amaranthus viridis* [19, 20]. 19 and 25 mg/100 g Vitamin C has been reported for *Amaranthus* species [21] as well. *Xanthosoma saggitifolium* in the present study had a higher vitamin C content than that which was reported (37 mg/100 g) by Agbemafle *et al*. [22]. The study has shown wide variations of vitamin C among the studied green leafy vegetables and in comparison, with reported values in literature [8, 11]. The variations could be attributed to differences in variety, method of analysis or pre-and post-harvest factors such as agronomic practices, climatic conditions, maturation, harvesting method and storage, these factors are known to cause differences in vitamin C content of food crops [23].

Some of the richest sources of vitamin C are guava (228 mg/100 g), yellow bell pepper (184 mg/100 g), broccoli stalk (93 /100 g), and mustard spinach 170 mg/100 g [24]. The raw Ghanaian TGLVs compared favourably to these well-known vegetables, especially the uncultivated tree green leaves, *Nephrolepis undulata* species.

3.2. Carotenoids: β-carotene and Lutein Content of Raw Vegetables

Main carotenoids in the green leafy vegetables were lutein and β-carotene (Table 2), with variable relative proportions (Lutein/ β-carotene ranging from 1.6 to 6). The leaves of *Solanum macrocarpon* were particularly rich in lutein whereas, those of *Amaranthus hybridus* were high in β-carotene. Lutein concentration was lowest for *Launaea taraxacifolia* and highest for *Solanum macrocarpon* while *Nephrolepis undulata* harvested from palm tree and *Amaranthus hybridus* recorded the least and highest β-carotene contents, respectively. With the exception of *Launaea taraxafolia* and *Amaranthus hybridus*, all the other investigated vegetables had higher lutein concentrations than spinach. Similar trend was observed for β-carotene where all the vegetables with the exception of *Nephrolepis undulate,* had higher β-carotene than spinach.

In a similar study by Steiner-Asiedu *et al*. [25], β-carotene was found to be the predominant carotenoid followed by lutein in *Solanum macrocarpon* and *Corchorus olitorius*, although comparatively higher values of β-carotene (140.05 ±0.13 and 196.32 ±0.08 mg/100 g) and lutein (90.38 ±0.08 and 112.75 ±0.05 mg/100 g) were obtained in the raw samples. Slightly lower β-carotene content has been reported for *Solanum macrocarpon* (5.5 mg/100 g) and *Corchorus olitorius* (4.3 mg/100 g) [11].

In Malaysia, green vegetables such as Chinese mustard leaves, kale, lettuce, spinach and swamp cabbage contribute between 1.8-4.8 mg β-carotene/100 g edible portion. Chang *et al*. [20] obtained a range of 0.08-9.2 mg/100 g β-carotene for green vegetables while Pakistan *et al*. [26] in their studies found out that dark green vegetables have more β-carotene than other vegetables. The authors obtained about twice the β-carotene content in spinach compared to the present study. The variations in carotenoid concentrations among the vegetables could be due to differences in moisture content, variety, maturity of samples and extraction method.

Daily recommended allowance (RDA) for vitamin A range from 500 to 600 µg retinol equivalent for female adults and adolescent/male adults. Using the FAO / IN FOODS [27] vitamin A and β-carotene conversion factor of 1: 12 and the maximum recommended safe intake of 600 RE, the TGLVs could provide between 40 to 140% of vitamin A RDA when consumed raw. Results for β-carotene content of the vegetables studied support the observation of Jaarsveld *et al.* [11], that green leafy vegetables are potential sources of β-carotene and could be utilized as a cheaper and a more sustainable means of mitigating vitamin A deficiency. The high concentrations of β-carotene in *Amaranthus hybridus*, *Launaea taraxifolia* and *Solanum macrocarpon* in particular could be exploited for provision of vitamin A in diets. In India, 90% of β-carotene is obtained from consumption of green leafy vegetables [11]. *Launaea taraxifolia* for instance is gradually gaining popularity for salads and in tea production (Personal communication), thus, conscious effort towards awareness creation for increase and diversified consumption of these vegetables is essential.

Information on the lutein content of TGLVs in Ghana is scarce [25]. Djuikwo *et al*. [28] studied the lutein content of tropical leafy vegetables from Cameroon and reported similar results to the values obtained in this study. Judging from the important function of lutein in protecting against the development of age-related macular degeneration (AMD) [29] and in improving visual function, consumption of the TGLVs in this study could be promoted for potential health benefits. Intake of 6-10 mg/day lutein is reported to have positive effects. Thus, the obtained 14 to 32 mg/100 g of lutein in the investigated vegetables shows high potential of obtaining enough daily needs of lutein provided the lutein is bio-available and the vegetables are consumed raw.

