

## Determination of *in vitro* Availability of Iron from Common Foods

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**KEYWORDS** Total Iron. Ionizable Iron. Foods. Ascorbic Acid. Tannin

**ABSTRACT** Some common foodstuffs of plant origin were analyzed spectrophotometrically using derivative spectroscopy for total and *in vitro* available iron (ionizable iron). Though rice had lowest total iron content ( $0.61 \pm 0.09$  mg/100g), the percent ionizable iron was highest ( $29.50 \pm 4.75\%$ ) as compared to all other cereals and millets tested and also comparable to some of the whole pulses analyzed. Similarly, maize with comparatively lower total iron content ( $2.73 \pm 0.14$  mg/100g), had a higher percent ionizable iron ( $25.30 \pm 1.46\%$ ). The whole pulses were found to contain total iron ranging from  $4.40 \pm 0.30$  mg/100g in blackgram to  $6.36 \pm 0.55$  mg/100g in rajmah, but except Bengal gram (white) ( $21.71 \pm 0.53\%$ ), pea ( $35.66 \pm 4.44\%$ ) and rajmah ( $24.05 \pm 1.42\%$ ), others showed very low ionizable iron ( $3.41 \pm 0.22$ - $6.53 \pm 1.31\%$ ). Split pulses had a better percentage of ionizable iron ( $13.80 \pm 0.73$ - $31.70 \pm 1.74\%$ ) compared to whole pulses, which could possibly due to removal of some inhibiting factors present in the seed coat. Though green leafy vegetables had comparatively lower amount of total iron ( $1.82 \pm 0.11$ - $3.76 \pm 0.23$  mg/100g), it had a high ionizable iron from  $30.22 \pm 1.10$  to  $52.13 \pm 1.90\%$ . Endogenous levels of ascorbic acid and tannin present in the food samples evaluated had no direct influence on iron availability. The results by spectrophotometric method were further compared with radioisotopic method by extrinsic tagging of a few samples with  $^{59}\text{Fe}$ . A significant positive correlation ( $r=0.986$ ;  $P<0.001$ ) was observed between the two methods.

### INTRODUCTION

Iron in general has poor availability from foods derived from plant sources compared to foods from animal sources. Consequently nutritional iron deficiency reaches its greater prevalence and severity in populations subsisting predominantly on cereal and legume based diets (Hallberg, 1981). However, the Food and Nutrition Board of the National Research Council (National Research Council, 1989) has also stated that – iron deficiency anaemia appears to be no more prevalent among vegetarian women than among non vegetarian women. This was because in many studies of vegetarians in Western societies have not found poorer iron status in vegetarian than in omnivores on the basis of measurements of haemoglobin, haematocrit, serum iron, iron binding capacity, or transferrin saturation (Anderson et al., 1981; Latta and Liebman, 1984; Donovan and Gibson, 1995). In this context further study of iron bioavailability in vegetarian diets is needed, because, several studies also suggested that vegetarians, compared to omnivores, have a greater risk of low iron status as indicated by lower concentration of serum ferritin (Donovan

and Gibson, 1995; Helman and Darnton-Hill, 1987; Worthington-Roberts et al., 1988; Legett et al., 1990; Reddy and Sander, 1990; Alexander et al., 1994; Shaw et al., 1995). This is because iron from plant derived foods are non-haem in nature which is markedly influenced by a greater number of dietary factors (Hallberg, 1981; Fairweather-Tait, 1992; Carpenter and Mahoney, 1992; Siegenberg et al., 1991; Reddy et al., 1996; Tuntawiroon et al., 1991). It was also reported that lactoovo-vegetarian diet had 70% lower nonheme iron absorption than from non-vegetarian diet (Hunt and Roughead, 1999).

Majority of the Indian population with lower socio-economic status have to depend on foods from plant origin to satisfy their iron requirement, because of their poor purchasing capacity. Their diet mostly constituted of cereals, millets, pulses and vegetables. However, information on availability of iron from widely varied Indian diets is very limited. In absence of information on composite diet, availability of iron from different single foods, may serve as a prerequisite for informations on availability of iron from composite diets.

