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Iron bioavailability in green leafy vegetables cooked in different utensils

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Abstract

The effects of cooking utensils on the total and bioavailable iron contents of five green leafy vegetables, along with related promoters and inhibitors, were investigated. The cooked and fresh greens were analysed for moisture, total and bioavailable iron, ascorbic acid, dietary fibre, tannins, total oxalates and soluble oxalates by standard techniques. Moisture content of fresh greens ranged from 80–90%, total dietary fibre (5–11 g/100 g), oxalates (0.022–1.37 g/100 g) and tannin (41–166 mg/100 g). Cooking in different utensils had no effect on these parameters. Ascorbic acid content ranged from 8.7 to 88.3 mg/100 g in fresh greens and was reduced by 18–64% on cooking. The total and ionisable iron contents of greens ranged from 3 to 13 mg/100 g and 0.43 to 2.7 mg/100 g, respectively, and increased on cooking in iron utensil to 9.7 to 17.5 mg/100 g and 1.50 to 8.56 mg/100 g, respectively. The availability of iron, in relation to total iron, of greens cooked in iron utensils was either comparable or marginally higher than those cooked in other metallic utensils. Since the total iron content of greens cooked in iron utensils was high, the actual amount of available iron also increased. It can be concluded that cooking in iron utensils increases the total as well as the available iron content of greens.

Keywords: Iron bioavailability; Green leafy vegetables; Iron utensils; Ascorbic acid; Ionisable iron; Dietary fibre

1. Introduction

Iron deficiency anaemia is a global nutritional problem, affecting nearly 1.78 billion people of which 358 million are from the developing world (WHO, 1998). Iron deficiency has a massive economic cost, adding to the burden on the health system, affecting cognitive performance of children and reducing adult productivity. The World Bank, WHO and Harvard University list iron deficiency anaemia as entailing a higher overall cost than any other disease except tuberculosis (Murray & Lopez, 1996). Iron deficiency anaemia can be prevented at low cost and the benefit/cost ratio of implementing preventive programmes is recognised as one of the highest in the realm of public health.

Inadequate dietary intake and poor bioavailability of iron from food are considered as prime etiological factors of anaemia. Despite implementation of large-scale nutritional intervention/prophylaxis programmes, iron deficiency anaemia still continues to be a public health problem in most developing countries (Allen & Sabel, 2001). Research has suggested that bioavailability of iron from food systems is an outcome/resultant of the interaction of its components. Of the dietary components, oxalates (Chawla, Saxena, & Seshadri, 1988), tannins (Narasinga Rao & Prabhavati, 1982) and phytates (Gillooly et al., 1983) are known to inhibit iron absorption whereas organic acids, such as ascorbic acid, citric acid, malic acid and lactic acid are known to enhance the absorption of iron (Ballot et al., 1987; Hazell & Johnson, 1987).

The food-based approach, including dietary diversification and modification to promote year-round availability, access to and utilization of foods which promote increased intake and absorption of dietary iron and cooking in iron utensils, is an important method for dietary enrichment. An array of studies conducted in India (Kakade & Agte, 1997; Reddy, Ingale, & Nalwade, 1997) and other countries (Martinez & Vannucchi,

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1986; Mistry, Brittin, & Stoecker, 1988) has shown that cooking in iron utensils increases the total and available iron of foods. Many of these studies show that addition of other components added, e.g. tomato or tamarind juice, might also be partially responsible for increase in the available iron reported (Arora, 2000). Cooking in cast iron utensils, traditionally used in Indian families until four decades back for boiling milk and cooking vegetables, provides extra dietary iron. This available dietary iron is well absorbed. WHO reports on prevalence of anaemia among pregnant women record that the lowest rates of anaemia among all the sub-regions of the developing world were observed in Southern Africa, due to widespread use of iron cooking pots by indigenous people (WHO, 1992). An improvement of the haematological parameters in human and rat assays on consumption of foods cooked in iron utensils clearly indicates that the iron added to foods by the iron pots is in the readily-soluble ferrous form (Adish, Esrey, Gyorkkos, Jean-Baptise, & Rojhani, 1999; Borigato & Martinez, 1998; Martinez & Vannucchi, 1986).

