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Effect of Cookware Material on the Iron Content in Green Leafy Vegetables

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Abstract: ***Iron is an essential component of haemoglobin which is responsible for carrying oxygen in various parts of the body. Deficiency of iron causes anemia which can further result in many serious health conditions. The focus of this study is to examine and suggest ways to improve cooking methods to increase iron content in daily diet. In this work, four green leafy vegetables (spinach, goosefoot, fenugreek leaves and mustard leaves) which are known to be good sources of iron and are also part of regular Indian diet have been selected. Their iron content was determined in uncooked and cooked form in iron and aluminium utensils. Since iron forms a coloured complex with 1, 10- phenanthroline, UV-visible spectroscopy has been used to determine iron content. The analysis employs Beer-Lambert law for the determination. The results show that the amount of ironcontent in vegetables increases significantly when cooked in iron utensils while it decreases in aluminium utensils. This study suggests that adopting Indian traditional ways of cooking involving iron utensils can contribute to fightiron deficiency problems. The use of aluminium utensils on the other hand, may cause aluminium to leach into food and consistent usage may cause harmful effects in body.***

Keywords: Green vegetables, Iron deficiency, Aluminium utensils, Iron utensils, Spectrophotometer

1. Introduction

Iron is not only the most common element on earth by mass but it is also very necessary for human body to function properly. This transition metal is found in red blood cells in human body and is necessary for the synthesis of proteins like hemoglobin and myoglobin (1). Iron also plays important role in variety of metabolic processes such as DNA synthesis and electron transport (2). It is also very essential for proper mental and physical growth (3, 4).Iron in human body stays in complex forms bound to protein as heme compounds, heme enzymes, or non-heme compounds. Iron is transported in body via transferrin and stored in ferritin molecules (5,6). After iron absorption, there is no apparent mechanism for excretion of iron other than blood loss. Hence, iron is conserved by the body (3,7). Not having enough amount of iron in body is known as iron deficiency which can also lead to koilonychia (spoon-shaped finger nails), malnutrition and some general or serious health problems like fatigue, chest pain, brittle nails, anemia, hypoxia (8,9,10). The major consequences of this are child morbidity and development, maternal mortality and reduced adult work efficiency (11,12).When the body doesn't have enough iron to produce haemoglobin, the condition is called iron deficiency anemia. Women, children and adolescent girls are more commonly affected by this disease (13,14). According to WHO report, up to 88% of pregnant and 74% of non-pregnant women are affected by anemia in India (15). The National Family Health Survey-3 (NFHS-3) data shows 56% adolescent girls (15-19 years) were found to be affected and as per National Nutrition Monitoring Bureau Survey (NNMBS) 2006, the number was 68.6% for the age group 12-14 years whereas it is 69.7% in 15-17 years age group (16). If a pregnant woman is anemic, she may carry this deficiency till the later pregnancy stage and the same deficiency may pass on to the baby.

Despite of many initiatives taken by the government of India to address this health issue; it has been continuously increasing (17, 18). The various possible reasons behind this can be unavailability of true data of anemic population, poor awareness about daily nutrition requirements, lack of access to various government launched food programs like the Integrated Child Development Scheme (ICDS) and the Public Distribution System (PDS), the Mid-day Meal (MDM) programme (under the 2013 National Food Security Act (NFSA)), National Rural Employment Guarantee Act (19). However, it is worth mentioning that excess iron in the body, which is also called secondary iron overload or secondary hemochromatosis can also have undesirable health effects like liver disease (leading to cirrhosis), skin pigmentation, diabetes, arthropathy, erectile dysfunction, and sometimes heart failure (20).Cereals, nuts, vegetables, beans, and fortified grains are good sources of iron. Meat and seafood are very rich in iron content and people who consume these are less prone to iron deficiency (21,22). On the other hand, vegetarians have to pay more attention towards iron intake in their food since they consume non-heme iron which needs to be altered before it can be absorbed by the body (2,9). In vegetarian food, green leafy vegetables are known to be good sources of iron (23,24).The right method of preparing food also impact the iron content in the diet. One of the methods that has been employed since ancient times to retain and even enhance iron content in food involves cooking in iron utensils (11, 25).

India has more vegetarians than the rest of the world put together (26). Survey and studies indicate that approximately 38% to 45% population in India is completely dependent on farm-based products and are strictly vegetarian (27). Iron is one of the most important components of the human body, so it must be consumed in sufficient quantities.

2. Materials and Methods

The methodology adopted in this work involves binding of iron with 1,10-phenanthroline to form a coloured complex. Once a coloured complex is formed, its amount can be detected using UV-visible spectroscopy(28,29). To reduce the iron present in the sample to Fe (II) state, $NH_2OH.HCl$(hydroxylamine hydrochloride) is added at pH 4. pH of the solution is maintained by addition of sodium acetate buffer solution. The complex develops brownishorange colour. The reactioninvolved between iron and hydroxylamine hydrochloride is:

$$4\,Fe^{3+} + 2NH_2OH.HCl \rightarrow 4Fe^{2+} + N_2O + 4H^+ + H_2O$$

The absorbance was noted in the visible range of electromagnetic spectrum (380 nm-740 nm). The wavelength at which iron-phenanthroline complex shows maximum absorbance (λ_{max}) was found to be 500 nm.