Although most reliable method for determining bioavailability is measurement of absorption in human volunteer using radioisotopic technique, adoption of such technique involves ethical clearance. It is also expensive and needs elaborate experimental arrangements. One approach to address this problem has been the development of *in vitro* measures of food iron availability in terms of ionizable iron (Narasinga Rao and Prabhavathi, 1978), dialyzable iron (Miller et al., 1981), iron uptake in the Caco-2 cell culture model (Glahn et al., 1998) etc. Earlier investigations also showed good correlation between measurement of available iron by *in vitro* and *in vivo* methods (Miller et al., 1981; Lynch et al., 1982).

Foodstuffs with relatively low amounts of iron need a sensitive method of estimation especially to measure the amounts of available iron which is much lower while compared to total iron. Microprocessor based spectrophotometers are capable of plotting derivative spectra with very low levels of iron. Derivative spectroscopy has additional advantage of being insensitive to turbidity (Worth, 1983; Soloni et al., 1986), which could be beneficial while measuring *in vitro* available iron.

Considering the above aspects, the present study was designed to analyze spectrophotometrically various samples of cereals, millets, pulses and green leafy vegetables for *in vitro* available iron. Endogenous levels of ascorbic acid and tannin present in the foods and their influence on iron availability were also planned to determine. The spectrophotometric results were further compared with radioisotopic method by extrinsic tagging of food samples with  $^{59}\text{Fe}$ .

## MATERIALS AND METHODS

**Selection of Samples:** Food sample elected for the study were as follows:

**Cereals and Hillets:** Rice (*Oryza sativa*), wheat (*Triticum aestivum*), sorghum (*Sorghum vulgare*), ragi (*Eleusine coracana*), bajra (*Pennisetum typhoideum*) and maize (*Zea mays*).

**Whole Pulses:** Bengalgram (brown) (*Cicer arietinum*), Bengalgram (white) (*Cicer arietinum*), blackgram (*Phaseolus mungo*), greengram (*Phaseolus aureus*), lentil (*Lens esculenta*), pea (*Pisum sativum*) and rajmah (*Phaseolus vulgaris*).

**Decorticated Split Pulses (Dals):** Bengalgram

(*Cicer arietinum*), blackgram (*Phaseolus mungo*), greengram (*Phaseolus aureus*), lentil (*Lens esculenta*) and redgram (*Cajanus cajan*)

**Green Leafy Vegetables:** Amaranth (*Amaranthus* sp.), 'agathi' (*Sesbania grandiflora*), 'bachali' (*Basella alba*), fenugreek (*Trigonella foenumgraecum*), 'gongura' (*Hibiscus canna binus*) and spinach (*Spinacia oleracea*). Samples were collected from 6 different market places of Hyderabad and the study was conducted at National Institute of Nutrition, Hyderabad. Samples were cleaned, washed with tap water followed by deionized distilled water, dried in an oven at 50-60°C. Using a 'Cyclotech' mill, samples were powdered to pass through 0.25 mm sieve.

All the glasswares were washed with teepol solution, rinsed thoroughly with tap water, drained completely and kept overnight in concentrated nitric acid. Next day it was washed thoroughly with tap water and then rinsed with deionized distilled water and dried.

**Digestion of Samples for Total Iron:** Samples were wet digested with a modified procedure of Gorusch (1959) and Schelenz (1977). Powdered food sample (250-500 mg) were digested by heating with nitric, perchloric and sulphuric acid (7:2:1 by volume), till the completion of digestion. Digested samples were cooled and made upto 25 ml with deionized distilled water.

**Digestion of Samples for In Vitro Available (Ionizable) Iron:**

**Reagents** i) 0.1 N hydrochloric acid solution.

ii) 0.5% pepsin-hydrochloric acid solution: Half a g of pepsin was dissolved in 0.1 N HCl and volume made up to 100ml with the same.

Samples were digested by a simulated *in vitro* gastrointestinal digestion procedure, which was a modification of two earlier methods (Narasinga Rao and Prabhavathi, 1978; Miller et al., 1981). A few samples were also measured for ionizable iron with extrinsic tag method using  $^{59}\text{Fe}$ .

Two g each of a sample was taken in two conical flasks and mixed with 25 ml of 0.5% pepsin-hydrochloric acid solution. One of the sets was spiked with 1m Ci of  $^{59}\text{Fe}$ , mixed thoroughly and an aliquot was taken for initial counting in a Packard Auto Gamma Counter. After the counting, the aliquot was transferred quantitatively to the respective flasks. Content of both the sets of flasks (spiked and unspiked with  $^{59}\text{Fe}$ ) were adjusted to pH 1.35. The flasks were then incubated at 37°C in a shaker water bath for 90 minutes. At the end of the incubation period, the

pH of the flasks was gradually adjusted to 7.5 using 10-20% NaOH. Trypsin, (5 mg/g of sample) was added to the content of the flasks and incubated for another 90 minutes under the same conditions described above. The contents were centrifuged at 15000 rpm for 30 min at 4°C. The supernatant was collected for *in vitro* available (ionizable) iron.