3.3. Folate

The studied vegetables had total folate concentrations between 18 and 99 µg/100 g for *Nephrolepis undulata* and *Xanthosoma saggitifolium* respectively (Table 2). Spinach, a well-known vegetable for high folate content, had concentrations of 147 µg/100 g. Similar results for raw spinach have been reported by other researchers; Delchier *et al*. [30] (1.49 mg/kg) and Konings *et al*. [31] (1 mg/kg). Similar folate concentrations have been reported by Ejoh *et al*. [32]. According to Ogle *et al*. [33], green leafy vegetables are considered good sources of dietary folate when their concentrations are higher than 50 µg/100 g edible portion of the fresh vegetable. All the vegetables studied, with the exception of *Nephrolepis undulate* (found on palm trees) and *Solanum macrocarpon,* could be considered good sources of folate. In fresh form, *Xanthosoma sagittifolium*, which had the highest folate concentration among the green leafy vegetables from Ghana, will contribute about 25% of folate RDA (400 µg/day for adults) when 100 g is consumed, while spinach will contribute about 36%. Shohag *et al*. [34] reported that for one cup serving of different vegetables, 5-25% of folate RDA was provided for adults while pregnant women would gain 3-17%. Folate supplementation has been the norm for Ghanaian pregnant women aimed at preventing neural tube defect of newborns. The studied TGLVs have the potential to contribute to folate needs of consumers.

3.4. Impact of Boiling on the Vitamin C, β-carotene, Lutein and Folate Content of Green Leafy Vegetables

3.4.1. Vitamin C Content of Boiled Vegetables

Vitamin C concentrations reduced drastically by 68% and 93% for *Solanum macrocarpon* and *Launaea taraxacifolia* respectively, when the vegetables were boiled for 5 min in vegetable to water ratio of 1: 4 (Table 3). Spinach had the least vitamin C loss (44%) when boiled under the same conditions. Similar huge losses of vitamin C by blanching boiling or cooking in water have been reported in the literature [9, 22, 25, 30]. As expected, the general trend was that samples with higher vitamin C in the raw vegetables retained higher vitamin C levels when boiled while *Lunea taraxacifolia* nearly lost all of its vitamin C content. Vitamin C is heat labile and can easily

oxidize to dehydroascobic acid, which has some vitamin C activity but can also be further hydrolysed to give diketogulonic acid with total loss of its biological activity [23]. Losses could however be minimized if vegetables are steamed [8] or boiled in smaller quantities of water [9].

Even with reduced vitamin C concentration, a 100 g portion of the cooked vegetables could contribute up to 90% of vitamin C RDA [35] with *Nephrolepis undulata* leaves contributing the highest. Knowing the wound healing properties of vitamin C [19], *Nephrolepis undulate* leaves (traditionally used in Ghana to prepare soups for lactating mothers) could contribute to general good health and healing of wounds from childbearing. Cooked *Xanthosoma saggitifolium* leaves are also widely consumed in Ghana, especially those in forest areas, could also contribute about 40 percent of vitamin C RDA [35]. Considering the vital roles of vitamin C in human nutrition and the associated diseases resulting from its deficiency, indigenous vegetables that have appreciable contents when cooked should be promoted as part of everyday meals.

Table 3. *Ascorbic acid, β-carotene, lutein and folate contents of boiled green leafy vegetables (nutrient per 100 g of edible portion (EP), fresh weight).*

Vegetable name	Moisture (g)	Vit C mg/100 g	% loss	β-carotene mg/100 g	% loss / gain	Lutein mg/100 g	% loss	Total folate µg/100 g	% loss / gain
Amaranthus hybridus	91.5±0.1	3.3±1.1	87	24.9±0.2	-76	9.6±0.4	41	46±1.8	-37
Corchorus olitorius	92.9±0.1	9.3±2.3	81	11.9±0.5	-81	4.4±0.1	80	n/d	n/d
Launaea taraxacifolia	93.8±0.1	0.7±0.7	94	43.0±3.0	-43	9.7±0.6	28	n/d	n/d
Nephrolepis undulate (found on palm trees)	86.8±0.1	36.1±16.1	78	24.3±2.6	-18	12.4±1.8	41	n/d	n/d
Nephrolepis undulata species (SP*)	83.4±0.4	15.5±9.4	84	34.4±4.7	-29	18.0±2.6	29	n/d	n/d
Solanum macrocarpon	90.7±0.2	6.6±0.3	68	69.7±0.8	+8	17.4±0.5	45	36±0.6	+2.3
*Spinach**	93.8±0.3	4.0±0.5	45	35.0±0.8	-15	15.8±0.3	17	n/d	n/d
Xanthosoma sagittifolium	90.3±0.1	15.6±4.0	74	25.6±1.2	-45	11.5±0.6	42	95±11.3	-4

