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RESEARCH ARTICLE

In-vitro dialysability of iron in green leafy vegetables and seasonal variation of total iron content

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Abstract: Green leafy vegetables are considered a good source of iron in Sri Lankan diets, particularly among populations dependant on plant based diets. The iron status of diets can be improved by including iron rich plant sources and enhancing the bioavailability by cooking/ processing.

The mean total iron content ± SD of the green leafy vegetables studied were as follows (expressed as fresh weight, mg/ 100g): sarana *(Trianthema portulacastrum* L.): 6.7±1.5, rathu thampala *(Amaranthus lividis.*L.*)*: 13.4±2.7; nivithi (*Basella alba* L.): 1.3±0.6; kathrumurunga {Sesbania grandiflora (L.) Poir.}: 3.2±0.7; mukunuwenna {*Alternanthera sessili* (L.)Dc.}: 1.8±0.3; *kankun* (*Ipomoea aquatica.* Forssk.): 1.9±0.9 and *gotukola* {*Centella asiatica* (L). Urban}: 5.2±1.6. The seasonal variation observed was not significant $p>0.05$ over the period of one year. These values indicate that the contribution of iron from green leafy vegetables to the diets is low.

The freshly cooked and dehydrated-cooked samples (hot-air drier at 55°C) were blended with water and subjected to simulated gastro-intestinal digestion procedures, following which the proportion of iron that diffused through a semi permeable membrane (molecular mass cut-off 10 kDa) determined-cooked as the dialyzable iron. This was quantified spectrophotometrically at 535 nm. The dialysable iron extracted from the freshly cooked and dehydrated - cooked green leafy vegetables ranged between 1.5-13.4 % and 3.6-17.6% respectively. Accordingly the best sources of available iron from green leafy vegetables are *sarana, kathrumurunga* and *rathu thampala.*

Key Words: Dialysable iron, green leafy vegetables, iron

INTRODUCTION

Green leafy vegetables are considered a good source of iron, calcium and B-carotene in Sri Lankan diets, particularly among populations that are largely dependant on plant based diets. Choice of the iron rich plant sources, market availability and improved bioavailability through cooking/ processing are factors that contribute towards improving the iron status of the population.

The bioavailability of iron from plant material is low [1] as compared to animal sources. It has been demonstrated that Fe^{2+} is more available than Fe^{3+}. Dietary constituents such as amines, ascorbic acid and citric acid enhance iron availability from the diet[2]. The absorption of iron from cereals and legumes however, is low due to the presence of inhibitory factors such as phytates[3] and tannins[4]. Literature reviews show that processing of cereals and legumes using traditional methods or other technologies enhance absorption of iron by destroying inhibitors or through the formation of beneficial complexes between food components and the metal ions, thereby enhancing its iron availability[5].

The iron available from food materials classified as elemental, soluble, complexed and ions, have been quantified by chemical methods [6,7]. In recent times *in-vitro* iron dialysability methods have been used for predicting the bioavailability of iron from food sources. This method is in reasonable agreement with human absorption data[8] and is far more cost effective when compared to *in vitro*-digestion/Caco-2 cell culture models used to demonstrate iron availability[9].

This study reports the seasonal variation in the total iron content of seven green leafy vegetables and evaluates their *in-vitro* availability of iron (as dialysable iron) for indexing the best sources of iron available from the same which are commonly consumed in Sri Lanka.

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METHODS AND MATERIALS

Materials: Seven green leafy vegetables, locally known as sarana (*Trianthema portulacastrum* L.), rathu thampala (*Amaranthus lividis* L.), nivithi (*Basella alba* L.), kathurumurunga {*Sesbania grandiflora (*L.*)* Poir}, mukunuwenna (*Alternanthera sessili* (L.) Dc.), kankun (*Ipomoea aquatica* Forssk.) and gotukola {*Centella asiatica* (L.) Urban.} were obtained from Piliyandala through a main supplier of green leafy vegetables to the Colombo district during the period of August 2002 to July 2003. Plants were identified at the National Herbarium of Botanical Gardens, Peradeniya. Five representative samples were drawn from a bulk (3000 g) of each green leafy vegetable, at 5-6 day intervals of each month, for quantifying the variability in the total iron content (n=60).

Digestive enzymes and bile salts were purchased from Sigma Chemical Co., (St.Louis, MO, USA). A pepsin digestion mixture was prepared by suspending 16 g pepsin (P-7000 from porcine stomach mucosa) in 100 mL 0.1M HCl. The pancreatin-bile extract contained 4g pancreatin (P-1750, from procine pancreas) and 25 g bile extract (B-8631, porcine) dissolved in 1 L of 0.1 M $NaHCO^3$.