#### **Estimation of Total and Ionizable Iron:**

##### **Reagents:**

- i) 2M sodium acetate solution.
- ii) **Protein Precipitant Solution:** Ten g of trichloroacetic acid, 10 g of hydroxylamine hydrochloride, and 10 ml of concentrated hydrochloric acid were dissolved in water and volume made up to 100 ml.
- iii) **Chromagen Solution:** Twenty five mg of bathophenathroline disulphonic acid was dissolved in 2M solution of sodium acetate and the volume was made upto 50 ml with the same.

Digested food samples were analyzed for total and *in vitro* available iron with a modification of procedure of Miller et al., 1981. Half to 1ml of digested samples were made upto 1ml wherever necessary, treated with 1ml protein precipitant, vortexed and centrifuged at 3000 rpm for 20 min. An aliquot of 0.5 ml was treated with equal volume of chromagen solution, mixed and incubated for 20 min at room temperature for the development of colour.

A series of standards with the concentration ranging from 25 ng to 150 ng were prepared using ferrous ammonium sulphate and treating similarly as in case of samples. The standards and the samples were scanned against a blank solution in an ultraviolet – visible Hitachi spectrophotometer (model 220S) using first-order derivative spectrophotometry. The instrument was set at first-order derivative pen function with the use of following parameters:- maximum wavelength 600nm, minimum wavelength 500nm, scan speed 120nm/min, chart format 40 nm/cm, response time 8 seconds, slit width 4 nm, scale from 0.01 to  $-0.03 \times 2$ , first-derivative with differentiating width (D1)<sup>2</sup>.

The peak height of standard solutions from zero crossing was measured in mm and a calibration curve was prepared. A linear relation was found between the concentration of the standards and the absorbance. The concentration of iron present in the sample was calculated using the slope of the standard curve.

To estimate the ionizable iron by radiosotopic method, an aliquot of 0.5 ml of each spiked samples was taken and counted as it was done for initial counting. From the initial and final counts the percentage of ionizable iron present in foods was calculated.

**Recovery of Added Iron:** Known amounts of iron were added to the samples from the stock solution of ferrous ammonium sulphate before wet digesting for total iron of the samples and analysed like other samples.

The performance of the method was also tested by analyzing a standard reference material of rice flour (NBS-SRM-1568) obtained from National Bureau of Standards, Washington D.C.

##### **Ascorbic Acid**

**Sample Processing:** Known amount of water soaked grain samples were ground to paste with 6% metaphosphoric acid, made upto a known volume with 3% metaphosphoric acid, centrifuged and supernatant was utilized for estimation.

Fresh samples of green leafy vegetables of known amount were processed with metaphosphoric acid as in case of grain samples.

Ascorbic acid content of foods was estimated following the method by Jagota and Dani (1982).

**Tannin:** Tannin content of the samples was estimated by vanillin reaction assay given by Price et al. (1978).

## **RESULTS AND DISCUSSION**

**Recovery of Added Iron:** The recoveries of standards were found to be  $97.27 \pm 0.38\%$  (mean  $\pm$  SEM) in case of rice and  $94.27 \pm 0.64\%$  (mean  $\pm$  SEM) in case of Bengalgram (white).

The performance of the method tested by analyzing a standard reference material of rice flour (NBS-SRM-1568) was found to be  $8.10 \pm 0.10$  mg/g ( $n = 6$ ) as against the certified value of  $8.70 \pm 0.60$  mg/g. Differences were not statistically significant.

**Total and Ionizable Iron in Cereals and Millets:** The results of total iron, ionizable iron, ascorbic acid and tannin content are presented in Table 1.

