A study to determine the total iron and extractable iron content available in ten indigenous vegetables of Ghana

ABSTRACT

AimsVegetables are used to substitute most meats in most meals of poor communities since they are the main source of iron. The availabilityof numerous vegetables in Ghana and little knowledge about their iron content do not help select the best food. This study aimed to determine the total and extractable iron content of ten indigenous vegetables commonly incorporated into various meals
Study design: The study was a cross-sectional sample conducted in June and August 2014.
Place and Duration of Study: Ten mostly used indigenous vegetables were randomly purchased from different vendors from the Kasoa market in the Central Region of Ghana and transported to the Department of Laboratory Technology Research laboratory, University of Cape Coast for lab analyses.
Methodology: Each sample was packaged in a separate clean polythene bag and labeled for transportation to the Chemistry laboratory, University of Cape Coast, Cape Coast for total and extractable iron determination. Total iron content and extractable iron content were examined after homogenization and the concentration of theironin the leaves was analyzed by Atomic Absorption Spectrophotometer to obtain the mean.
Results:*Manihotesculenta*leaves had the highest mean iron content of 336.77 mg/kg, followed by*Abelmoschusesculentus*leaves (319.30 mg/kg),*Phaseolus vulgaris* leaves (159.90 mg/kg),*Moringaoleifera leaves* (*133.72 mg/kg*), and *Amaranthuscruentus* leaves (129.96 mg/kg). The results showed the total iron contents were significantly different (P<0.0001) among the different vegetables, except for *Xanthosomasagittifolium* and *Corchorusolitorius*.The mean extracted iron from each vegetable was lower than the total iron content. *Manihotesculenta* leaves had the highest mean extractable iron of 143.60 mg/kg followed by *Phaseolus vulgaris* leaves with a value of (79.37 mg/kg). Statistics indicated the mean extractable iron contents were significantly different (P<0.0001) among the different vegetables.The total iron and extractable iron contents of the ten vegetables were significantly different (P<0.00001) from each other.**Conclusion:**The presence of iron in indigenous vegetables and their extractable nature was uncovered. The optimal sequence for choosing vegetables for the highest possible iron advantage is:*Solanummelongena<Talinumtriangulare leaves<Xanthosomasagittifolium leaves<Abelmoschusesculentus leaves <Corchorusolitorius leaves < Ipomoea batatas leaves <Amaranthuscruentus leaves <Moringaoleifera leaves <Phaseolus vulgaris leaves<Manihotesculenta*leaves.Public awareness should go on about the high benefits of iron

from*Phaseolusvulgaris* leaves and*Manihotesculenta*leaves.

Keywords: indigenous vegetables; total iron, extractable iron; nutrition

1. INTRODUCTION

Iron is a vital element that is required in the diet for three distinct roles in humans namely: hemoglobin and myoglobin formation, and components of some enzymes such as catalase, cytochrome enzymes, and others [1]. It is involved in oxygen transfer from the lungs to the rest of the body, and carbon dioxide from the body to the lungs for removal. Secondly, iron as a component of enzymes such as catalase, peroxidase, and cytochrome enzymes is involved in energy production [1]. Finally, iron is involved in maintaining the body's immune system [2].

Iron deficiency is a prevalent nutritional defect globally that affects about two billion people. The greatest consequences are fatigue, lethargy, more frequent infections, ill health, reduced resistance to cold, hypoferrimia, premature death, and lost earnings to individuals, communities, and nations. In addition to the above, iron deficiency also retards growth and intellectual development in children [3]. Since iron is necessary for the formation of hemoglobin, anemia represents the predominant clinical manifestation of iron deficiency. Given the critical role of oxygen transport in sustaining human life, severe anemia can adversely affect or even be fatal by depriving organs of an adequate oxygen supply [4].

Iron is found in the diet in two forms, namely: haem iron and non-haem iron. Non-haem iron is delivered by both plant and animal-derived foods, while haem iron is delivered only by animal-based products like meat, fish, poultry, and eggs. Haem iron has higher bioavailability than non-haem [1].

