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SPECTROPHOTOMETRIC DETERMINATION OF IRON CONTENT IN SIX INDIGENOUS GREEN LEAFY VEGETABLES CONSUMED IN MUAK LEK, THAILAND

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ABSTRACT

This study was undertaken to determine the iron and moisture content of six popular green leafy vegetables (GLV) consumed in Muak Lek, Thailand. The six different green leafy vegetables were *Holy basil* **(**Bai Kaprow), *Sweet basil* (Bai horapa), *Amaranthus spinosus* (Pak khoum), *Lettuce* (Pak Salad), *Chinese kale* (Pak khana), *Coccinia* (Pak tumlun). The spectrophotometric method was used to determine the concentration of iron from uncooked (raw), cooked, and their pot liquor at 480 nm. Among these green leafy vegetables, the pot liquor of *Coccinia* (21.41 ppm) had the highest iron content, whereas the least concentration was found in cooked *Lettuce* (0.235 ppm). Besides, raw *Lettuce* showed the highest moisture content of 94.74%, and raw *Amaranthus* appeared to have the least moisture content, 66.18%. All the investigated GLV samples contain iron; however, there were variations among the uncooked, cooked, and pot liquor. Moreover, the GLV pot liquor had a higher concentration of iron than cooked and uncooked (raw) green leafy vegetables. Therefore, cooked GLV with its pot liquor consumption would be the best option to obtain large iron quantities.

Keywords: Vegetables, Iron, Moisture, Spectrophotometer, UV, Green, Pot Liquor, GLV.

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INTRODUCTION

Green leafy vegetables (GLV) play a substantial role in human nutrition and are essential for a healthy life.[1] They provide an adequate amount of dietary fibers, minerals, vitamins, and other nutrients[2], and they are beneficial for the maintenance of good health and prevention of various diseases.[3] GLV have low energy densities, and thus, they are recommended for weight management[4], and some leaves have unique properties; to overcome the swelling of the human body due to sprains.[5]

GLV is essential from both economic and nutritional perspectives. The presence of fiber in green vegetables has been reported to have beneficial health effects on blood cholesterol and to aid in preventing major bowel diseases. At the same time, in diabetic subjects, it progresses their glucose intolerances. To be healthy, minerals are necessary, and they are required for essential body functions such as heartbeat, muscle contraction, movement, growth, and regulatory processes.[4] Numerous plants and their products play an imperative role in human health, and they help prevent and cure many sicknesses.[6,7]

GLV contains various minerals such as calcium, iron, copper, phosphorous, zinc, chloride, and sodium, and these minerals play a significant role in growth and metabolism. Elements such as sodium, potassium, iron, and calcium provide an alkalizing effect to the acidity produced by other foods.[8] One essential transition metal in the living system is iron, which carries oxygen to the tissues and is responsible for the appropriate protection against microbes.[9] Vegetables are an essential part of the diet since they contribute proteins, vitamins, iron, calcium, and other valuable nutrients to us.[10] The total iron present in an average adult is forty grams and is mostly stored in the spleen, liver, and bone marrow.[11] Moreover, vegetables contain essential and toxic elements over a wide range of concentrations.[12]

Iron deficiency is the most common and widespread nutritional deficiency globally, and it has been estimated that 30 to 40 percent of the world's population is iron deficient.[13] Iron is the fourth most abundant element on the earth; however, its deficiency in humans is common. It is a component of hemoglobin, present in the ubiquitous RBC in the body, that conveys oxygen throughout it; thus, iron is essential to make healthy RBCs. Non-vegetarians are rarely a victim of iron deficiency; however, vegetarians are more susceptible to the lack of iron.[14]

The contribution of iron in humans is dominantly through our diet. Heme and non-heme are the two primary forms of iron, and these two forms differ significantly in the molecular mechanisms of their absorption and bioavailability. Heme is mainly obtained from meat, fish, and poultry, whereas non-heme is usually obtained from plants. The absorption of heme is not much affected by other food components eaten in the same meal; however, the absorption of non-heme is greatly influenced by them. Hence, the total amount of iron and its two forms in food nutrition are significantly essential.[15]