Total iron ranged from  $0.61 \pm 0.09$  mg/100g in rice to  $8.00 \pm 0.92$  mg/100g in bajra which are comparable to values reported in earlier studies (Gopalan et al., 1989; Sankara Rao and Deosthale, 1981; Sankara Rao and Deosthale, 1983). The absolute amount of ionizable iron ranged from  $0.18 \pm 0.03$  mg/100g in rice to  $1.10 \pm 0.17$  mg/100g in bajra. Although rice has lowest amount of

**Table 1: Total iron, ionizable iron, ascorbic acid and tannin contents in cereals and millets**

| <i>Foods</i>                           |     | <i>Total iron</i><br><i>mg/100g</i> | <i>Ionizable iron</i><br><i>mg/100g</i> | <i>Ionizable iron</i><br><i>%</i> | <i>Ascorbic acid</i><br><i>mg/100g</i> | <i>Tannin</i><br><i>mg/100g</i> |
|----------------------------------------|-----|-------------------------------------|-----------------------------------------|-----------------------------------|----------------------------------------|---------------------------------|
| Rice ( <i>Oryza sativa</i> )           | (6) | 0.61 ± 0.09 <sup>a</sup>            | 0.18 ± 0.03 <sup>a</sup>                | 29.50 ± 4.75 <sup>a</sup>         | 1.50 ± 0.13 <sup>a</sup>               | ND                              |
| Wheat ( <i>Triticum aestivum</i> )     | (6) | 5.87 ± 0.26 <sup>cd</sup>           | 0.45 ± 0.08 <sup>bc</sup>               | 7.70 ± 1.36 <sup>a</sup>          | 16.00 ± 0.61 <sup>b</sup>              | 21.90 ± 1.35 <sup>a</sup>       |
| Bajra ( <i>Pennisetum typhoideum</i> ) | (6) | 8.00 ± 0.92 <sup>d</sup>            | 1.10 ± 0.17 <sup>d</sup>                | 13.75 ± 2.12 <sup>b</sup>         | 31.71 ± 1.10 <sup>d</sup>              | 12.90 ± 1.41 <sup>b</sup>       |
| Sorghum ( <i>Sorghum vulgare</i> )     | (6) | 4.44 ± 0.50 <sup>bc</sup>           | 0.67 ± 0.04 <sup>c</sup>                | 15.10 ± 0.90 <sup>b</sup>         | 14.11 ± 0.23 <sup>b</sup>              | 3.33 ± 0.40 <sup>c</sup>        |
| Ragi ( <i>Eleusine coracana</i> )      | (6) | 7.85 ± 1.66 <sup>d</sup>            | 0.38 ± 0.02 <sup>ab</sup>               | 4.84 ± 0.25 <sup>a</sup>          | 32.40 ± 1.06 <sup>d</sup>              | 598.20 ± 62.50 <sup>d</sup>     |
| Maize ( <i>Zea mays</i> )              | (6) | 2.73 ± 0.14 <sup>b</sup>            | 0.69 ± 0.04 <sup>c</sup>                | 25.30 ± 1.46 <sup>c</sup>         | 20.30 ± 0.66 <sup>c</sup>              | 24.10 ± 3.13 <sup>a</sup>       |

i. Values in parentheses indicates the number of samples.

ii. Values are mean ± SEM.

iii. Variation in superscripts between mean values for given parameters indicate significant differences (ANOVA) ( $P < 0.05$ ).

iv. ND indicates values are not detectable.

ionizable iron in terms of absolute iron, while expressing it as percentage of total iron it showed highest level (29.50±4.75%). The percentage ionizable iron content of maize (25.30±1.46%) was found to be comparable with rice.

Most of the cereals and millets had higher percentage of ionizable iron than reported values (Narasinga Rao and Prabhavathi, 1978; Narasinga Rao and Prabhavathi, 1982; Udayasekhara Rao and Deosthale, 1988), which could be because of methodological or varietal differences of the samples. Differences in the percent ionizable iron in present study and in earlier studies were also because of the differences in total iron and absolute amounts of iron. For example Narasinga Rao and Prabhavathi (1978) reported a total iron content amounting 1.8 mg/100 g and percent ionizable iron 15.0% in case of rice that indicates an absolute amount of ionizable iron in rice was 0.27 mg/100 g. Whereas in the present study rice had a much lower total iron of 0.61±0.09 mg/100 g (Table 1) and absolute amount of ionizable iron of 0.18±0.03 mg/100 g. While expressing the absolute amount of ionizable iron in percentage of total iron, ionizable iron percentage was found to be much higher (29.50±4.75%) compared to earlier study (Narasinga Rao and Prabhavathi, 1978). Variation in percentages of ionizable iron due to variation in total and absolute amounts of iron were also observed in other foods like ragi, bajra, sorghum when results of the present study was compared with earlier reported values (Narasinga Rao and Prabhavathi, 1978; Sankara Rao and Deosthale, 1983).