Typical Ghanaian meals of low-standard communities consist mainly of starchy foods obtained from roots or tubers, cereals or fruits, and vegetables with or without meat. Vegetable plants are defined as herbaceous species that are cultivated for human consumption and whose edible parts consist of leaves, roots, hypocotyls, stems, petioles, and flower buds [5]. Vegetable serves as a source of most nutrients (minerals, vitamins, and proteins) except carbohydrates. Since meat is almost absent in the meals of poor communities, vegetables serve as the main source of iron. However, the availability of numerous vegetables in Ghana and little knowledge about their iron content do not help select the best food. In addition, inherent characteristics of the various plants may also influence the availability of iron from these

vegetables.

UNICEF Ghana report of 2011[6] indicated that malnutrition was responsible for one-third of all child deaths, while iron deficiency anemia, in particular, was a major threat to children's health and the leading cause of maternal mortality in young women.

Food supplies nutrients for the maintenance and development of our bodies. When our diet does not contain the right proportion of nutrients our bodies need, it results in malnutrition, which could be either undernutrition or overnutrition. An inadequate supply of nutrients in food leads to undernutrition in humans and is seen as a deficiency disease. Deficiency diseases are common in developing countries, persist in poor communities, and are very severe in children [7].

The objective of this study was to determine the iron content of ten indigenous vegetables commonly used in meals, the extractable iron content of these vegetables, and to calculate their iron extractability so that the best source of iron from these vegetables could be communicated to consumers and dieticians/nutritionists on how to get significant iron from their diet.

2. MATERIAL AND METHODS

2.1 Study Area and Design

The study was conducted at Kasoa Market, a major local market located in the Awutu Senya district of central Ghana.Agriculture and agribusiness are among the most important economic activities for Kasoa workers. Kasoa has one of the most important markets in the central region. The city is located on the Accra-Cape Coast Road, approximately 36 kilometers (22 miles) by road west of Kotoka International Airport (5.320° North and 0.253° West). The study was a cross-sectional sample conducted in June and August 2014.

2.2 Materials

Materials and equipment were acquired from the Research Laboratory of the Department of Laboratory Technology at the University of Cape Coast. Porcelain, ceramic, and glass wares were soaked in aqua regia for three hours and washed with pipe-borne water. Finally, the porcelain, ceramic, and glass wares were rinsed with deionized water followed by drying in a hot dry oven.

2.3 Methods

2.3.1 Sampling Technique and Pretreatment

Fresh samples of ten mostly used indigenous vegetables: garden egg (*Solanummelongena)*, leaves of cocoyam (*Xanthosomasagittifolium)*, water leaf (*Talinumtriangulare),* "aleefu" leaves (*Amaranthuscruentus),Moringaoleifera* leaves*,* cowpea leaves (*Phaseolus vulgaris*), cassava leaves (*Manihotesculenta),* sweet potatoes leaves (*Ipomoeabatatas),* "ayoyo*" leaves (Corchorusolitorius)* and okra leaves (*Abelmoschusesculentus*) were randomly procured from different vendors in Kasoa market in the Central Region of Ghana. Each sample was placed in a separate clean polythene bag and labeled for transportation to the laboratory for analysis. The extraneous and decomposing matter were removed from all the fresh vegetables. They were rinsed with pipe-borne water several times to ensure that all contaminants were removed and then finally rinsed with deionized water. Each sample was ground using a dry mortar and pestle that had been cleaned as described previously and then in a warring blender to make the sample homogenous. Fresh homogenous samples were used for the extraction of iron and the determination of total iron content.