The deficiency of iron causes many serious issues such as fatigue and reduced work performances.[16] Moreover, extremely high iron doses can also cause health issues such as organ failure, coma, convulsions, and even death. Those people with hereditary hemochromatosis can develop severe liver cirrhosis, liver cancer, and heart disease, and all these complications can be avoided by taking iron and vitamin C supplements.[15-17] Typical iron deficiency symptoms include reduced work capacity, impaired cognitive development, increased parterre delivery rates, and low birth weight. Iron deficiency in the human body results from low dietary intake, inadequate absorption, or iron loss.[18–21]

The RDA (Recommended Dietary Allowance) for iron has been set at 8 mg for men, 18 mg for women, and 27 mg for pregnant women per day, and these values are based on a person consuming 10 percent of dietary iron from heme sources.[22] GLV can be a good source of some minerals and may provide adequate intakes of iron in vegetarian diets.[23] GLV contain inhibitors that limit their potential as a source of iron, and evidence suggests that GLV may constitute a bioavailable source of iron. Also, GLV has been proved to be better source of bioavailability iron than cereals and legumes. The isocaloric substitution of a portion of grains and legumes with GLV can increase iron density and increase iron absorption from meals.[24]

Food-based approaches are advised as the priority in strategies intended to meet iron requirements.[20] The essential basic strategies that have been used to combat iron deficiency include iron supplementation, food fortification, and the consumption of high iron content foods.[25-26] A green leafy vegetable that contains greater than 3.6 mg of iron per RACC (Reference Amounts Customarily Consumed per eating occasion) is an excellent source of iron and is labeled as a good or excellent source of nutrients.[27]

The main objective of this research was to determine the concentration of iron and moisture composition of the locally available green leafy vegetables such as *Holy basil* **(**Bai Kaprow), *Sweet basil* (Bai horapa), *Amaranthus spinosus* (Pak khoum), *Lettuce* (Pak Salad), *Chinese kale* (Pak khana), *Coccinia* (Pak tumlun).

EXPERIMENTAL

Method and Materials

The research was done at the Asia-Pacific International University, Muak Lek, Thailand. Samples of healthy and commonly consumed six different Green Leafy Vegetables (GLV) were purchased from the local market, Muak Lek. Six GLV investigated were: *Holy basil* **(**Bai Kaprow), *Sweet basil* (Bai horapa), *Amaranthus spinosus* (Pak khoum), *Lettuce* (Pak Salad), *Chinese kale* (Pak khana), *Coccinia* (Pak tumlun). Three preparations were used: uncooked vegetables (raw), cooked vegetables, and their pot liquors.

Cleaning of Apparatus

All the glassware apparatus were cleaned by soaking them in 10%(v/v) HNO_3 for 24 hours, rinsed with deionized water, and dried in an oven before using them for experimental purposes.[15]

Preparation of the Stock Solution

1. **100 ppm Iron (III) Nitrate Solutions:** The 100 ppm (100 μg/ml) iron (III) nitrate stock solution was prepared by dissolving 0.217 g of ferric nitrate in 100 ml distilled water followed by the addition of 2 ml concentrated HCl. The content was diluted to 500 ml using distilled water in a 500 ml volumetric flask.
2. **0.1M HCl Solution:** The 0.1M HCl was prepared by dissolving 8.2 ml of Con. HCl in 1000 ml using distilled water.

3. **0.1M KCSN (Potassium thiocyanate) Solution:** A 0.1M KCSN solution was prepared by dissolving 4.859 g of KCSN in 500 ml using distilled water.

