# Dark green leafy vegetables: Nutrient and total polyphenols contents, and estimation of iron bioavailability using *in vitro* digestion/Caco-2 cell model

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# Background

- Dark green leafy vegetables (DGLV) are generally considered to be important sources of micronutrients such as iron and vitamin A
  - DGLV were reported to contribute about 19-39% of iron and 42-68% of vitamin A*
- Iron deficiency in SSA is public health concern, especially for people living rural communities where DGLV form a significant part of diet
  - Sauce prepared from amaranth or Jew's mallow/corchorus, examples of DGLV, eaten with a thick maize paste by young Burkinabe women did not increase the amount of iron absorbed**

* Faber, M., P. Van Jaarsveld, and R. Laubscher, *The contribution of dark-green leafy vegetables to total micronutrient intake of two-to five-year-old children in a rural setting.* Water SA, 2007. **33**(3): p. 407-412.

** Cercamondi, C.I., et al., *A higher proportion of iron-rich leafy vegetables in a typical Burkinabe maize meal does not increase the amount of iron absorbed in young women.* Journal of Nutrition, 2014. **144**(9): p. 1394 - 1400.

# Background *Contd.*

- An inadequate dietary intake of bioavailable iron and vitamin A could be the primary cause of iron and vitamin A deficiencies.

- Concentration of micronutrients MAY NOT indicate bioavailability

# Background *Contd.*

- Polyphenols and phytates in cereal and leguminous foods have been shown to limit bioavailability of essential micronutrients including iron, calcium and zinc, and these staples are usually consumed with these DGLV that may also contain significant levels of these inhibitors*❉

- Ascorbic acid and β-carotene, are enhancers of iron absorption from food.
  - Relatively high in DGLV


- Davies, N.T. and H. Reid, An evaluation of the phytate, zinc, copper, iron and manganese contents of, and Zn availability from, soya-based textured-vegetable-protein meat-substitutes or meat-extenders. British Journal of Nutrition, 1979. 41(03): p. 579-589.

❉ Gautam, S., K. Platel, and K. Srinivasan, Promoting influence of combinations of amchur, β-carotene-rich vegetables and Allium spices on the bioaccessibility of zinc and iron from food grains. International Journal of Food Sciences and Nutrition, 2011. 62(5): p. 518-524.

# Background *Contd.*

- The *in vitro* digestion/Caco-2 cell model has been suggested to an appropriate physiological tool to predict iron availability [#]
  - Thus, it was used to measure iron bioavailability of selected greens available in Ghana in comparison with 5 varieties of SP

[#] Glahn, R.P., et al., *A comparison of iron availability from commercial iron preparations using an in vitro digestion/Caco-2 cell culture model.* Journal of Nutritional Biochemistry, 2000. **11**(2): p. 62-68.

# Study focus

- To compare the nutrient contents and iron bioavailability using the *in vitro* digestion/Caco-2 cell model of five different cultivars of sweetpotato with five other commonly consumed DGLV in Ghana: cocoyam, corchorus, baobab, kenaf and moringa.

# Materials and methods

## Experimental Design

- Completely Randomised Design

# Materials and methods *Contd.*

- Compositional analyses were carried
- *In vitro* digestion/Caco-2 cell model for iron

