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ORIGINAL ARTICLE **OPEN ACCESS**

Obtainability of Total and Ionizable iron in Drumstick leaves cooked vegetable (sabzi) by adding tamarind.

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ABSTRACT
Iron deficiency anaemia (IDA) is one of the most common public health problems generally affecting women, children, and adolescent girls. India is a predominantly vegetarian country, and the prevalence of anaemia is heightened because non-haem iron present in vegetarian diets is less absorbed by the body. To determine if total and ionisable iron in Moringa oleifera Lam. (drumstick) leaves cooked vegetable food item (sabzi) is affected by adding tamarind. Drumstick leaves were used to prepare two types of sabzis, firstly with the addition of bengal gram flour (preparation 1), and secondly with bengal gram flour and tamarind paste (preparation 2). The sabzis were then analyzed for moisture (Ranganna 2014), total ash (AOAC, 2000), total iron (ICP-OES), and ionizable iron (Rao and Prabhavathi, 1978). Moisture, ash, and total iron content was found to be 73.5 g/100g, 2 g/100g, and 8.45 mg/100g for raw drumstick leaves, 76.1± 0.1 g/100g, 2.0±0.0 g/100g and 7.15±0.29 mg/100g for preparation 1 and 73.9±0.4 g/100g, 2.0±0.1 g/100g and 7.04±0.62 mg/100g for preparation 2 respectively. The % ionizable iron was 1.55±0.29, 0.83±0.00, and 1.16±0.18 for raw drumstick leaves, preparation 1 and 2 respectively. This study shows that in-vitro iron bioavailability of non-haem iron from Moringa leaves is poor and is further reduced during cooking. Adding tamarind seems to improve the availability slightly. The use of tamarind needs to be tested with other foods/recipes.
Keywords: *Drumstick leaves, Tamarind, Non-haem iron, Iron Deficiency Anaemia (IDA), Sabzi(cooked vegetable).*

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INTRODUCTION

Iron, the element with the fourth most copious supply in the Earth's crust, claims its position as an essential part of almost all biological components.[1] Iron is an essential micronutrient for almost all living organisms as it participates in various physiological and metabolic functions in the body such as oxygen transport and storage, cellular growth and differentiation, deoxyribonucleic acid (DNA) synthesis, electron transport, enzymatic reactions, physical and cognitive development, and pathogen destruction, etc.[1,2] Both developing and developed nations have concerns regarding iron deficiency and women are at higher risk of diseases relating to iron deficiency.[3] The Egyptians, Hindus, Greeks, and Romans used iron for various early medicinal uses. During the 17th century, iron was used to treat chlorosis (green disease), a condition often resulting from iron deficiency.[2] Anaemia results in a condition in which our body lacks enough healthy red blood cells to carry adequate oxygen to our body's tissues resulting in insufficiency of iron for meeting physiological needs.[4] Anaemia is the most frequently appearing major public health issue in the life of a woman, which in turn leads to unfavourable situations concerning pregnancy.[5] Anaemia particularly affects children aged 0-5 years, women of childbearing age, and pregnant women; which in turn leads to maternal and child morbidity, reduced physical action, and referral to healthcare professionals.[6] According to NFHS 5 data (2019-21), the percentage of the population in India who are anaemic are as follows- children aged 6-59 months (67.1%), non-pregnant women aged 15-49 years (57.2%), pregnant women aged 15-49 years (52.2%), all women aged 15-49 years (57.0%), all women aged 15-19 years (59.1%), men aged 15-49 years (25.0%) and men aged 15-19 years (31.1%), amongst which rural population is more affected followed by the urban population. It is seen from the data that pregnant women and adolescent children, particularly adolescent girls are more vulnerable to anaemia and its