Green leafy vegetables are good sources of iron, providing around 5–10 mg per 100 g on an average (Gopalan et al., 1996). A daily intake of 100 g of greens is recommended in an adult's diet (NIN, 1998). However, bioavailability of iron in greens may depend upon ascorbic acid content, which is a promoter and dietary fibre, oxalates and tannins, which are inhibitors of iron absorption. Greens are generally low-cost and cooking in iron pots could be an effective strategy to increase iron intake. The present investigation was planned with an aim to investigate the role of cooking utensils on the total and available iron from greens. In addition, dietary fibre, ascorbic acid, oxalates and tannins, which influence the iron bioavailability were also analysed to study their effects.

2. Materials and methods

Five commonly consumed greens, namely Amaranth (*Amaranthus gangeticus*), Shepu (*Peucedanum graveolens*), Kilkeerai (*Amaranthus tricolor*), Fenugreek (*Trigonella foenum graecum*) and Chakotha (*Chenopodium album*), were selected. They were procured in a single lot from a local market, cleaned and washed with glass-distilled water after separating the non-edible portion. The thoroughly drained greens were divided into four equal portions and were cut into 1 cm pieces using a stainless steel knife on a wooden cutting board. One portion was retained, as such, for analysis, which served as control. The other three portions were cooked in iron, stainless steel and aluminium vessels, respectively, using the open-pan method of cooking. The times taken for cooking and water required for different greens are presented in Table 1. Greens were cooked until they became soft, i.e. judged by fingerfeel. Time taken for cooking depends on the gauge, heat penetration and retention by the metal. Iron utensils required comparatively shorter cooking times due, to better heat retention while in the aluminium utensil, water uptake was high due to faster heat penetration.

The cooked and fresh greens were analysed for moisture (AOAC, 1999), ascorbic acid by the visual titration method of reduction of 2,6-dichlorophenol-indophenol dye (Ranganna, 1986), total oxalates (extraction with hydrochloric acid) and soluble oxalates (aqueous extraction) by precipitation with calcium oxalate from deproteinized extract and subsequent titration with potassium permanganate (Baker, 1952). The cooked and fresh samples were dried in glass dishes in a hot air oven at 50 ± 5 °C. The samples were finely powdered, using porcelain pestle and mortar, and stored in airtight containers. The dried samples were used for the estimation of total iron, bioavailable iron, dietary fibre and tannins. Total iron in ash solution was determined colorimetrically by the α–α dipyridyl method (AOAC, 1965), dietary fibre by separation of non-starch polysaccharides by enzymatic gravimetric method (Asp, Johansson, Hallmer, & Siljestrom, 1983) and tannins by the vanillin hydrochloride method (Burns, 1971). Bioavailable iron was estimated by an in vitro method (Narasinga Rao & Prabhavati, 1978). The ionisable iron determined by this method has been shown to correlate highly with the in vivo iron absorption in humans by isotope studies.

Data were subjected to a suitable statistical test. One-way ANOVA was used to calculate the differences between the constituents of the green leafy vegetables

Table 1
Cooking conditions of green leafy vegetables

Greens	Utensils used for cooking					
	Aluminium		Iron		Steel	
	Time (min)	Water (ml)	Time (min)	Water (ml)	Time (min)	Water (ml)
Amaranth	25	450	23	350	26	350
Chakotha	26	100	24	100	32	100
Fenugreek	30	350	26	300	34	300
Kilkeerai	41	300	37	300	52	350
Shepu	46	400	44	200	51	200

cooked in different utensils. Probability level was set at $P < 0.05$.

3. Results and discussion

The results of the study are summarized in Tables 2–6. The iron bioavailability and related constituents analysed in all the fresh greens listed in Table 2 indicate that the moisture content of fresh greens ranged from 80.9 to 92.1 g/100 g of fresh greens. The dietary fibre contents ranged from 4.85 to 10.7 g/100 g. For the total oxalate content, a wide range of values was encountered. Fenugreek and Shepu had very low values of 0.031 g and 0.092 g/100 g, respectively, whereas Amaranth had a very high value of 1.37 g/100 g. These values were similar to those reported by Chawla et al. (1988). The soluble oxalate content of greens also differed considerably with Chakotha having the maximum amount of soluble oxalates (0.816%) and Fenugreek having the least (0.017%). Tannin content was highest in Fenugreek (166 mg) followed by Shepu and Amaranth. Chakotha and Kilkeerai had similar amounts of 41.9 and 40.9 mg/100 g. The ascorbic acid content also showed a wide variation, from 9 to 88 mg. The total iron content was in lower ranges in Kilkeerai, Shepu and Chakotha (3.56–4.30 mg/100 g) and high in Amaranth and Fenugreek (10.6 and 13.2 mg/100 g, respectively). The available iron content, which actually represents the physiologically available iron, was highest for Fenugreek, followed by Shepu, Kilkeerai, Chakotha and Amaranth. It may be noted here that Fenugreek had very low amounts of total and soluble oxalates and high amounts of ascorbic acid, as well as a higher content of total iron, thereby increasing the actual amount of iron available, despite a higher content of tannin.