2.3.2Determination of Total Iron

Total iron determination was carried out by the method of Food Safety and Standards Authority of India [8] with slight modifications. Approximately 3.0 g of the homogenized sample was weighed and placed in a porcelain crucible that had been previously ignited, cooled, and weighed. The homogenized sample was oven-dried in the crucible, then the crucible and its content were ignited gently over a low flame (bunsen burner) until charred. The charred material was transferred to a muffle furnace at 550 °C until ash had formed. The crucible and its contents were allowed to cool and then the ash was dissolved in 5 ml of concentrated nitric acid in a conical flask and allowed to stand overnight. After this, the solution was heated gently on a hot plate until brown fumes disappeared. The flask was left to cool at room temperature and then 15 ml of concentrated hydrochloric acid was added. The mixture was heated again until a colorless solution was obtained. Then 20 ml of deionized water was added and filtered through Whatman filter paper Grade 42 into a 100 ml volumetric flask. The conical flask was rinsed with deionized water and added to the volumetric flask, then the content of the volumetric flask was made up to 100 ml mark with deionized water and shaken to mix well. The above procedure was repeated for all samples in triplicate and the mean was calculated.

2.3.3 Determination of Extractable Iron

Iron in the samples was extracted by the method described by Chauhan and Mahajan [9]. Approximately 1.0 g of fresh homogenized sample was weighed into a clean conical flask. 10 ml of 0.03 M HCl was added and shaken for 3 hours at 37 $^{\circ}$C on a shaker. At the end of the period, the mixture was filtered using Whatman paper Grade 42. The clear extract obtained was oven-dried at 100 $^{\circ}$C to obtain a residue. The residue from oven drying was acid digested in a cleaned dried beaker with concentrated nitric acidand concentrated hydrochloric acid as in total iron determination. The above procedure was

repeated for all samples in triplicate and the mean was calculated.

HCl extractability (%) (an index of bioavailability) was calculated as follows:

$$\text{Iron extractability (\%)} = \frac{\text{Iron extractable in 0.03 M HCl (mg /100 g)}}{\text{Total iron content (mg/100 g)}} \times 100$$

2.3.4 Preparation of the Blank

A blank for total iron determination was prepared without the sample. 5 ml of concentrated nitric acid and 15 ml of concentrated hydrochloric acid were heated until a colorless solution was obtained and filtered through Whatman filter paper Grade 42 into a 100 ml volumetric flask. Deionized water was added to make the flask's contents up to 100 ml.

A blank for extractable iron was prepared with 10 ml of 0.03 M hydrochloric acid, 5 ml concentrated nitric acid, and 15 ml hydrochloric acid. These chemicals were transferred into a conical flask and heated on a hot plate until red fumes of nitrogen (IV) oxide ceased. The solution was then brought off the hot plate and allowed to cool after which deionized water was added to the conical flask, transferred into a 100 ml volumetric flask, and made up to the mark with deionized water.

2.3.5 Standard Preparation

Standard iron solution (iron (II) sulfate) of the following concentrations was prepared; 2 mg/L, 4 mg/L, and 6 mg/L from a stock standard iron solution of 20 mg/L concentration using serial dilution. 10 % nitric aciddiluent was usedto dissolve and digest the standard to free the iron.

2.3.6 Atomic Absorption Spectrophotometer (AAS)

An atomic absorption spectrophotometer was used to analyze the concentration (mg/L) of the iron in the samples. The AAS fitted with a source lamp with a characteristic wavelength of 248.3 nm was used in the analysis of iron.

2.3.7Statistical Analysis

Data were analyzed by using GraphPad Prism v9. One-way ANOVA was conducted to determine the significance differences ($P<0.05$) in the various vegetables for the total and extractable iron content. Student-independent t-test was performed between the total and extractable iron for each vegetable. Differences of ($P<0.05$) were considered statistically significant. Tukey's test was used for multiple comparison analyses.