n/d- not determined; P* and SP* represent location where the *Nephrolepis undulate* was found: 'on palm tree' and 'swampy area'
Spinach* This spinach is not a traditional vegetable from Ghana, it was obtained from an open market in Avignon to compare
All samples were analyzed in triplicates for ascorbic acid, duplicate for carotenoids and folate

3.4.2. β-carotene and Lutein Content of Boiled Vegetables

Similar to vitamin C, losses were observed in β-carotene upon cooking the vegetables. However, losses were less pronounced in majority of the vegetables compared to vitamin C losses. Spinach comparatively recorded lower β-carotene losses (15%) when boiled than the other studied vegetables (Table 3) whose losses ranged from 18 to 80%, for *Nephrolepis undulata* harvested from palm tree and *Corchorus olitorius,* respectively. An exception was observed for *Solanum macrocarpon* where the β-carotene rather increased (8%) when boiled. Pressure cooking, microwaving, steaming and blanching has resulted in increased extractability of carotenoids.

Up to 130% increased carotenoids as a result of boiling, steaming, blanching and microwaving green leafy vegetables have been reported by Okpalamma *et al*. [36] and Bureau *et al*. [9]. The higher β-carotene concentrations in boiled *Solanum macrocarpon* could be due to difficulty in extracting carotenoids in its raw samples. Unlike the raw vegetables, boiling might have caused break down of the plant cell wall which allows for the leakage of cell contents thus releasing more β-carotene into solution [37]. According to Rodriguez-Amaya [38], lower β-carotene in raw vegetables is attributed to food component complexes which hinders the rate of extraction as compared to boiled vegetables. It could also result from loss of water and soluble solids, which can lead to release of more carotenoids per unit weight of sample [9]. Moderate cooking may increase the availability of β-carotene in vegetables but repeated cooking at high temperature rather destroys most pro-vitamins.

Hundred-gram (100g) portions of β-carotene in the studied boiled vegetables, could contribute to between 17 to 97% of RDA for vitamin A. Vitamin A deficiency (VAD) continues to be a severe health problem in Ghana especially for children and women. The studied traditional green leafy vegetables could play a significant role in addressing VAD health problems if consumed frequently, together with some fat component [38].

Lutein concentrations in boiled vegetables had minimal losses with the exception of *Corchorus olitorius*, which had about 80% loss. Spinach had the least lutein loss after boiling, the percentage loss being lower than the observed loss for β-carotene. This was not the expectation as lutein is more water soluble than β-carotene. However, Granado et al. [39] also found higher retention of lutein in boiled spinach and amaranth samples. Chang et al. [20] observed that lutein in spinach increased after boiling for 4 min but reduced after 8 min of boiling while Okpalamma et al. [36] reported up to 76% increase in lutein content with similar cooking conditions for *Telfaria occidentalis*.

3.4.3. Folate Content in Boiled Vegetables

Folate concentration remained virtually unchanged when boiled for *Solanum macrocarpon* and *Xanthososma sagittifolium* as compared to *Amaranthus hybridus*, which recorded about 37% losses. The obtained losses in these vegetables to boiling are comparable to the reported for green beans but comparatively lower in many of the reported cases by other authors. Boiling caused folate loss of 26% in green beans and 94% in hashed spinach [9]. Similarly, Mckillop *et al*. [40] observed significant decreases of folate (51% and 56% respectively) for spinach and broccoli when boiled.

Retention between 14-99% as a result of cooking has been observed depending on both the type of food involved and the method of processing [40]. Steam blanching has been observed to have higher folate retention compared to water blanching [40]. According to Delchier *et al*. [30], folates readily leach into water due to their hydrophilic property and

the losses during boiling were found to be accounted for in the boiling liquid. Generally, cooking methods with indirect contact with the food such as pressure cooking are better than boiling in terms of folate retention [41]. Thus, in advocating for increased consumption of vegetables with high folate content, practical advice on cooking methods especially those that limit water contact with the vegetables should be emphasized for optimal benefits.