# Findings

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

| DGLV | Moisture (g)[¥] | Calcium (mg)[§] | Iron (mg) | Zinc (mg) | ß-carotene (µg) | Ascorbic acid (mg) | Total Polyphenols (mg GAE)[†] |
|---|---|---|---|---|---|---|---|
| OFSP1 | 84.09±0.34$^{cd}$ | 95.61±8.01$^{cd}$ | 3.41±0.36$^{ab}$ | 0.44±0.01$^{b}$ | 10533$^{ab}$ | 0.74±0.16$^{c}$ | 506.93±86.76$^{b}$ |
| OFSP2 | 84.76±0.75$^{bcd}$ | 81.04±3.24$^{d}$ | 1.89±0.29$^{b}$ | 0.42±0.02$^{b}$ | 8280$^{abc}$ | 0.50±0.15$^{c}$ | 356.69±79.60$^{c}$ |
| OFSP3 | 87.24±0.13$^{a}$ | 103.25±2.59$^{cd}$ | 2.58±0.21$^{ab}$ | 0.36±0.03$^{b}$ | 7053$^{bc}$ | 0.45±0.07$^{c}$ | 336.38±63.15$^{cde}$ |
| PFSP | 84.30±0.26$^{cd}$ | 84.75±8.83$^{cd}$ | 2.04±0.36$^{b}$ | 0.44±0.04$^{b}$ | 4472$^{bc}$ | 0.48±0.03$^{c}$ | 231.44±49.77$^{cde}$ |
| WFSP | 83.91±0.26$^{d}$ | 87.02±6.80$^{cd}$ | 3.27±0.34$^{ab}$ | 0.40±0.03$^{b}$ | 9501$^{abc}$ | 0.34±0.10$^{c}$ | 234.86±0.16$^{cde}$ |
| Baobab | 85.97±0.53$^{b}$ | 535.63±22.93$^{a}$ | 4.59±1.28$^{a}$ | 0.65±0.03$^{b}$ | 7166$^{bc}$ | 25.50±0.01$^{b}$ | 1646.75±69.44$^{a}$ |
| Cocoyam | 85.23±0.64$^{bc}$ | 166.39±15.13$^{b}$ | 2.64±0.16$^{ab}$ | 1.49±0.47$^{a}$ | 3911$^{c}$ | 1.14±0.01$^{c}$ | 196.05±10.96$^{e}$ |
| Corchorus | 78.99±0.38$^{f}$ | 121.41±3.61$^{c}$ | 2.48±0.23$^{ab}$ | 0.45±0.02$^{b}$ | 9298$^{abc}$ | 3.53±0.58$^{c}$ | 337.94±16.44$^{cde}$ |
| Kenaf | 80.68±0.18$^{e}$ | 90.24±17.76$^{cd}$ | 2.94±0.25$^{ab}$ | 0.35±0.05$^{b}$ | 8959$^{abc}$ | 21.79±1.54$^{b}$ | 202.42±9.29$^{de}$ |
| Moringa | 78.81±0.42$^{f}$ | 186.22±23.81$^{b}$ | 4.55±1.88$^{a}$ | 0.77±0.06$^{b}$ | 14169$^{a}$ | 46.30±4.78$^{a}$ | 347.38±14.59$^{cd}$ |
| *P-value* | *<0.001* | *<0.001* | *0.002* | *<0.001* | *<0.001* | *<0.001* | *<0.001* |