Preparation of the Standard Solution

Five different standard solutions were prepared, each having a concentration of 0, 5, 10, 15, 20, and 25 ppm (μg/ml). A 5 ppm solution was prepared by diluting 5 ml of 100 ppm iron(III)nitrate stock solution in 95 ml of 0.1M HCl solution and 10 ppm by diluting 10 ml of 100 ppm iron(III)nitrate stock solution in 90 ml of 0.1M HCl solution. Similarly, the other standard solutions were prepared by diluting 15 ml, 20 ml, and 25 ml of 100 ppm iron(III)nitrate stock solution to 100 ml using 0.1M HCl solution. The zero-ppm solution was prepared without ferric nitrate solution.[14,28]

Calibration Curve

A volume of 10 ml of standard solution (0, 5, 10, 15, 20, and 25 ppm) was pipetted out into a six separate test tube and then 5 ml of 0.1M KCSN solution and stirred well. After adding KSCN, the absorbance was measured without delay using a UV-Vis spectrophotometer at 480 nm because the absorbance value can be affected as the color of the solution fades within 15-20 minutes. The 0.1M HCl solution was used as the blank.

Finding the Absorbance of Fresh GLV Samples

The Green leafy vegetables (GLV) samples were washed using deionized water and cut into small pieces. They were air-dried at room temperature in the laboratory for two weeks. Two grams of dried GLV were taken in a crucible and heated (200-250˚C) over a Bunsen burner until the sample was completely turned into ash, and then grounded into a fine powder. To 1 gram of ash sample, 20 ml of a 0.1M HCl solution was added carefully, stirred and mixed well. The ware was then filtered to collect the filtrate, and then 10 ml of the filtrate was mixed with 5 ml of 0.1M KCSN solution and shaken well. The sampling solutions were taken in separate cuvettes, and the absorbance was measured immediately using a UV-Vis spectrophotometer at 480 nm. The blank used was 0.1M HCl.[14,28]

Finding the Absorbance of Cooked GLV Samples and Their Pot Liquors

Green leafy vegetables were rinsed in deionized water and then boiled in deionized water. Each green leafy vegetable was boiled separately in a stainless steel pot using fresh deionized water. Cooking times for each leaf ranged from 5-10 minutes. After cooking, samples were removed and immediately placed in ice for up to 5 minutes to prevent further cooking and then filtered. The cooked GLV residue and its filtrate (pot liquor) were analyzed separately to determine iron content as follows:

The cooked GLV residue was air-dried at room temperature for two weeks. Two grams of dried, cooked GLV sample were taken in a crucible and heated over a Bunsen burner (200 - 250˚C) until the sample was completely turned into ash, and then grounded into a fine powder. To 1 gram of ash sample, 20 ml of a 0.1M HCl solution was carefully added, stirred, and mixed well. The ware was then filtered to collect the filtrate, and then 10 ml of the filtrate was mixed with 5 ml of 0.1M KCSN solution and shaken well. The testing solutions were taken in different cuvettes, and the absorbance was measured immediately using a UV-Vis spectrophotometer at 480 nm. The blank used was 0.1M HCl.

To 5 ml of each of GLV pot liquor samples, 10 ml 0.1M HCl was added carefully, and mixing was well done, and then 5 ml of 0.1M KCSN solution was added. The testing solutions were taken in separate cuvettes, and the absorbance was measured immediately using a UV-Vis spectrophotometer at 480 nm. The blank used was 0.1M HCl.

Determination of Moisture Content

Fifty grams of fresh GLV samples were weighed and dried at room temperature for two weeks, and then their moisture content was calculated:

$$\text{Percent Moisture Content (w/w)} = \frac{(X-Y)}{X} \times 100$$

Where, X - Weight of wet GLV sample, and Y - Weight of dry GLV sample

RESULTS AND DISCUSSION

Figure-1 shows the standard calibration curve, which was established using various concentrations of standard ferric nitrate solution, and the absorbance was measured at 480 nm. The correlation coefficient R^2 = 0.9984 indicated a highly positive correlation between absorbance and concentration. The concentration (ppm) of all the uncooked (raw), cooked, and pot liquor of GLV samples were tabulated in Table-1, determined by the interpolation and extrapolation of the standard calibration curve.