3.5. Impact of Microwave Cooking on the Vitamin C, β-carotene, Lutein and Folate Content of the Leafy Vegetables

Table 4 presents the results of the microwaved samples. Unlike boiling the vegetables for 5 min, they were microwaved for 10 min to obtain similar cooked texture. The losses due to microwave cooking varied for the different micronutrients determined. Vitamin C losses for *Amaranthus hybridus, Solanum macrocarpon* and *Xanthosoma sagittifolium*, were higher than the losses due to boiling (Tables 3, 4). Losses between 78 and 91% were observed for *Solanum macrocarpon* and *Amaranthus hybridus*. Losses of β-carotene between 0.62 and 61% were observed for *Xanthoéoma sagittifolium* and *Amaranthus hybridus* respectively due to microwave cooking, and these were lower than losses due to boiling.

Table 4. *Ascorbic acid, beta carotene, lutein and folate contents of microwaved Ghanaian traditional leafy vegetables (fresh weight).*

Vegetable name	Moisture (g)	Vit C mg/100 g	% loss	β-carotene mg/100 g	% decrease	Lutein mg/100 g	% loss/gain	Total folate µg/100 g	% gain
Amaranthus hybridus	81.8±0.1	2.3±0.3	91	4.1±0.0	61	14.6±0.4	-11	80±1.9	+9
Launaea taraxacifolia	87.2±0.1	n/a	n/a	6.9±0.5	8	13.7±1.3	+1	n/a	n/a
Solanum macrocarpon	84.6±1.9	4.5±0.5	78	3.5±0.5	45	16.1±1.2	-49	104±1.3	+196
Xanthosoma sagittifolium	77.4±0.0	8.4±1.8	86	4.6±0.1	1	20.4±2.6	+3	124±4.6	+26

nd- not determined
All samples were analyzed in triplicates for ascorbic acid, duplicate for carotenoids and folate

Microwaving can profoundly affect both the texture and nutritional value of vegetables**.** Thermal treatment leads to various carotenoid losses and increasing the temperature in particular leads to significantly higher losses of some carotenoids [42]. The observed losses could be due to the high temperatures used in microwave ovens and the relatively longer time (10 min) in which the vegetables were microwaved to acceptably cooked levels.

In the case of lutein, microwave cooking had less impact with the highest loss of 49% in *Solanum macrocarpon* while Launaea *taraxacifolia* and *Xanthosoma saggitifolium* had negligible changes (Table 2) in comparison to the 90 and 78% losses detected in boiling (Table 3). Lutein as observed in this study is reported to be more resistant to heat treatment and it is not liberated in aqueous phase [30]. Given the good concentrations of lutein in the vegetables, promoting their consumption is important, especially where age-related mucular diseases (AMDs) is prevalent.

Folate concentrations measured in the microwaved vegetables were higher than in the raw vegetables, probably as a result of water loss during microwaving (Table 4), which leads to more folate per unit weight of the vegetable. All the three microwaved samples assessed for folate concentrations had increases ranging from about 9 to as high as 200% for *Amaranthus hybridus and Solanum macrocarpon* (Table 4) compared to the raw samples (Table 2). Microwave cooking, which is a cooking method with indirect contact with food, was reported to be better than boiling in terms of folate retention [43].

The variation in retention or losses of micronutrients is influenced by type of vegetables, environmental conditions, cooking method and interaction between the cooking method and the type of vegetables [20]. In general, losses of the micronutrient to microwave cooking were lower when compared to the losses due to boiling. The minimal losses compared to boiling was expected as many studies have shown similar or better retention of nutrients for microwave cooking than the same product prepared conventionally. Using recommended procedures for microwave cooking and reheating would result in products that are satisfying from both a sensory and nutritional standpoint [43]. Contradictory results have however been reported on the retention of nutrients by microwave cooking due to different conditions (time, power, and added water) that are employed. Vitamin C is reported to have the highest losses probably as a result of thermal degradation during microwave cooking [44] as observed in this study.

4. Conclusion

The traditional green leafy vegetables studied had high and varied micronutrient contents, comparable to established good sources. *Amaranthus hybridus*, *Lunear taraxifolia* and *Solanum macrocarpon* had high concentrations of β-carotene, whereas all the vegetables had appreciable levels of lutein. *Xanthosoma sagittifolium* and *Nephrolepis undulate* could represent good or non-negligible sources of vitamin C and folate, respectively. Both boiling and microwave cooking led to drastic losses of the micronutrient; however, cooked vegetables could contribute significantly to RDA of the various tested micronutrients. Consistent and varied consumption of indigenous vegetables stand a huge potential to solving micronutrient deficiency health problems, especially that of vitamin A. Improved cooking methods for enhanced micronutrients retention should be advocated for.

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