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

# Findings *Contd*.

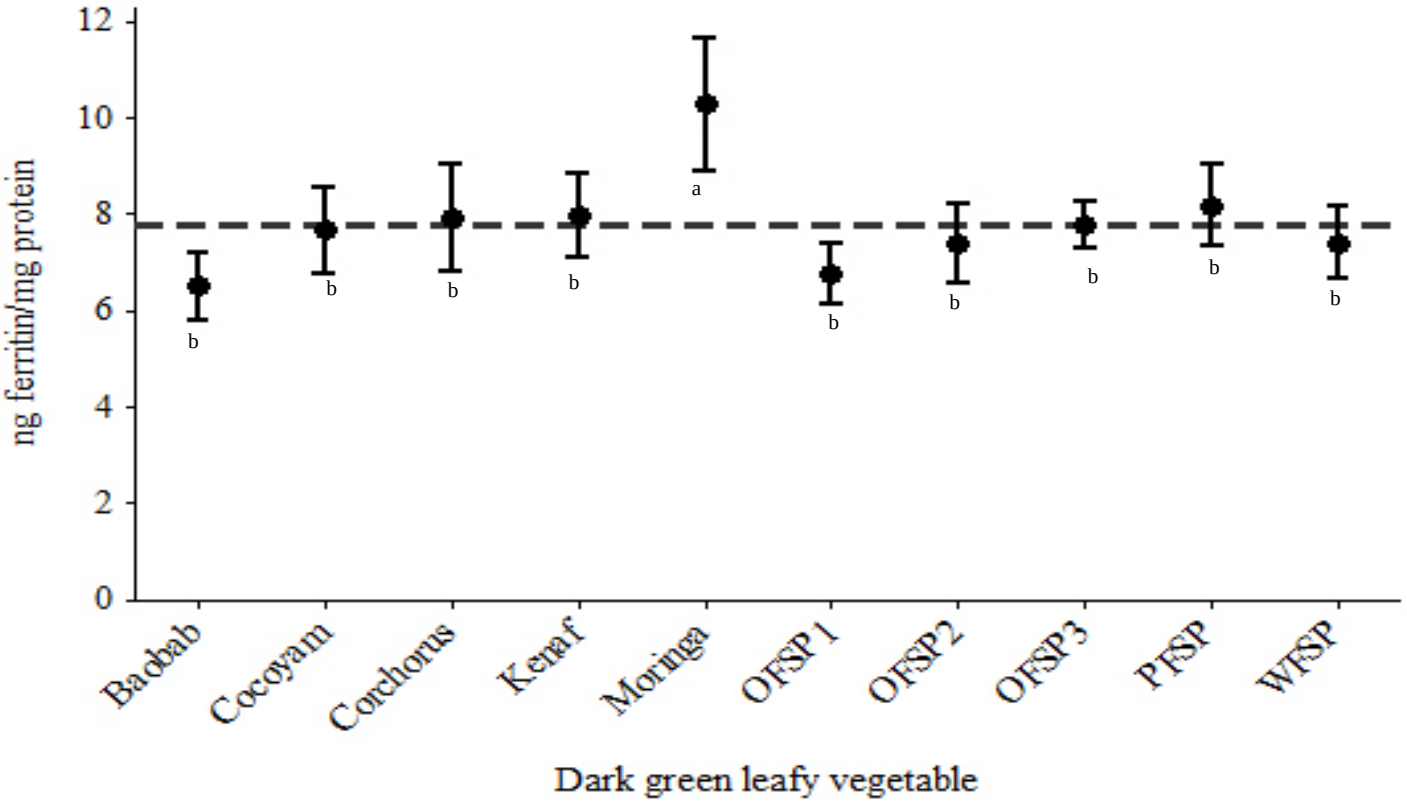


**Figure 1.** Ferritin formation per half a gram of freeze-dried green leafy vegetable

Vertical lines are means with 95% confidence interval of ng ferritin/mg protein from the various greens ($n$=12 for Corchorus; $n$=18 for OFSP1, PFSP, Baobab, Kenaf and Moringa; $n$=21 for OFSP2, OFSP3, WFSP and Cocoyam) normalised to the blank digest ferritin level;

Horizontal line indicates the overall mean of ng ferritin/mg protein;

Mean with 95% confidence interval with a different letter is significantly different ($p < 0.0001$).

# Summary of key findings

- Among the DGLV investigated, iron content does not explicitly indicate its bioavailability

- The sweetpotato leaves and cocoyam had similar iron bioavailability; thus the sweetpotato leaves could be used in culinary preparations, and have an added advantage of increasing dietary intake of β-carotene compared to that of cocoyam

- Generally, the level of iron bioavailability from the DGLV was relatively low (6 - 10 ng ferritin/mg protein) compared with our previous work from the same laboratory on complementary food (12 - 34 ng ferritin/mg protein)

# Summary of key findings

- The composition of nutrients in moringa compared with the other DGLV contributed to the highest bioavailability of iron as obtained from the in vitro Caco-2 cells model study

- OFSP1 had significantly similar levels of β-carotene and iron to moringa, and a third of the total polyphenols of baobab, its iron bioavailability was lower than moringa, indicating that the reported caffeoylquinic acid derivatives in sweetpotato leaves may have limited the bioavailability of iron

- In addition, the level of total polyphenols irrespective of the concentrations of ascorbic acid, β-carotene and iron appeared to be the major factor limiting iron bioavailability of OFSP1 and Baobab

# Conclusions & Recommendation

- Within the limits of this study, iron bioavailability is influenced by a complex interplay of several components in DGLV including protein, ascorbic acid, β-carotene and total polyphenols.

- Moringa had the best iron bioavailability, and the lowest was found in baobab and one of the orange-fleshed sweetpotato with purplish young leaves

- Estimating iron bioavailability in greens based on the mineral concentration may lead to incorrect conclusions

- Based on the similarity of the iron bioavailability of the sweetpotato leaves and cocoyam leaf, the widely promoted "nutritious" DGLV in Ghana, the former greens have an added advantage of increasing dietary intake of provitamin A.

# Acknowledgements


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Healthy, Happy Ghanaian kid

# Thank you