To the prepared Mohr's salt solutions, 5 mL buffer, 5 mL hydroxylamine hydrochloride and 10 mL 1, 10-phenanthroline solution were added. The addition of 1,10-phenanthroline in all five flasks causes colour to change to brownish orange. However, the intensity of colour in every flask was different. The final concentration of each solution was calculated. The absorbance of each of the solutions at different concentrations was recorded at λ_{max}(500 nm), as shown in Table 1.

Table 1: Absorbance of complex by varying concentration of Mohr's salt solutions

S. no.	Volume of Mohr's salt solution taken (mL)	Concentration of Mohr's salt (moles/L)	Absorbance
1	1.0	0.23×10^{-4}	0.250
2	5.0	1.00×10^{-4}	0.702
3	8.0	1.42×10^{-4}	1.110
4	10.0	1.66×10^{-4}	1.325
5	25.0	2.77×10^{-4}	1.990

Molar absorptivity coefficient $\mathcal{E}$ was calculated as below
Slope of the graph= 0.7443×10^4 (from Figure 1)

Therefore, Molar absorptivity coefficient $\mathcal{E} = 0.7443\times10^4\ L\ mol^{-1}cm^{-1}$
Path length here is $l = 1\ cm$
The value of $\mathcal{E}$ was further used for the calculation of concentration of iron in vegetable samples.

Concentration $C = (Absorbance/\ 0.7443\times10^4)\ mol/L$

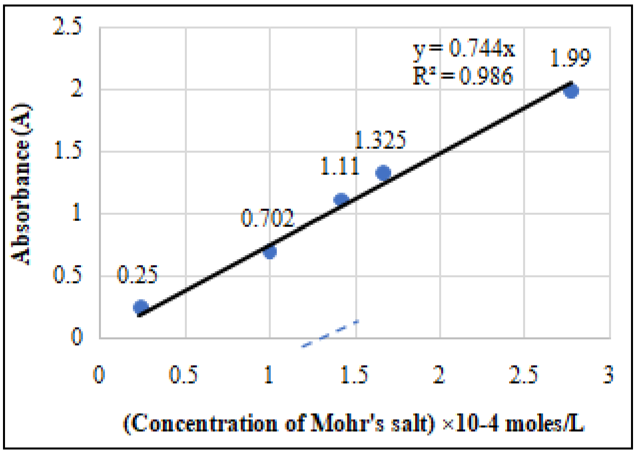

Figure 1: Plot of Absorbance vs concentration of Mohr's salt solution for the calculation of molar extinction or absorptivity coefficient ($\mathcal{E}$)

Vegetables sample preparation
Four commonly used vegetables spinach, goosefoot, fenugreek and mustard leaves were selected for this study. Equal amounts (100 g) of these vegetables were taken and cut into small pieces. 250 mL of water was added into each sample. All four samples were heated at a temperature of 100 ºC for 15 minutes in the iron and aluminium utensils of equal sizes. These two metals were selected because of their popularity in daily usage in most Indian households. After 15 minutes of cooking, samples were allowed to cool down to room temperature and were then filtered out in conical flasks. Four samples of uncooked vegetables were also prepared. For uncooked samples, 250 g of each vegetable was taken and crushed. 100 mL of water was added and the solution was filtered multiple times to remove any pulp. All the 12 samples were labelled as S1, S2, S3, S4, S5, S6, S7, S8, S9, S10, S11 and S12. Details of the samples are shown in Table 2.

Table 2: Samples of different green leafy vegetables taken in this study

Common Name	Indian Name	Scientific Name	Sample Type	Sample Name
Spinach	Palak	*Spinacia oleracea*	uncooked	S1
			Cooked in iron	S2
			Cooked in aluminium	S3
Goosefoot	Bathua	*Chenopodium album*	uncooked	S4
			Cooked in iron	S5
			Cooked in aluminium	S6
Fenugreek	Methi	*Trigonella foenum-graecum*	uncooked	S7
			Cooked in iron	S8
			Cooked in aluminium	S9
Mustard	Sarso	*Brassica juncea*	uncooked	S10
			Cooked in iron	S11
			Cooked in aluminium	S12

Analysis of sample solutions

After the preparation of all vegetable samples, the absorbance was recorded. For this purpose, 5 mL of the samples S1, S2, S3……S12 were taken and 5 mL buffer was added followed by the addition of 5 mL 0.1M hydroxylamine hydrochloride solution and 10 mL 0.1M 1,10-phenanthroline solution. A blank solution was also prepared by taking same volume of each vegetable sample, buffer and hydroxylamine hydrochloride. The prepared solutions were subjected to spectrophotometric analysis. The absorbance for the different samples is presented in Table 3.