Conversely, Amaranth had a very high content of total oxalates, tannins and dietary fibre and comparatively lower contents of ascorbic acid, thereby lowering the iron bioavailability. Chakotha also exhibited a low iron bioavailability due to high total as well as soluble oxalate contents, whereas Shepu and Kilkeerai had intermediate values. Shepu and Kilkeerai, with a lower iron content, had 2–10 times more bioavailable iron than other greens (Chakota, Fenugreek and Amaranth, respectively) because of low fibre, oxalate and higher ascorbic acid in Shepu and low tannin and fibre in Kilkeerai. In terms of percent of total iron available, it was found to be very low for Amaranth (2.5%), followed by Chakotha (12.8%), Fenugreek (14.5%), Kilkeerai (25%) and Shepu (27.2%). The relationship between the oxalate content and in vitro availability of iron in Amaranth, observed in the present study, is similar to that of Chawla et al. (1988), who observed that iron

Table 2
Iron availability and related constituents in fresh green leafy vegetables[a] (per 100 g fresh weight basis)

Constituent	Amaranth	Chakotha	Fenugreek	Kilkeerai	Shepu	Average
Moisture (g)	86.4	92.1	80.9	90.2	89.8	87.8 ± 4.42
Dietary fibre (g)	8.44	4.85	10.7	5.90	5.65	7.11 ± 2.42
Total oxalates (g)	1.37	0.909	0.031	0.638	0.092	0.061 ± 0.57
Soluble oxalates (g)	0.289	0.816	0.017	0.216	0.033	0.273 ± 0.32
Tannins (mg)	60.1	41.9	166	40.9	68.7	75.4 ± 51.80
Ascorbic acid (mg)	18.3	39.2	88.3	8.7	56.7	42.2 ± 31.8
Total iron (mg)	10.6	3.66	13.2	4.30	3.56	6.82 ± 4.70
Ionisable iron (mg)	0.43	0.92	2.69	1.91	1.90	1.49 ± 0.90
Bioavailable iron (mg)	0.27	0.47	1.92	1.07	0.97	0.88 ± 0.60
% availability of total iron	2.5	12.8	14.5	25.0	27.2	16.4 ± 10.0

[a] Represents mean values of four replicates.

Table 3
Effect of cooking green leafy vegetables in aluminium utensil on iron availability and related constituents[a] (per 100 g fresh weight basis)

Constituent	Amaranth	Chakotha	Fenugreek	Kilkeerai	Shepu	Average
Moisture (g)	83.0	91.0	89.1	91.3	87.7	88.4 ± 3.40
Dietary fibre (g)	9.35	5.90	12.30	6.61	6.85	8.20 ± 2.64
Total oxalates (g)	1.10	0.987	0.026	0.515	0.048	0.536 ± 0.50
Soluble oxalates (g)	0.316	0.661	0.014	0.214	0.042	0.249 ± 0.30
Tannins (mg)	81.0	38.1	139	37.1	66.0	72.3 ± 1.75
Ascorbic acid (mg)	3.2	16.7	56.3	3.3	14.2	18.7 ± 21.9
Total iron (mg)	11.86	4.52	12.65	4.30	3.20	7.31 ± 4.55
Ionisable iron (mg)	0.24	1.81	1.75	1.91	1.66	1.47 ± 0.70
Bioavailable iron (mg)	0.18	0.96	0.89	1.07	0.83	0.79 ± 0.35
% availability of total iron	1.50	21.2	7.04	24.9	25.9	16.5 ± 9.20

[a] Represents mean values of four replicates.