3. RESULTS AND DISCUSSION

3.1 Total Iron Content

The results from the study as presented in Figure 1below showed the mean total iron contents among the different vegetables. *Manihotesculenta* leaves had the highest iron content of 336.77 mg/kg, followed by *Abelmoschusesculentus* leaves (319.30 mg/kg), *Phaseolus vulgaris* leaves (159.90 mg/kg), *Moringaoleifera* leaves (133.72 mg/kg), and *Amaranthuscruentus* leaves (129.96 mg/kg). The rest of the vegetables had an iron content of less than 100 mg/kg. The iron content in the vegetables increased in the order: *Solanummelongena*<*Talinumtriangulare* leaves<*Corchorusolitorius* leaves<*Xanthosomasagittifolium* leaves<*Ipomoea batatas* leaves<*Amaranthuscruentus* leaves<*Moringaoleifera* leaves<*Phaseolus vulgaris* leaves <*Abelmoschusesculentus* leaves<*Manihotesculenta* leaves. The iron content of *Solanummelongena* in this work was higher than that of Bukva et al. [10]. Iron content determined by Atuna et al. [11] in *Amaranthuscruentus* leaves, *Xanthosomasagittifolium* leaves, and *Talinumtriangulare* leaves was higher, however, *Moringaoleifera* leaves was lower when compared to this work. Oduro et al [12] reported a higher iron content in *Moringaoleifera* leaves and *Ipomoea batatas*leave than in this work. Anyawu et al. [13] and Khairah et al. [14] observed that differences in iron content may be due to several factors that influence the concentration of mineral elements on and within plants; these factors included climate, atmospheric deposition, the nature of the soil on which the plant was grown, and irrigation with wastewater. However, Adotey et al. [15] indicated that the contrast in mean total iron contents among different vegetables may be attributed to the iron content in the soil where the plants are cultivated, the preferential uptake of iron by plants, ambient climate conditions, and the amount of fertilizer and pesticides applied on the crops or land. The results obtained from this study showed that the total iron contents were significantly different ($P<0.0001$) among the different vegetablesexcept for *Xanthosomasagittifolium* and *Corchorusolitorius*.

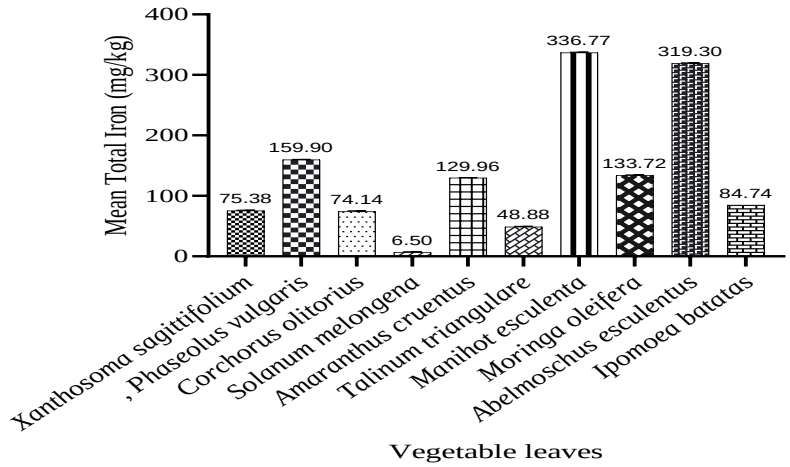

Fig. 1. Mean total iron (mg/kg) content among different vegetables

3.2 Extractable Iron Content

The results from the study as presented in Figure 2 below showed the mean total iron contents among the different vegetables.*Manihotesculenta* leaves had the highest iron content of 336.77 mg/kg, followed by *Abelmoschusesculentus* leaves (19.30 mg/kg),*Phaseolus vulgaris* leaves (159.90 mg/kg), *Moringaoleifera* leaves (133.72 mg/kg), and*Amaranthuscruentus* leaves (129.96 mg/kg). The rest of the vegetables had an iron content of less than 100 mg/kg. The iron content in the vegetables increased in the order: *Solanummelongena*<*Talinumtriangulare leaves*<*Corchorusolitorius* leaves<*Xanthosomasagittifolium* leaves<*Ipomoea batatas* leaves<*Amaranthuscruentus* leaves<*Moringaoleifera* leaves<*Phaseolus vulgaris* leaves <*Abelmoschusesculentus* leaves<*Manihotesculenta* leaves. The iron content of *Solanummelongena* in this work was higher than that of Bukva et al. [10]. Iron content determined by Atuna et al. [11] in *Amaranthuscruentus* leaves,*Xanthosomasagittifolium*leaves, and *Talinumtriangulare leaves* was higher, however, *Moringaoleifera* leaves was lower when compared to this work. Oduro et al [12] reported a higher iron content in *Moringaoleifera* leaves and *Ipomoea batatas*leave than in this work. Anyawu et al. [13] and Khairah et al. [14] observed that differences in iron content may be due to several factors that influence the concentration of mineral elements on and within plants; these factors included climate, atmospheric deposition, the nature of the soil on which the plant was grown, and irrigation with wastewater. However, Adotey et al. [15] indicated that the contrast in mean total iron contents among different vegetables may be attributed to the iron content in the soil where the plants are cultivated, the preferential uptake of iron by plants, ambient climate conditions, and the amount of fertilizer and pesticides applied on the crops or land.The results obtained from this study showed the mean extractable iron contents were significantly different ($P<0.0001$) among all the different vegetables.