3. Results and Discussion

The concentrations and the amount of iron present in one litre of vegetable samples were calculated using the following method. The data has been reported in Table 3.

Molarity of unknown solution= C moles/L

Amount of iron present in 1 L of the given sample = (C × molar mass of iron) g/L

= Z g/L

Table 3: Amount of iron present in each sample

S. no.	Sample	Absorbance	Concentration of iron ($C\times10^{-4}$ moles/L)	Amount of iron ($Z\times10^{-4}$ g/L)
1	S1	0.050	0.0672	3.762
2	S2	0.494	0.6637	37.168
3	S3	0.0071	0.0095	0.534
4	S4	0.027	0.0363	2.031
5	S5	0.202	0.2714	15.198
6	S6	0.004	0.0054	0.301
7	S7	0.157	0.2109	11.812
8	S8	0.062	0.0833	4.665
9	S9	0.041	0.0551	3.085
10	S10	0.141	0.1894	10.609
11	S11	0.335	0.4501	25.205
12	S12	0.089	0.1196	6.696

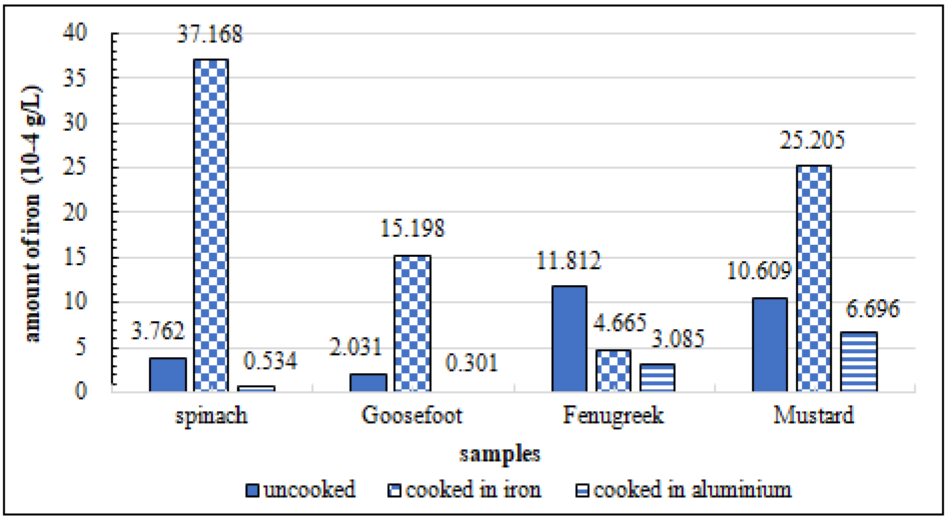

Figure 2: Amount of iron in various uncooked samples and samples cooked in iron and aluminium utensils

Amongst the 12 samples, it is evident that the results are best for the samples cooked in iron utensils. The significant surge in iron content in the case of spinach, goosefoot, and mustard can be seen from Figure 2. The reduction in iron content of fenugreek in cooked samples is not as per the trend. Spinach cooked in iron utensil was found to be the richest in dietary iron amongst all the tested samples, whereas fenugreek leaves are good source of iron in uncooked form. The reason for the increased iron content in the samples that were cooked in iron utensils is that Fe from the utensils is leached into the samples while cooking. On the other hand, the amount of iron decreased in the samples which were cooked in the aluminium utensils. The reason is that aluminium is a stronger reducing agent than iron, hence when the food is cooked in aluminium utensils, iron is replaced by aluminium, and iron gets precipitated, as shown in the reaction below:

$$2FeSO_4 + 2Al \rightarrow Al_2(SO_4)_3 + 3Fe$$

As a result, not only food becomes iron deficient, but also aluminium enters our body and excess aluminium causes genotoxicity, neurotoxicity, and several other major diseases (30-32).

4. Conclusion

A large portion of the Indian population follows a vegetarian diet. For such a population, green leafy vegetables are very good source of iron and other essential nutrients required in the body. The present study deals with addressing the issue of providing required amount of iron in daily meals. In this context, thedetermination of iron content in four green leafy vegetables,spinach, goosefoot, fenugreek leaves and mustard leaves in raw form and when cooked in iron and aluminium utensils was done. The results show that the iron content in food is enhanced when vegetables are cooked in iron utensils and it drastically decreases when cooked in aluminium utensils. Due to abundance of aluminium metal on the earth and it's properties like light weight, high melting point and good thermal conductivity makes it

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desirable for crafting cooking utensils. However, aluminium doesnothave any significant function in human body. Also, studies reveal aluminium may be a possible reason for causing Alzheimer's disease (33).The traditional method of cooking in iron utensils can help in preserving nutritional value of food and retain or enhance the iron content of food. On the other hand, the use of aluminium utensils should be avoidedbecause of its possible health concerns.

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Conflict of Interest
There is no conflict of interest between authors.

Contributions
All authors contributed equally.

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