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
In order to contribute to the micronutrient status of Ghanaians, the study also 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, E lancourt, France). The frozen liquid was subsequently kept at -20°C and the frozen cooked vegetable was ground in liquid nitrogen. The frozen cooked vegetable was ground in liquid nitrogen and 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.

Table 1: Selected Ghanaian Traditional Green Leafy Vegetables Studied.

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Family</th>
<th>English/Local name</th>
<th>Status of cultivation</th>
<th>Edible part and use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>Amaranthaceae</td>
<td>Efan</td>
<td>Cultivated</td>
<td>Leaves used in soups&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Corchorus olitorius</em></td>
<td>Malvaceae</td>
<td>Jute mallow/Ayoyo</td>
<td>Cultivated</td>
<td>Leaves used in soups&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Launaea taraxacifolia</em></td>
<td>Asteraceae</td>
<td>Dandelion/African lettuce</td>
<td>Uncultivated</td>
<td>The leaves-raw, cooked or dried are used in soup, sauces, salads and teas&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Nephrolepis undulate</em></td>
<td>Oleandraceae</td>
<td>Fern in swampy/Aya</td>
<td>Tree, uncultivated</td>
<td>Leaves used in soups for lactating mothers&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Nephrolepis undulate</em></td>
<td>Oleandraceae</td>
<td>Fern on palm tree/Aya</td>
<td>Tree, uncultivated</td>
<td>Leaves used in soups for lactating mothers&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Solanum macrocarpon</em></td>
<td>Solanaceae</td>
<td>Bona</td>
<td>Cultivated</td>
<td>Both fruit and leaves of parent plant are used in soups, sauces&lt;sup&gt;1&lt;/sup&gt; Used in sauces and soups&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>Xanthosoma sagittifolium</em></td>
<td>Araceae</td>
<td>Cocoyam leaves/Kontomire</td>
<td>Cultivated</td>
<td>Tubers and leaves are used. Leaves for soups, stews&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Duru et al. [12, 13], <sup>2</sup>Osonwa et al. [14], <sup>3</sup>Personal communication, <sup>4</sup>Oke [15], <sup>5</sup>Mepha et al. [17]
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. The 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 Delclier 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-CH₃THF: 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. Folate quantification: With the use of HPLC equipped with fluorimetric detection (RF-10AXL, Shimadzu Inc., Kyoto, Japan), Column Licospher ® RP-18 Column 250 x 4.6 mm, 5 µm; Pre-column 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% acetic acid (v/v) and acetonitrile.

2.3. Statistics

At least two replicate analyses of two or three separate

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

<table>
<thead>
<tr>
<th>Vegetable name</th>
<th>Moisture (g)</th>
<th>Vit C mg/100 g</th>
<th>β-carotene mg/100 g</th>
<th>Lutein mg/100g</th>
<th>Total folate µg/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaranthus hybridus</td>
<td>87.3±1.5</td>
<td>24±1.7</td>
<td>10.4±0.85</td>
<td>16±1.4</td>
<td>73±2.3</td>
</tr>
<tr>
<td>Corchorus olitorius</td>
<td>82.0±0.1</td>
<td>48±4.1</td>
<td>6.4±0.18</td>
<td>22±0.3</td>
<td>89±5.8</td>
</tr>
<tr>
<td>Launaea taraxacifolia</td>
<td>90.8±0.9</td>
<td>11±0.7</td>
<td>7.5±0.19</td>
<td>14±1.1</td>
<td>60±6.5</td>
</tr>
<tr>
<td>Xanthosoma sagittifolium</td>
<td>92.5±0.1</td>
<td>7×0.8</td>
<td>4.1±0.04</td>
<td>19±0.1</td>
<td>146±5.2</td>
</tr>
</tbody>
</table>

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 sagittifolium* 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 sagittifolium* in the present study had a higher vitamin C content than that which was reported (37 mg/100 g) by Agbemamele 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 taraxacifolia 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 undulata, 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 sagittifolium 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 undulata (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 Launaea taraxacifolia nearly lost all of its vitamin C content. Vitamin C is heat labile and can easily
oxidize to dehydroascorbic 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 undulata* leaves (traditionally used in Ghana to prepare soups for lactating mothers) could contribute to general good health and healing of wounds from childbirth. Cooked *Xanthosoma sagittifolium* 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).