The ascorbic acid content of dry seeds was reported to be undetectable or negligible (Gopalan et al., 1989). In the present study when the grains were soaked overnight in water a considerable amount of ascorbic acid was detected. Similar

observation was also reported by Narasinga Rao and Prabhavathi (1982).

Tannin content of ragi was highest (598.20±62.50 mg/100g) in ragi compared to other cereals and millets analyzed. A higher tannin content in ragi was also reported by Narasinga Rao and Prabhavathi (1982) compared to some other cereals and millets. Sorghum used in the present study was of white variety had lowest amount of tannin of 3.33±0.40 mg/100 g. Earlier studies (Narasinga Rao and Prabhavathi, 1982) also showed much lower tannin content (77 mg/100 g) in white variety compared to red variety (973 mg/100 g). In general endogenous amounts of ascorbic acid and tannin were not found to influence the percent ionizable iron content. Studies with composite meals had also indicated that influence of ascorbic acid on iron absorption was less than commonly assumed. The investigation were unable to detect a significant increase in iron absorption when 1500 mg vitamin C / day was added to the diet (Hunt et al., 1994; Cook and Reddy, 2001). Various other associated factors may have influence which needs to be investigated.

#### **Total and Ionizable Iron in Whole Pulses:**

Except blackgram, the total iron content of all whole pulses evaluated were comparable whereas, the ionizable iron content of whole pulses varied widely both in terms of absolute (mg/100g) and relative (%) amounts (Table 2). Most of the values are within the range of earlier reported values (Narasinga Rao and Prabhavathi, 1982; Gopalan et al., 1989).

Like cereals and millets most of the whole pulses were found to contain considerable amounts of ascorbic acid (Table 2). Similar observations were also reported by earlier investigators (Narasinga Rao and Prabhavathi,

**Table 2: Total iron, ionizable iron, ascorbic acid and tannin contents in whole pulses**

| Foods                                            |     | Total total<br>mg/100g   | Ionizable<br>iron mg/100g | Ionizable<br>iron %       | Ascorbic<br>acid mg/100g  | Tannin<br>mg/100g           |
|--------------------------------------------------|-----|--------------------------|---------------------------|---------------------------|---------------------------|-----------------------------|
| Bengalgram (white)<br>( <i>Cicer arietinum</i> ) | (6) | 5.62 ± 0.40 <sup>a</sup> | 1.22 ± 0.03 <sup>b</sup>  | 21.71 ± 0.53 <sup>b</sup> | 5.50 ± 0.13 <sup>a</sup>  | 31.10 ± 3.47 <sup>a</sup>   |
| Bengalgram (brown)<br>( <i>Cicer arietinum</i> ) | (6) | 5.71 ± 0.20 <sup>a</sup> | 0.36 ± 0.02 <sup>c</sup>  | 6.30 ± 0.35 <sup>c</sup>  | 5.60 ± 0.23 <sup>a</sup>  | 41.40 ± 2.76 <sup>ab</sup>  |
| Greengram<br>( <i>Phaseolus aureus</i> )         | (6) | 6.12 ± 0.12 <sup>a</sup> | 0.40 ± 0.08 <sup>c</sup>  | 6.53 ± 1.31 <sup>c</sup>  | 29.80 ± 2.53 <sup>b</sup> | 100.40 ± 7.70               |
| Blackgram<br>( <i>Phaseolus mungo</i> )          | (6) | 4.40 ± 0.30 <sup>b</sup> | 0.15 ± 0.01 <sup>c</sup>  | 3.41 ± 0.22 <sup>c</sup>  | 50.00 ± 0.98 <sup>c</sup> | 394.31 ± 18.30 <sup>d</sup> |
| Lentil ( <i>Lens esculenta</i> )                 | (6) | 6.30 ± 0.44 <sup>a</sup> | 0.22 ± 0.01 <sup>a</sup>  | 3.50 ± 0.24 <sup>a</sup>  | 27.00 ± 0.85 <sup>b</sup> | 71.70 ± 1.54 <sup>bc</sup>  |
| Pea ( <i>Pisum sativum</i> )                     | (6) | 6.31 ± 0.30 <sup>a</sup> | 2.25 ± 0.30 <sup>a</sup>  | 35.66 ± 4.44 <sup>a</sup> | 8.60 ± 0.74 <sup>a</sup>  | 16.80 ± 2.09 <sup>a</sup>   |
| Rajmah ( <i>Phaseolus vulgaris</i> )             | (6) | 6.36 ± 0.55 <sup>a</sup> | 1.53 ± 0.10 <sup>b</sup>  | 24.05 ± 1.42 <sup>b</sup> | 51.30 ± 1.34 <sup>c</sup> | 897.31 ± 27.68 <sup>e</sup> |