consequences.[7] Anaemia is considered the major cause of disability in the world; and it is also involved with issues of morbidity and mortality, particularly in women's inability to react to postpartum blood loss thereby leading to serious health consequences.[5] Iron deficiency anaemia is frequently related to several chronic diseases, particularly chronic kidney disease, cancer, chronic heart failure, and inflammatory bowel disease.[6] IDA in infants and toddlers is seen to have a relation with potentially irreversible long-term neurodevelopmental sequelae.[8] The most common etiological factors of anaemia include poor nutrition, iron deficiency, micronutrient deficiencies including vitamin A, vitamin B12, and folic acid; parasitic infections such as malaria, hookworms infestation, schistosomiasis, HIV infection and genetic conditions like thalassemia. Iron requirement during pregnancy is insufficient due to improper diet which accounts for 75% of all types of anaemia during pregnancy.[5] Three major approaches may be adopted in developing countries for combatting iron deficiency: dietary diversification, food fortification (preferably staple foods), and iron supplementation.[9] Humans consume their food from both plant and animal sources. Iron from these foods comes in basically two forms- non-heme iron from both plant and animal sources and heme iron from animal sources; out of which heme iron is better absorbed and the absorption of non-heme iron in the person consuming it is dependent on the iron status of the individual and composition of the meal. Foods such as liver, red meat, beans, nuts, green leafy vegetables, fruits, vegetables, and cereals are relatively high in iron content having variable iron absorption capacity.[1,10] Non-heme iron has lower iron bioavailability when compared to heme iron because non-heme iron in plant foods is found in the chelated state with tannin, phytate, and phenols. Polyphenols and phytate form insoluble complexes with iron thereby reducing its bioavailability.[10] Green leafy vegetables are an excellent source of vitamins, phenolic compounds, and minerals, particularly iron and calcium. It is also seen that green leafy vegetables are the only natural source of folic acid, out of which *Moringa oliefera* Lam. has the richest constituent.[11] The bioavailability of iron from non-heme sources can be improved by consuming it with foods rich in tartaric acid.[12] Tamarind (*Tamarindus indica* L.) referred to as the backbone of Indian medicine contains tartaric acid throughout all the stages of its development and is shown to increase iron absorption in human beings.[13] Dietary diversification can be a potential source of increased micronutrient intake including iron which will in turn help in combating iron deficiency anaemia.[14].

To determine if total and ionisable iron in *Moringa oleifera* Lam. (drumstick) leaves cooked vegetable food item (sabzi) is affected by adding tamarind.

Hypothesis of the study:

Null hypothesis (H_0): Tamarind is not effective in enhancing total and ionisable iron absorption of drumstick leaves sabzi.

Alternative hypothesis (H_1): Tamarind is effective in enhancing total and ionisable iron absorption of drumstick leaves sabzi.

MATERIAL AND METHODS

The present study examined the *in vitro* availability of iron from two different types of sabzis (cooked vegetable food items) prepared using drumstick leaves with the addition of besan (*Cicer arietinum* L. flour) and tamarind (*Tamarindus indica* L.) paste. Figure 1 depicts the different preparations of drumstick leaves sabzi analyzed in this study.

Standardization and preparation of samples (drumstick leaves sabzi):

Standardization of drumstick leaves sabzi:

For standardization of the sabzi, 3 bunches approximate of 140.20 g weight of drumstick leaves were purchased from a local vendor in Lower Parel, Mumbai. The standardization was done twice for both the finalized recipes.

Preparation of samples (drumstick leaves sabzi):

For the preparation of the sabzi, 1 bunch of drumstick leaves weighing 300gms was procured from Lower Parel and the sabzis were prepared with the addition of besan and tamarind paste. The standardized recipe was formulated using 100 g of raw drumstick leaves in 16.6 ml of oil and 3.16 g of salt as constant for both the recipes and thereafter adding onion (60 g), green chillies (4.53 g), bengal gram flour (34.5 g) for recipe 1 and onion (60 g), green chillies (4.53 g), bengal gram flour (34.5 g), tamarind paste (18.6 g) (tamarind paste was prepared by soaking 5 g of seedless tamarind in 30 ml of water for 1 h. and then mashing it slightly using hands and straining the mixture to obtain the liquid) for recipe 2. The bengal gram flour weighing 34.5 g was mixed with 82.6 ml of water to form a paste. Both recipes were cooked for 60 minutes. After the cooking was completed, the sabzis were allowed to cool down completely. Once cooled, each of the sabzis was transferred into a stainless-steel plate that had been rinsed thoroughly with deionized water and dried. Each sabzi was grounded to a fine paste and was transferred to an airtight plastic container

(previously acid-washed and dried). The containers were then labelled and stored in the freezer at -20°C for further use.

Chemical analysis of the products:

The sabzis were analyzed for the following:

- Moisture and total ash content
- Total iron by ICP-OES (Inductively coupled plasma optical emission spectroscopy) of the product
- Ionisable iron content of the product

Determination of moisture content: Five grams of the homogenized sample were dried at 70°C using the thermogravimetric method in a hot air oven for 5h as per the method given by Ranganna, 2014.[15]

Determination of total ash: Two grams of the sample were weighed accurately in a crucible and heated in a muffle furnace at 500°C until grey ash was obtained and the difference between two consecutive readings was less than 1 mg, as per the Association of Official Analytical Chemists (AOAC, 2000) method.[16]

Estimation of total iron using ICP-OES (Inductively coupled plasma-optical emission spectroscopy): Two grams of the sample was weighed accurately in a crucible and ashed as described *vide supra*. After that 2 drops of conc. Nitric acid was added to the crucible and the crucible was heated again in the muffle furnace for another 2 h 30 min until the ash was white in colour. The obtained ash was then dissolved in 1ml of conc. nitric acid and made to 50 ml in a volumetric flask using deionized water, using the method given by the Association of Official Analytical Chemists (AOAC, 2000).[16] The total iron content was measured using ICP- OES (Perkin Elmer Model Optima 7000DV, Shelton CT, USA).