<table>
<thead>
<tr>
<th>Vegetable name</th>
<th>Moisture (g)</th>
<th>Vit C mg/100 g</th>
<th>% loss</th>
<th>β-carotene mg/100 g</th>
<th>% loss</th>
<th>Lutein mg/100 g</th>
<th>% loss</th>
<th>Total folate µg/100 g</th>
<th>% loss</th>
<th>% loss/gain</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus hybridus</em></td>
<td>91.5±0.1</td>
<td>3.3±1.1</td>
<td>87</td>
<td>24.9±0.2</td>
<td>-76</td>
<td>9.6±0.4</td>
<td>41</td>
<td>46±1.8</td>
<td>-37</td>
<td></td>
</tr>
<tr>
<td><em>Corchorus olitorius</em></td>
<td>92±0.1</td>
<td>9.3±2.3</td>
<td>81</td>
<td>11.9±0.5</td>
<td>-81</td>
<td>4.4±0.1</td>
<td>80</td>
<td>n/d</td>
<td>n/d</td>
<td></td>
</tr>
<tr>
<td><em>Launaea taraxacifolia</em></td>
<td>93.8±0.1</td>
<td>0.7±0.7</td>
<td>94</td>
<td>43.0±3.0</td>
<td>-43</td>
<td>9.7±0.6</td>
<td>28</td>
<td>n/d</td>
<td>n/d</td>
<td></td>
</tr>
<tr>
<td><em>Nephrolepis undulata</em> (found on palm trees)</td>
<td>86.8±0.1</td>
<td>36.1±16.1</td>
<td>78</td>
<td>24.3±2.6</td>
<td>-18</td>
<td>12.4±1.8</td>
<td>41</td>
<td>n/d</td>
<td>n/d</td>
<td></td>
</tr>
<tr>
<td><em>Nephrolepis undulata</em> species (SP*)</td>
<td>83.4±0.4</td>
<td>15.5±9.4</td>
<td>84</td>
<td>34.4±4.7</td>
<td>-29</td>
<td>18.0±2.6</td>
<td>29</td>
<td>n/d</td>
<td>n/d</td>
<td></td>
</tr>
<tr>
<td><em>Solanum macrocarpon</em></td>
<td>90.7±0.2</td>
<td>6.6±0.3</td>
<td>68</td>
<td>69.7±0.8</td>
<td>18</td>
<td>17.4±0.5</td>
<td>45</td>
<td>36±0.6</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td><em>Spinach</em></td>
<td>93±0.3</td>
<td>4.0±0.5</td>
<td>45</td>
<td>35.0±0.8</td>
<td>-15</td>
<td>15.8±0.3</td>
<td>17</td>
<td>n/d</td>
<td>n/d</td>
<td></td>
</tr>
<tr>
<td><em>Xanthosoma sagittifolium</em></td>
<td>90.3±0.1</td>
<td>15.6±4.0</td>
<td>74</td>
<td>25.6±1.2</td>
<td>-45</td>
<td>11.5±0.6</td>
<td>42</td>
<td>95±11.3</td>
<td>-4</td>
<td></td>
</tr>
</tbody>
</table>

n/d: not determined; P* and SP* represent location where the *Nephrolepis undulata* 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 *Xanthosoma 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, \(\beta\)-carotene, Lutein and Folate Content of the Leafy Vegetables

Table 4 presents the results of the microwaved samples.

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

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 sagittifolium 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 macular 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. 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 \(\beta\)-carotene between 0.62 and 61% were observed for Xanthoëoma sagittifolium and Amaranthus hybridus respectively due to microwave cooking, and these were lower than losses due to boiling.

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 \(\beta\)-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.
Acknowledgements

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References


Beans (Phaseolus vulgaris) and spinach (Spinacea oleracea) are commonly used vegetables in Vietnam. They are rich in vitamins and minerals, making them a valuable source of micronutrients in the diet. The micronutrient composition and nutritional importance of these vegetables are crucial for understanding their role in human nutrition.

Determining the nutritional value of these vegetables is important for dietary planning and public health. For instance, a study by Ogle, B., Dao, H., Generose, M and Leif, H. (2001) found that traditional leafy vegetables in Vietnam are a rich source of various micronutrients, which are essential for maintaining good health.

Different cooking methods can influence the nutrient content of these vegetables. For example, Delchier N, Reich M, Renard C M G C (2012) investigated the impact of cooking methods on folate retention in various foods. They found that different cooking methods can significantly affect folate retention, with baking and steaming being less detrimental to folate content compared to other methods.

Moreover, the World Health Organization and Food and Agriculture Organization of the United Nations (2004) emphasize the importance of micronutrients in human nutrition, highlighting the need for a joint FAO/WHO expert consultation to address the dietary needs of different populations globally.

In conclusion, the study of the micronutrient composition and nutritional importance of beans and spinach in Vietnam is essential for developing effective dietary guidelines and improving public health. Further research is needed to explore the specific nutritional benefits of these vegetables under different cooking conditions and to assess their role in meeting the dietary requirements of the local population.