i. Values in parentheses indicates the number of samples.

ii. Values are mean ± SEM.

iii. Variation in superscripts between mean values for given parameters indicate significant differences (ANOVA) (P&lt;0.05). Differences of significance greater or less than 5% are mentioned appropriately in the text.

1982). Variation in the tannin content of whole pulses observed in the present and earlier studies could be due to varietal or locational differences of the samples.

No correlation was observed between the levels of ascorbic acid and tannin and the availability of iron of the whole pulses evaluated in the study. For example, Bengalgram (white) and rajmah had comparable amounts of ionizable iron (%), despite very high tannin content in rajmah. It cannot be due to higher ascorbic acid content in rajmah, since blackgram with comparable ascorbic acid and lower tannin content had lower ionizable iron. Therefore endogenous levels of these factors in whole pulses may not have any influence on the availability of iron. It was reported from some cereal based diets that even with a higher content of tannin, percent ionizable iron was higher compared to some other diets with lower tannin (Narasinga Rao and Prabhavathi, 1982).

**Total and Ionizable Iron in Decorticated split Pulses (dals) :** The total iron contents of

decorticated split pulses (*dals*) (Table 3) were not much different from their respective whole forms (Table 3), and also comparable to the reported values (Narasinga Rao and Prabhavathi, 1978). Significantly higher amounts of ionizable iron were observed in '*dals*' compared to whole pulses. This could be because of removal of some inhibiting factors during decortication, which needs further investigations.

The ascorbic acid content of the '*dals*' was found to be negligible (Table 3). Tannin content of a grain is mostly accounted by seed coat (Udayasekhara Rao and Deosthale, 1982). As a result decorticated '*dals*' contained very little or negligible amounts of tannin.

**Total and Ionizable Iron in Green Leaf Vegetables:** The total iron content of green leafy vegetables ranged from 1.82±0.11 mg/100g to 3.76±0.23 mg/100g (Table 4) and was comparable with the literature value (Layrisse et al., 1969; Zhang et al., 1985 and Gopalan et al., 1989).

The amount of ionizable iron from these green leafy vegetables was found to be considerably

**Table 3: Total iron, ionizable iron, ascorbic acid and tannin contents in decorticated split pulses (dals).**

| Foods                                         |     | Total iron<br>mg/100g  | Ionizable iron<br>mg/100g | Ionizable iron<br>%     | Ascorbic acid<br>mg/100g | Tannin<br>mg/100g       |
|-----------------------------------------------|-----|------------------------|---------------------------|-------------------------|--------------------------|-------------------------|
| Bengalgram (brown) ( <i>Cicer arietinum</i> ) | (6) | 5.63±0.30 <sup>b</sup> | 1.33±0.10 <sup>b</sup>    | 23.62±1.80 <sup>b</sup> | 5.31±0.20 <sup>c</sup>   | 11.20±1.23 <sup>a</sup> |
| Greengram ( <i>Phaseolus aureus</i> )         | (6) | 5.50±0.25 <sup>b</sup> | 1.18±0.04 <sup>b</sup>    | 21.45±0.73 <sup>b</sup> | 8.30±0.24 <sup>e</sup>   | 17.80±1.38 <sup>b</sup> |
| Blackgram ( <i>Phaseolus mungo</i> )          | (6) | 4.13±0.11 <sup>c</sup> | 0.57±0.03 <sup>c</sup>    | 13.80±0.73 <sup>c</sup> | 3.60±0.24 <sup>b</sup>   | 24.84±1.06 <sup>b</sup> |
| Redgram ( <i>Cajanus cajan</i> )              | (6) | 3.62±0.12 <sup>d</sup> | 0.85±0.03 <sup>d</sup>    | 23.50±0.83 <sup>b</sup> | 6.71±0.23 <sup>d</sup>   | 44.00±2.36 <sup>c</sup> |
| Lentil ( <i>Lens esculenta</i> )              | (6) | 6.31±0.21 <sup>a</sup> | 2.00±0.11 <sup>a</sup>    | 31.70±1.74 <sup>a</sup> | 2.80±0.14 <sup>a</sup>   | 41.83±3.51 <sup>c</sup> |

i. Values in parentheses indicates the number of samples.