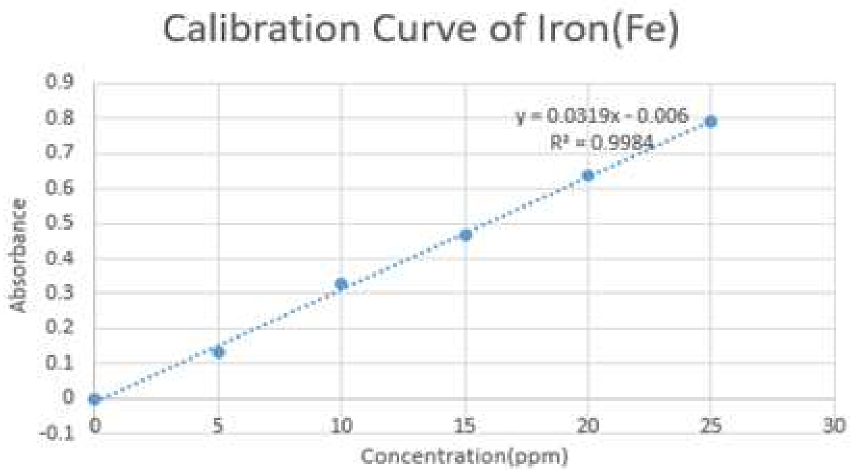

Fig.-1

Table-1: The Concentration (ppm) of the GLV Samples

Vegetable Samples	Concentration (ppm)		
	Uncooked	Cooked	Pot Liquor
Coccinia	6.74	3.82	21.41
Holy Basil	1.69	1.63	8.385
Chinese kale	3.42	2.58	3.793
Lettuce	4.18	0.235	10.61
Sweet Basil	8.44	1.536	6.34
Amaranths	3.94	2.06	8.23

The minimum and maximum iron concentrations; for the raw green leafy vegetables *Holy basil* and *Sweet basil* were 1.69 and 8.44 ppm (parts per million), cooked GLV *Lettuce* and *Coccinia* were 0.235 and 3.82 ppm, and GLV pot liquors, *Chinese Kale* and Coccinia were 3.793 and 21.41 ppm respectively. The GLV pot liquors had higher iron content than uncooked (raw) and cooked green leafy vegetables of all the analyzed samples. The comparative concentration of iron in uncooked, cooked, and GLV pot liquors were summarized in Fig.-2, and there were variations found in them. The percentage differences were summarized in Table-2, and it was observed that there was a difference in iron concentration in GLV foods compared to the conventional consumption method of cooking.

Table-2: Percentage Difference of Cooked, Uncooked, and GLV Pot Liquor

GLV Samples	Percent Differences from Cooked GLV		
	Cooked (Raw)	Uncooked	Pot liquor
Coccinia	3.82	76.44%	460.47%
Holy Basil	1.63	3.68%	414.42%
Chinese kale	2.58	32.56%	47.02%
Lettuce	0.235	1678.72%	4414.89%
Sweet Basil	1.536	449.48%	312.76%
Amaranths	2.06	91.26%	299.51%

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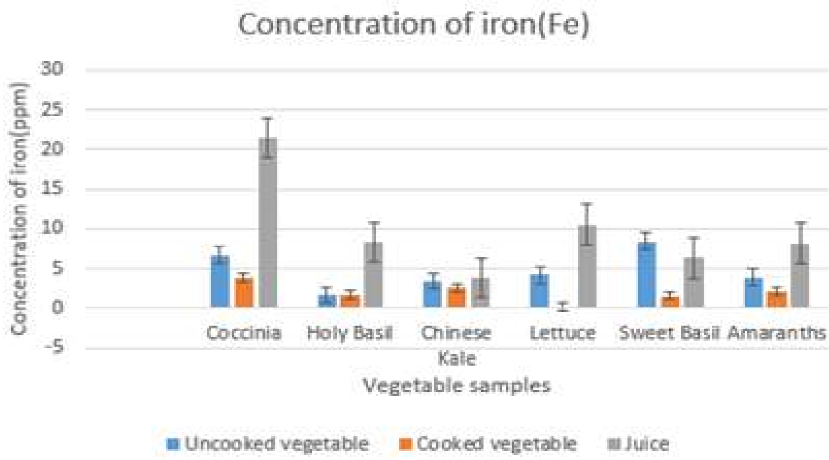