Table 4
Effect of cooking green leafy vegetables in steel utensil on iron availability and related constituents[a] (per 100 g fresh weight basis)

Constituent	Amaranth	Chakotha	Fenugreek	Kilkeerai	Shepu	Average
Moisture (g)	79.1	91.5	87.8	91.4	87.6	87.5 ± 5.05
Dietary fibre (g)	9.10	6.20	12.1	6.72	5.75	7.96 ± 2.62
Total oxalates (g)	1.18	0.750	0.026	0.774	0.048	0.566 ± 0.50
Soluble oxalates (g)	0.487	0.739	0.014	0.211	0.039	0.298 ± 0.30
Tannins (mg)	82.5	43.4	132	37.4	61.9	71.4 ± 38.06
Ascorbic acid (mg)	4.0	21.6	35.7	4.4	23.3	17.8 ± 13.6
Total iron (mg)	10.4	3.02	11.8	4.47	3.32	6.60 ± 4.20
Ionisable iron (mg)	0.67	1.07	3.31	2.06	1.73	1.77 ± 1.00
Bioavailable iron (mg)	0.40	0.56	1.63	1.02	0.77	0.88 ± 0.50
% availability of total iron	3.8	18.5	13.8	22.8	23.2	16.4 ± 8.00

[a] Represents mean values of four replicates.

Table 5
Effect of cooking green leafy vegetables in iron utensil on iron availability and related constituents[a] (per 100 g fresh weight basis)

Constituent	Amaranth	Chakotha	Fenugreek	Kilkeerai	Shepu	Average
Moisture (g)	82.7	92.2	88.3	90.2	83.1	87.3 ± 4.30
Dietary fibre (g)	9.02	5.35	11.9	6.52	6.62	7.88 ± 2.61
Total oxalates (g)	1.39	1.05	0.026	0.711	0.049	0.644 ± 0.60
Soluble oxalates (g)	0.278	0.696	0.023	0.210	0.031	0.248 ± 0.27
Tannins (mg)	64.6	40.4	144	30.1	64.0	68.6 ± 44.70
Ascorbic acid (mg)	3.2	16.7	28.2	4.4	29.2	16.3 ± 12.50
Total iron (mg)	16.8	13.9	15.4	9.70	17.5	14.7 ± 3.08
Ionisable iron (mg)	1.50	6.12	6.15	4.24	8.56	5.31 ± 2.63
Bioavailable iron (mg)	0.76	2.94	2.96	2.05	4.10	2.56 ± 1.24
% availability of total iron	4.5	21.1	19.3	21.1	23.5	17.9 ± 7.64

[a] Represents mean values of four replicates.

availability in Amaranth was lower, due to high oxalate content, than Fenugreek or Carrot greens (Chawla et al., 1988). Thus it can be said that bioavailable iron and percent availability are the outcome of the inherent composition of foods, which is clearly reflected, in Table 2.

The greens cooked in an aluminium vessel, had moisture contents in the range of 83–91 g%, dietary fibre – 5.9–12.3 g, total oxalates – 0.26–1.10 g, soluble oxalates – 0.014–0.661 g/100 g and tannins – 37.1–139 mg/100 g (Table 3). These values were comparable to the values of fresh greens. The ascorbic acid contents of all the samples were reduced considerably by cooking. Similar results were also reported by Yadav and Sehgal (1995) who showed loss of ascorbic acid in spinach and Amaranth leaves on cooking in an open pan. The decrease was highest for Amaranth, corresponding to 83%, followed by Shepu (75%), Kilkeerai (62%), Chakotha (57%) and Fenugreek (36%). Slight variations were encountered in total iron content in comparison with control, which could be attributed to the samples themselves. The bioavailable iron was highest in Kilkeerai, followed by Chakotha, Fenugreek, Shepu and Amaranth. This difference in the extent of bioavailable iron can be attributed to the total iron content of samples. In the case of Chakotha, the soluble oxalate (as percent of total oxalate) and dietary fibre contents were less than Shepu greens, which may account for a higher available iron. However, these differences were due to the greens themselves and cannot be attributed to the aluminium vessel.

When the greens were cooked in steel vessels, major differences were not found in moisture, dietary fibre or oxalate contents of samples, which were comparable to fresh greens (Table 4). Ascorbic acid was reduced in all samples to the extent of 45–78% due to the cooking. The available iron was slightly higher in Amaranth, Chakotha and Kilkeerai and lower in Fenugreek and Shepu in comparison with fresh greens.