All chemicals were of Analar Grade and purchased from BDH, Poole, England. A protein precipitation solution was prepared by mixing 100 g trichloroacetic acid, 100 g hydroxylamine and 100 g of 370 g L HCl and made up to 1 L. The chromogen solution contained 250 g of bathophenanthroline sulphate dissolved in 1L of 2M Sodium Acetate. Iron standards, containing 0.1, 0.2, 0.4, 0.8 and 1.6 μg Fe(II) mL^{-1} in 10 g L HCl were used for establishing a calibration curve (R^2= 0.9906). Iron was determined spectophotometrically using a Hach spectrophotometer- Model DR/4000 (Hach Co. Colarado, USA).

Distilled water used in the assay was Milli Q water. Dialysis tubing Spectra (Por 7) with a molecular cut of 10 000 Da were obtained from Cole Parmer Instrument Co. Ltd, USA. A Colworth Stomacher Lab-Blender Model 400 (Seaward Medical, London, U.K.) was used for blending the samples. A drier ("Mitchel" Model, Prachitt Brothers Ltd., Carlisle, U.K.) was used for hot-air drying of samples.

Stimulated digestion method: In the determination of dialyzable iron content, freshly purchased green leafy vegetables were washed, shredded and the material was cooked with water (1:5) for 10 min. Dehydrated green leafy vegetables were prepared after washing and drying in a hot-air drier at 55°C. The dehydrated product was powdered and cooked with water (1:10) for 10 min.

The cooked samples of both freshly processed and dehydrated powders of all seven green leafy vegetables were homogenized separately and an aliquot of the samples (in triplicate) were subjected to simulated gastrointestinal digestion as described[8]. In the gastric stage, the sample (20 g) was adjusted to pH 2.0 by adding 6 M HCl. Freshly prepared pepsin solution (5 g) was added and made up to 100 g with water. The sample was mechanically digested in a stomacher and was incubated at 37 °C, for 2 h in a shaking water bath. A homogenized pepsin digest (20 g) was taken in an Erlenmeyer flask and the pH was adjusted to 7.5 with O.5 M NaOH (after determining the titratable acidity). Freshly prepared pancreatin mixture (5 g) was added. A calculated amount of sodium bicarbonate (after determining the titratable acidity) dissolved in 25 g of water contained in a semi-permeable membrane (molecular mass cut-off 10 kDa) was introduced to the flask. The contents of the flask were incubated at 37 °C, for 2 h in a shaking water bath. The dialyzed bags were rinsed with water, carefully dried and weighed. The contents of each dialysed bag was transferred into acid-washed containers and analysed for its iron content.

The aliquots of the dialysate or standard solution (10 mL) were pipetted into test tubes and mixed with 5 ml of protein solution. Each tube was covered and kept in a boiling water bath for 10 min. The sample was then centrifuged (3500 g 10 min). The supernatant (5 mL) was mixed with 2.5 mL of chromogen solution and the colour was allowed to develop for 10 min. The amount of dialyzable iron (mg/kg) was determined spectrophometrically at 535 nm.

The moisture content, total ash and total iron content in all samples were determined according to AOAC methods[10].

RESULTS

The moisture content in the green leafy vegetables varied between 76.8- 92.8%. The mean total iron content ± SD of the green leafy vegetables (expressed as fresh weight, mg/ 100 g) were as follows: sarana: 6.7±1.5, rathu thampala: 13.4±2.7; nivithi: 1.3±0.6; kathrumurunga: 3.2±0.7; mukunuwenna: 1.8±0.3; kankun: 1.9±0.9 and gotukola: 5.2±1.6. The monthly variations (n=60) in the total iron content in the seven green leafy vegetables were not significantly different ($p>0.05$). The results are presented in Figure 1.

The dialyzable iron extracted from the seven green leafy vegetables varied between the plant materials (Table 1). The dialyzable iron extracted from the dehydrated - cooked plant material was higher as compared to that of the freshly cooked leafy vegetables, except for sarana and nivithi.