ii. Values are mean ± SEM.

iii. Variation in superscripts between mean values for given parameters indicate significant differences (ANOVA) (P&lt;0.05). Differences of significance greater or less than 5% are mentioned appropriately in the text.

**Table 4: Total iron, ionizable iron, ascorbic acid and tannin content in green leafy vegetables (fresh weight basis).**

| Foods                                         |     | Total iron<br>mg/100g  | Ionizable iron<br>mg/100g | Ionizable iron<br>%      | Ascorbic acid<br>mg/100g  | Tannin<br>mg/100g          |
|-----------------------------------------------|-----|------------------------|---------------------------|--------------------------|---------------------------|----------------------------|
| Amaranth ( <i>Amaranthus</i> sp.)             | (4) | 3.17±0.36 <sup>a</sup> | 1.60±0.07 <sup>a</sup>    | 50.50±2.21 <sup>a</sup>  | 135.31±17.00 <sup>b</sup> | 198.50±12.90 <sup>bc</sup> |
| Agathi ( <i>Sesbania grandiflora</i> )        | (4) | 3.76±0.23 <sup>a</sup> | 1.75±0.10 <sup>b</sup>    | 46.54±2.40 <sup>a</sup>  | 118.71±3.45 <sup>b</sup>  | 183.05±1.35 <sup>b</sup>   |
| Spinach ( <i>Spinacia oleracea</i> )          | (4) | 1.90±0.10 <sup>b</sup> | 0.67±0.02 <sup>c</sup>    | 35.26±1.06 <sup>bc</sup> | 20.92±4.26 <sup>a</sup>   | 185.33±12.26 <sup>b</sup>  |
| Bachali ( <i>Basella alba</i> )               | (4) | 1.82±0.11 <sup>b</sup> | 0.55±0.02 <sup>c</sup>    | 30.22±1.10 <sup>c</sup>  | 105.25±8.46 <sup>b</sup>  | 192.30±6.20 <sup>bc</sup>  |
| Gongura ( <i>Hibiscus cannabinus</i> )        | (4) | 2.10±0.13 <sup>b</sup> | 0.95±0.05 <sup>d</sup>    | 45.24±2.40 <sup>ab</sup> | 100.00±31.00 <sup>b</sup> | 80.10±7.18 <sup>a</sup>    |
| Fenugreek ( <i>Trigonella foenumgraecum</i> ) | (4) | 2.11±0.10 <sup>b</sup> | 1.10±0.04 <sup>d</sup>    | 52.13±1.90 <sup>a</sup>  | 22.55±4.18 <sup>a</sup>   | 226.10±8.32 <sup>c</sup>   |

i. Values in parentheses indicates the number of samples.

ii. Values are mean ± SEM.

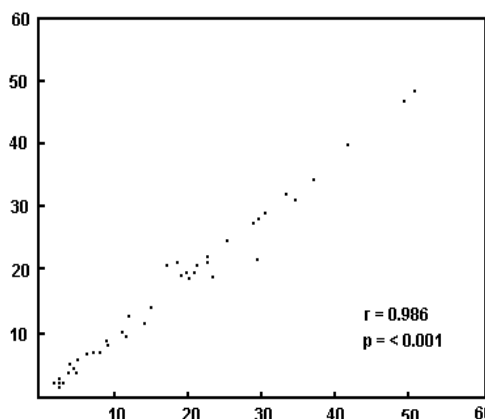
iii. Variation in superscripts between mean values for given parameters indicate significant differences (ANOVA) ( $P < 0.05$ ). Differences of significance greater or less than 5% are mentioned appropriately in the text.

high ranging from 30.22±1.10% to 52.13±1.90%. This is contrary to the reported values for ionizable iron by Narasinga Rao and Prabhavathi (1978) which was between 4-5%. Similarly Layrisse et al. (1969) reported 1.7% of available iron by *in vivo* measurements from spinach. The discrepancies could be because of varietal or methodological. However, Van Campen and Welch (1980) showed a very high absorption of iron from spinach, amounting to 58.8% in rat. High bioavailability in green leafy vegetables were also reported by several other investigations (Gordon and Chao, 1984; Zhang et al., 1985).