The total and bioavailable iron contents of greens cooked in an iron vessel, along with the related constituents, are presented in Table 5. As seen with the other samples, cooking in different vessels did not influence the moisture, dietary fibre, tannin or oxalate contents of different greens. A lowering of ascorbic acid value was observed in comparison with fresh greens. However, a significant increase was observed in total iron content of greens. The increase was by 1.6, 3.8, 1.2, 2.3 and 4.9 times for Amaranth, Chakotha, Fenugreek, Kilkeerai and Shepu, respectively. This is similar to the observations of Reddy et al. (1997) who reported that total iron increased by 1–3 times in Ambat Chuka,

Table 6
Analysis of variance for the analysed data

Constituent	DF	MS	F Ratio	*P* value
Moisture				
Between groups	3	1.7	0.09	0.963
Within groups	16	18.5		
Dietary fibre				
Between groups	3	3.12	0.38	0.770
Within groups	16	8.26		
Total oxalate				
Between groups	3	0.011	0.04	0.991
Within groups	16	0.301		
Soluble oxalate				
Between groups	3	0.0046	0.05	0.983
Within groups	16	0.0873		
Tannins				
Between groups	3	48	0.02	0.995
Within groups	16	2017		
Ascorbic acid				
Between groups	3	762	0.167	0.214
Within groups	16	457		
Total iron				
Between groups	3	74.3	4.33	0.021*
Within groups	16	17.2		
Ionisable iron				
Between groups	3	16.68	6.79	0.004**
Within groups	16	2.46		
Bioavailable iron				
Between groups	3	3.480	5.78	0.007**
Within groups	16	0.602		
Availability (%)				
Between groups	3	2.5	0.03	0.993
Within groups	16	83.2		

DF – degrees of freedom; MS – mean sum of squares.
* Significant ($P < 0.05$).
** Highly significant ($P < 0.01$).

Coriander leaves and Ambuta greens cooked in an iron pan compared to other metallic utensils. The observations of Reddy et al. (1997) has also shown that, irrespective of the method of cooking (boiling or frying), bioavailability of iron from greens increased on cooking in an iron pan.

Another interesting observation about the extent of available iron was that it was very sample-specific. Within a given green, there was not much variation in bioavailable iron with respect to percentage of total iron. Thus, for example in the case of Amaranth, 1.5–4.5% of total iron was available, for Chakotha, it was 12.8–21.2%, for Fenugreek 13.8–19.3%, for Kilkeerai 21.1–25.0% and for Shepu 23.2–27.3%. As stated earlier, the differences observed in available iron content of greens could be attributed to the presence of inhibitors and promoters in the greens themselves. Hence the bioavailability in Amaranth was extremely low, due to a high content of dietary fibre and oxalates and low content of ascorbic acid, whereas in Kilkeerai and Shepu it was higher due to a low content of oxalates. Martinez and Vannucchi (1986) showed that foods cooked in iron pan were more effective in improving the haematological parameters than those cooked in non-iron pans through an animal experiment indicating that iron released by cooking pan was available. Observations of Mistry et al. (1988) have shown that iron added to foods by cooking utensil (determined by colorimetric and radio assay) was equally as dialyzable as native iron of foods.

Analysis of variance revealed that the differences in total ($P < 0.05$) and bioavailable iron ($P < 0.01$) were statistically significant between the greens cooked in different metallic utensils. The differences among promoters/inhibitors, which were analysed, were not significant between greens cooked in different utensils, implying that the cooking utensil has no role in altering these components.

4. Conclusion

Though it is well established that iron availability is a function of the proportion of inhibitors and enhancers of a food material, an increase in the total (1.2–10.8 times) and bioavailable iron (4 times) from greens cooked in an iron utensil compared to fresh and those cooked in other metallic utensils clearly indicates that the cooking utensil also has a role in determining the availability of iron. The total iron availability was higher (by 9%) in samples cooked in iron utensils than in samples cooked in non-iron utensils, while the averages of all other components were comparable. It can be concluded that cooking in iron vessels significantly increases the total iron content of foods, and a part of this iron is also bioavailable. Although the percent availability of iron is similar in utensils, the actual amount of iron available from the sample increases considerably from the sample cooked in an iron vessel. Hence, cooking in iron pans can definitely increase the iron content of the diet and can be considered as an economic and convenient way of improving iron status. Further research in the field is required to confirm the findings in other foods as the availability varies from one food to another. This can be considered as a strategy in reducing iron deficiency anaemia, a major health problem.

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