**keep all the iron contents under bracket except of the first one i.e. *Manihotesculenta*

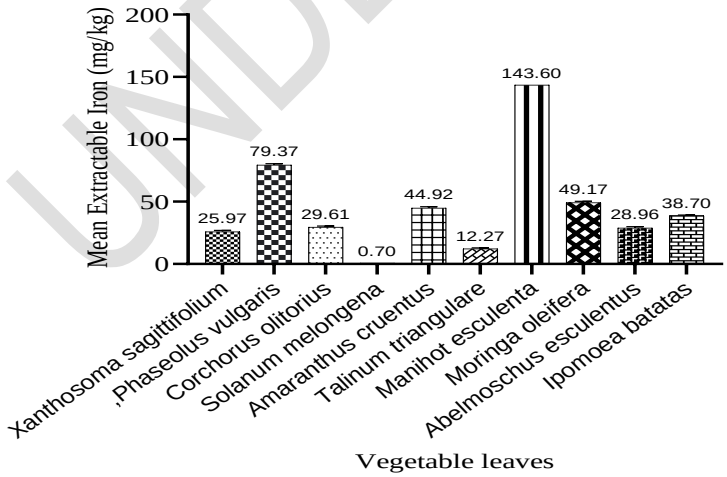

Fig 2. Mean extractable iron (mg/kg) among different vegetables

3.3Percentage of Iron Extractability

From Figure 3 below, iron in the vegetables was extractable and the level of extractable iron in each vegetable was different. The iron extracted from each vegetable was lower than the total iron content. *Manihotesculenta* leaves had the highest extractable iron of 143.60 mg/kg followed by*Phaseolus vulgaris* leaves with a value of (79.37 mg/kg). The other vegetables had extractable iron of less than (50.00 mg/kg). The order of increasing concentration of extractable iron occurred as follows: *Solanummelongena*<*Talinumtriangulare* leaves <*Xanthosomasagattifolium*leaves <*Abelmoschusesculentus* leaves <*Corchorusolitorius* leaves <*Ipomoea batatas* leaves <*Amaranthuscruentus* leaves <*Phaseolus vulgaris* leaves <*Manihotesculenta* leaves. The results from Figure 3 showed the mean extractable iron contents among different vegetables. The variation in the extractable iron content among the vegetables may be attributed to the effect of iron inhibitors (phytate, oxalate, and phosphate) and enhancers (ascorbic acid, citric acid, and mucin). Inhibitors form complexes with the iron which involves strong bonds that render the iron insoluble. Hence, extraction of iron in this state will not occur. Enhancers on the other hand solubilize the iron and enhance non-haem iron extraction [16]. The net effect of inhibitors and enhancers determined the extractable iron content in vegetables. Statistical analysis showed the percent extractability iron content of the ten vegetables was significantly different (P<0.0001) from each other, except for *Xanthosomasagittifolium* and *Amaranthuscruentus*.