Fig.-2

The moisture content percentage of raw GLV was investigated and presented in Table-3. The moisture content of analyzed raw GLV contains the highest for *Letture* (94.74%) and lowest value for *Amaranths* (66.18%), while raw GLV such as *C. kale*, *S.basil*, and *H. basil* had the moisture content of 87.72%, 84.04%, and 83.86%, respectively. The four green leafy vegetables, *Amaranth, Kilkeerai, Shepu, and Spinach,* ranged between 85.7%-92.2%[29], and the five dehydrated green leafy vegetables, *Amaranthus gangeticus, Chenopodium album, Centella asiatica, Amaranthus tricolor,* and *Trigo*nella *foenum graecum* ranged between 90.4%-94.7%.[30]

The high moisture content in the leaves of species exposed that their leaves were more prone to deterioration since food with high moisture contents was susceptible to perishability.[31,32] The high moisture contents of GLV may reduce a more significant activity of water-soluble enzymes and co-enzymes involved in metabolic activities.[1,33]

Table-4: Percent Moisture Content (w/w) of Uncooked Green Leafy Vegetables

Uncooked GLV	Moisture (%)
Holy Basil	83.56
Chinese Kale	87.72
Lettuce	94.74
Sweet Basil	84.04
Amaranths	66.18

Green leafy vegetables have been playing a vital role in those with underprivileged nutrition and health statuses; cooked indigenous vegetables with *lye* (traditional salt) had higher iron content than cooked vegetables in urban and rural settings.[28,34] The extractability percent of iron increased from 64.74 to 69.38 with an increase in cooking time. Long blanching times increased ionizable and available iron from 6.47 to 7.74 and 3.53 to 4.13 percent within five to fifteen minutes.[35] The iron concentration increased in cooking *Spinach*; raw *Spinach* contained 5.7 mg, whereas steamed *Spinach* contained 6.3 mg of iron per 100 g. They hypothesize that increased iron due to steaming may be caused by the decreased moisture content relative to the increase in solids.[36]

The iron content in GLV can vary widely depending on various factors, including cooking. The iron content in *Kale* has been affected by its cooking method and freezing process. Many of the cooked green leafy vegetables were good sources of many minerals. Six of the GLV studied contained excellent manganese levels, five were good in calcium content, and two had good levels of irons.[27] The iron content of *Kale* depends on many factors and is somewhat dependent on whether it is fresh or cooked.[37] Cooking GLV decreases the amount of oxalates, phytates, and tannins, and, therefore theoretically increases iron bioavailability by minimizing the inhibitory effects.[38,39] The bioavailability of iron density in meals could be increased by including a GLV salad sprinkled with added lemon juice.[40]

When we intake a sufficient quantity of iron, we can live with better health and prevent various diseases. It is especially true for infants and those facing iron deficiency or anemia. During the first 6 to 12 months of life, infants who experience iron deficiency are likely to experience persistent effects of the deficiency that alter functioning in adulthood[41]. In summary, the effects of cooking have led to variability in GLV iron content and may be based on the cooking method used.

CONCLUSION

Green leafy vegetables are good sources of iron content. However, there were variations of iron content in uncooked, cooked, and pot liquor. The concentration of iron in the green leafy vegetables was in the order of *Coccinia>Lettuce>Holy basil>Sweet basil>Amaranths>Chinese Kale*. In addition, the green leafy vegetables contained an appreciable percentage of moisture content. Among the six investigated green vegetable samples, *Coccinia* pot liquor was found to contain maximum iron content, and the least was found in cooked *Lettuce*. After comparing the differently prepared green leafy vegetables, pot liquor appeared to be the best iron source. Moreover, further research needs to be done to confirm the presence of other active compound and minerals in the tested sample.

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