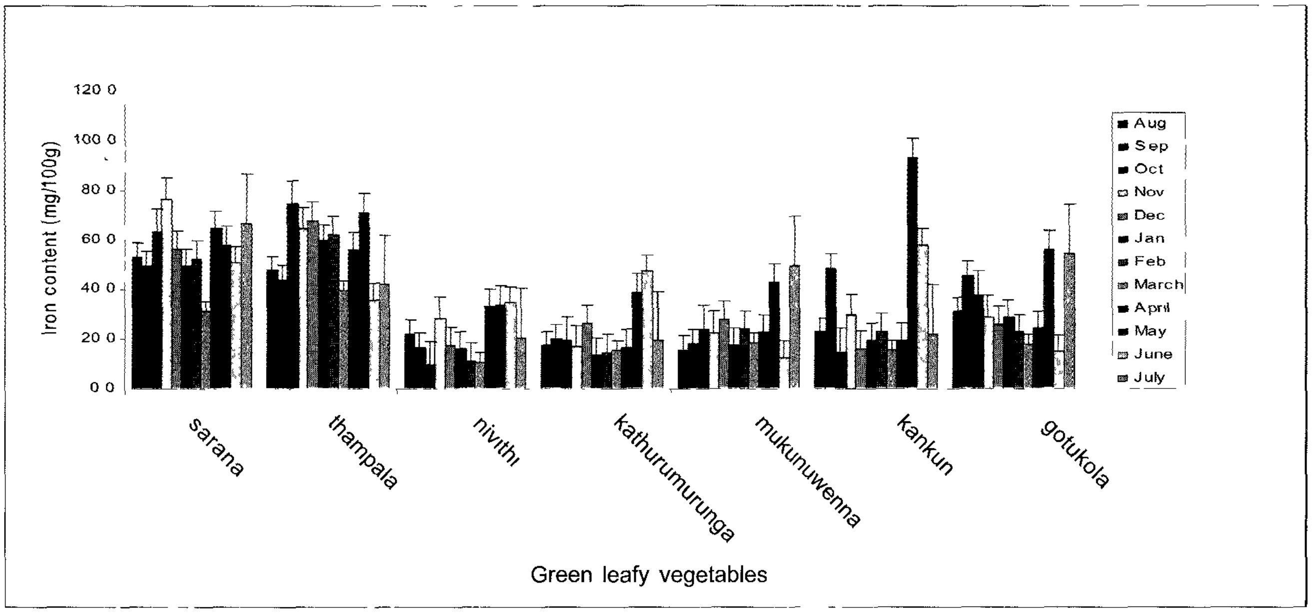

Figure 1: Seasonal variation in total iron content of green leafy vegetables (dry basis ± SE, n=60)

Table 1: % dialysable iron extracted from fresh /dehydrated and cooked green leafy vegetables

Green leafy vegetable	Freshly - cooked			Dehydrated - cooked		
	% Moisture Content	Dialysable iron (Dry basis, mg/kg) ± SD)	% Dialysable iron of total iron	% Moisture Content	Dialysable iron (Dry basis, mg/kg) ± SD)	% Dialysable iron of total iron
sarana	87.9 ± 1.4	38.0 ± 1.4	6.8 ± 2.6	1.9 ± 0.1	24 4 ± 1.2	4.3 ± 0.4
rathu thampala	76.8 ± 1 7	11.7 ± 1.3	2.0 ± 0.6	1.8 ± 0.2	20.6 ± 1.4	3.6 ± 0.6
nivithi	92.8 ± 1.8	23.8 ± 1.7	13.4 ± 3.9	2.1 ± 0.1	7.3 ± 1.2	4.1 ± 2.0
kathurumurunga	82.3 ± 2.4	20.4 ± 1.1	11 3 ± 3.9	2.2 ± 0.1	31.7 ± 1.4	17.6 ± 5.8
mukunuwenna	91.5 ± 2.3	16 5 ± 0.3	7.8 ± 0.6	2.1 ± 0.1	18.0 ± 0.7	8.5 ± 1.2
kankun	91 9 ± 2.3	8.5 ± 0.3	3.7 ± 0.6	2.2 ± 0.1	9.6 ± 0.4	4.1 ± 2.6
gotukola	81.8 ± 1.5	4.5 ± 0.2	1.5 ± 0.7	2.4 ± 0 3	20.5 ± 0.3	7.0 ± 2.7

Values are means of ± SD, n =5.

DISCUSSION

The total iron content of the green leafy vegetables monitored over a period of twelve months was not significantly different ($p > 0.05$). The soil properties such as pH, redox conditions, cation exchange capacity, the activity of microorganisms, soil structure and water content govern the mineral availability to the plant. The availability of iron to the plant depends on its spatiality in the soil. Iron can be present in the soil in different physicochemical forms such as free ionic form, chelates, absorbed onto mineral or organic surfaces, as precipitates, or in lattice structures or as a part of soil biomass.

According to the results the best source of iron was from rathu thampala, which provided 1/3 of the daily requirement of iron per 100 g fresh weight per adult (22 µg iron), while sarana and gotukola were good sources of iron providing approximately 1/4 of the Recommended Daily Allowances (RDA) The low iron content of some leafy vegetables could have a negative effect on the population of the Colombo district that consume green leafy vegetables as a source of dietary iron

In this study an attempt was made to quantify the bioavailable iron as dialyzable iron using an *in vitro* method The dialyzable iron was high in sarana, kathurumurunga and rathu thampala Literature also reports that the iron availability from green leafy vegetables can be enhanced by the addition of enhancers [11] such as citric acid, into the food matrix or meal The increase in the dialyzable iron observed in some dehydrated green leafy vegetables may be a result of the processing method

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