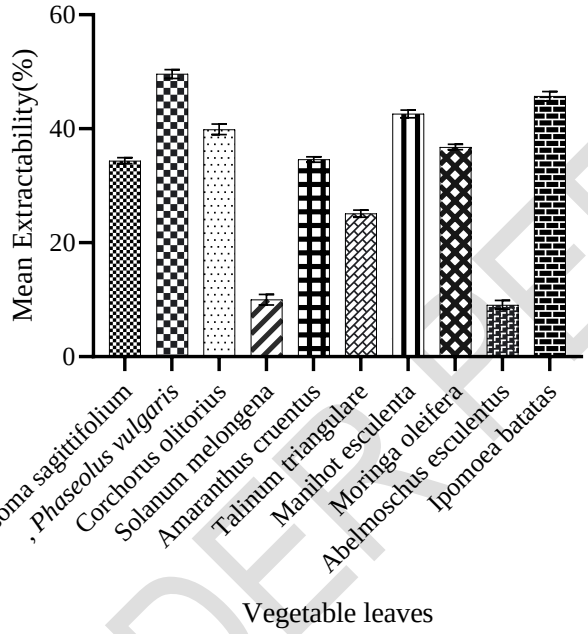

Fig. 3. Percentage of iron extractability among different vegetables

4. CONCLUSION

This study's results depicted the presence of iron in indigenous vegetables and their extractable nature. Considering this, the order of vegetable selection for maximum iron benefit is:*Solanummelongena*<*Talinumtriangulare* leaves<*Xanthosomasagittifolium*leaves<*Abelmoschusesculentus* leaves <*Corchorusolitorius* leaves < *Ipomoea batatas* leaves <*Amaranthuscruentus* leaves <*Moringaoleifera* leaves <*Phaseolus vulgaris* leaves<*Manihotesculenta* leaves. The total iron and extractable iron contents of the ten vegetables were significantly different (P<0.00001) from each other.Education should be intensified about the high benefits of iron from *Phaseolus vulgaris* leaves and*Manihotesculenta* leaves.

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Table 1. Comparison of total iron, extractable iron, and percent iron extractability contentin the individual vegetables

Vegetable leaves	Total Iron(mg/kg)	Total Extractability(mg/kg)	Percent Extractability (%)
Xanthosomasagittifolium	75.38±0.77[c]	25.97±0.98[a]	34.40±0.52[a]
Phaseolus vulgaris	159.90±0.18[b]	79.37±1.01[b]	49.60±0.79[b]
Corchorusolitorius	74.14±1.09[c]	29.61±0.82[c]	39.90±0.92[c]
Solanummelongena	6.50±0.96[d]	0.7±0.001[d]	10.00±0.91[d]
Amaranthuscruentus	129.96±0.25[a]	44.92±1.04[g]	34.60±0.45[a]
Talinumtriangulare	48.88±0.54[e]	12.27±0.67[h]	25.10±0.62[e]
Manihotesculenta	336.77±0.87[f]	143.60±0.03[j]	42.60±0.66[f]
Moringaoleifera	133.72±0.85[g]	49.17±1.11[k]	36.80±0.47[g]
Abelmoschusesculentus	319.30±0.67[h]	28.96±0.99[m]	9.10±0.79[h]
Ipomoea batatas	84.74±0.024[j]	38.70±0.48[n]	45.70±0.81[k]
	<0.0001	<0.0001	<0.0001

Mean values along the column with different superscripts are significantly different(p<0.05) while mean values with the same superscripts are not significantly different.

Table 2. Total iron content, total extractability, and statistical significance in various vegetables

Vegetable leaves	Total Iron(mg/kg)	Total Extractability(mg/kg)	*p*-value
Xanthosomasagittifolium	75.38±0.77	25.97±0.98	<0.00001
Phaseolus vulgaris	159.90±0.18	79.37±1.01	<0.00001
Corchorusolitorius	74.14±1.09	29.61±0.82	<0.00001
Solanummelongena	6.50±0.96	0.7±0.001	0.000471
Amaranthuscruentus	129.96±0.25	44.92±1.04	<0.000001
Talinumtriangulare	48.88±0.54	12.27±0.67	<0.000001
Manihotesculenta	336.77±0.87	143.60±0.03	<0.000001
Moringaoleifera	133.72±0.85	49.17±1.11	<0.000001
Abelmoschusesculentus	319.30±0.67	28.96±0.99	<0.000001
Ipomoea batatas	84.74±0.024	38.70±0.48	<0.000001