All the green leafy vegetables were found to have considerable amount of ascorbic acid and tannin (Table 4). As in case of other food samples evaluated, in green leafy vegetables also, the endogenous levels of enhancers and inhibitors were not found to have any impact on the availability of iron, because fenugreek with lower ascorbic acid and highest tannin showed highest levels of per cent ionizable iron.

**Comparison of Spectrophotometric and Radioisotopic Methods:** One of the major advantages of derivative spectrophotometry is, turbidity does not interfere in the determination of the element under consideration, hence gives more accurate results. In the present context of the simulated digestion for ionizable iron, extracts particularly from pulse samples give considerable turbidity even after protein precipitation. Besides, absolute amounts of ionizable iron of some samples are considerably low, i.e., 0.18±0.03 mg/100g in rice (Table 1) and 0.15±0.01 mg/100g in black gram (Table 2). Further, the levels were only in nanograms when an aliquot of the samples was taken. Therefore, adoption of derivative spectrophotometry could be of help for accuracy in estimation. While comparing the results of ionizable iron by the derivative spectro-

photometry and radioisotopic method, percent ionizable iron content of the samples were similar (Table 5), with a significant positive correlation ( $r = 0.986$ ;  $P < 0.001$ ) (Fig. 1). Miller et al. (1981) also reported a strong positive correlation of *in vitro* availability of iron from meals estimated by colorimetric and isotopic method. This indicates that added iron ( $^{59}\text{Fe}$ ) mixes completely with non-

**Fig. 1. Correlation between chemical and isotopic methods of estimation of ionizable iron in foods.****Table 5: Comparison of ionizable iron content estimated by chemical and isotopic method using  $^{59}\text{Fe}$** 

| Foods                                         |     | Ionizable iron % |                                  |
|-----------------------------------------------|-----|------------------|----------------------------------|
|                                               |     | Chemical method  | Isotopic method $^{59}\text{Fe}$ |
| Rice ( <i>Oryza sativa</i> )                  | (6) | 29.50±4.75       | 29.00±4.42                       |
| Wheat ( <i>Triticum aestivum</i> )            | (6) | 7.70±1.36        | 7.70±1.04                        |
| Sorghum ( <i>Sorghum vulgare</i> )            |     | 15.10±0.90       | 16.83±1.91                       |
| Bengalgram (white) ( <i>Cicer arietinum</i> ) | (6) | 21.71±0.53       | 21.73±1.32                       |
| Greengram ( <i>Phaseolus aureus</i> )         | (6) | 6.53±1.31        | 6.63±1.25                        |
| Lentil ( <i>Lens esculenta</i> )              | (6) | 3.50±0.24        | 3.08±0.37                        |

haem iron pool and behaves as intrinsic iron.

The present study on iron bioavailability serves as a basis for calculating actual iron requirement or recommendation for various segments of the population. Iron is a very important element in human health, and in the same time iron deficiency continues to be one of the most common nutritional deficiencies in the world. An estimated 30% of world's population is anaemic, with just under half of these – approximately 600 million cases- due to impaired iron status (Carpenter and Mahoney, 1992; Cook et al., 1994). The health consequences of iron deficiency, like decreased immune function, diminished work capacity, increased risk of delivery of pre term and low birth weight infants, diminished cognitive development and learning capacity are well known (Cook et al., 1994; National Research Council, 1989). Given the potential consequences of iron deficiency, determination of iron intakes is a useful strategy for assessing the adequacy of the diet to meet iron requirements. Importantly, however, absorption or bioavailability of iron is highly variable, dependent on various factors like, iron status of the individuals, dietary enhancers and inhibitors of iron absorption etc. As such, calculating iron availability is as important as determining total iron intakes when evaluating the adequacy of iron in the diet. Indeed, adjustment for bioavailability may provide a more realistic picture of whether or not iron requirements are met in a population than would a simple assessment of total iron intake. For this, amount of bioavailable iron from different foods are need to be known so that, requirements can be calculated on the basis of availability and not only on the basis of total iron content of foods or diets. Therefore, the present study is of importance of human health in calculating actual dietary recommendation of iron for a population, depending on the bioavailable amount of iron.

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