Estimation of ionizable iron content: Two grams of sample were accurately weighed and treated with a pepsin-HCl solution followed by adjusting the pH to 1.35 and 7.5. The analysis method was carried out using the in vitro method given by Rao and Prabhavathi (1978).[17] The ionizable iron content was determined using the spectrophotometric method given by Rao and Prabhavathi, 1978.[17] Absorbance was measured at 510 nm in a UV-Spectrophotometer (LabIndia, UV-Vis Spectrophotometer 3000).

RESULTS AND DISCUSSION

Moisture, ash, and total iron content of the raw leaves:

The moisture, ash, and total iron content of the raw leaves collected from Lower Parel were estimated. The values were then compared with the Indian Food Composition Table (IFCT) value given by National Institue of Nutrition (NIN), 2017.[18] The results of the study are shown in Figure 2. From Fig. 2, it is seen that the moisture and ash content of raw leaves collected from Lower Parel is almost at par with the value of moisture and slightly lower than the ash content of raw drumstick leaves given by IFCT, respectively. Also, from the above figure, it is seen that the total iron content of raw drumstick leaves is almost twice the total iron content of raw drumstick leaves given by IFCT. Variations in the nutritional composition of raw agricultural commodities are largely seen and it occurs generally during pre-harvest conditions of the plant as a result of various factors such as genetics, maturity of the edible product during harvesting, postharvest handling, storage conditions of the product, climatic and edaphic factors, cultivation method used, the age of the plants, maturity of the leaves and geographic location. [19,20]

Moisture, ash, and total iron content of the drumstick leaves sabzi:

The moisture, ash, and total iron content of the two different types of preparations were estimated and presented in Table 1. From Table 1 it is seen that the moisture and ash content of both the sabzi preparations are slightly higher and almost equal to the moisture and ash content of the raw drumstick leaves respectively. The added ingredients namely bengal gram flour paste (made with water) and water used during cooking could have contributed to the higher moisture content. The increasing moisture content after cooking may be the result of water absorption during processing.[21] Also from Table 1, it is seen that the total iron content on a dry-weight basis of the sabzis prepared from drumstick leaves is lower for both preparations than the total iron content on a dry-weight basis of the raw leaves. Significant losses of iron in drumstick leaves may be seen during wet frying or frying of drumstick leaves using water for more then 10 minutes, and this may be due to the leaching of mineral iron into the boiling water.[22]

Ionisable iron content of the raw leaves and their sabzi:

Both preparations' ionizable iron content on a dry weight basis was estimated. The results for the dry weight basis of this study are shown in Table 2.

From Table 2 it is seen that the ionizable iron content/100g dry weight basis at pH 1.35 and pH 7.5 of raw drumstick leaves is relatively higher than the sabzis prepared with the same drumstick leaves by adding bengal gram flour and tamarind. A decrease in the ionizable iron content at pH 7.5 is seen in the raw drumstick leaves as well as in both preparations when compared to the same at pH 1.35. It is also seen that

sabzi preparation done by adding tamarind paste has higher ionizable iron content when compared to the bengal gram flour added. The addition of tamarind containing tartaric acid is shown to have positive impacts on iron absorption in the human body.[23] In the present study also the addition of tamarind to the sabzi is seen to have a positive impact on increasing the ionizability of iron that is lost during cooking. The addition of legume-based food ingredients during cooking is also seen to have a positive impact on the iron bioavailability of green leafy vegetables.[24] In this study too, the addition of besan to the preparations has shown positive impacts on the iron ionizability of the sabzis slightly.

Table 1 : Moisture, ash and total iron content of drumstick leaves sabzi.

Preparations	Moisture (g/100g) Mean ± SD	Ash (g/100g) Mean ± SD	Total iron (mg/100g) Mean ± SD
1	76.1± 0.1	2.0±0.0	7.15±0.29
2	73.9±0.4	2.0±0.1	7.04±0.62

Table 2 : Ionisable iron content of raw drumstick leaves and their sabzi preparations.

Prepar--ations	Ionisable iron/100g dry weight basis			
	pH 1.35 (mg/100g) Mean ± SD	% Ionisable Iron* Mean ± SD	pH 7.5 (mg/100g) Mean ± SD	% Ionisable Iron** Mean ± SD
Raw leaves	43.78±2.7	5.17±0.11	13.14±0.54	1.55±0.29
1	7.6±0.42	1.08±0.01	5.95±0.22	0.83±0.00
2	12.48±0.32	1.77±0.18	8.16±0.77	1.16±0.18

*% Ionisable iron of total iron at pH 1.35
** % Ionisable iron of total iron at pH 7.5

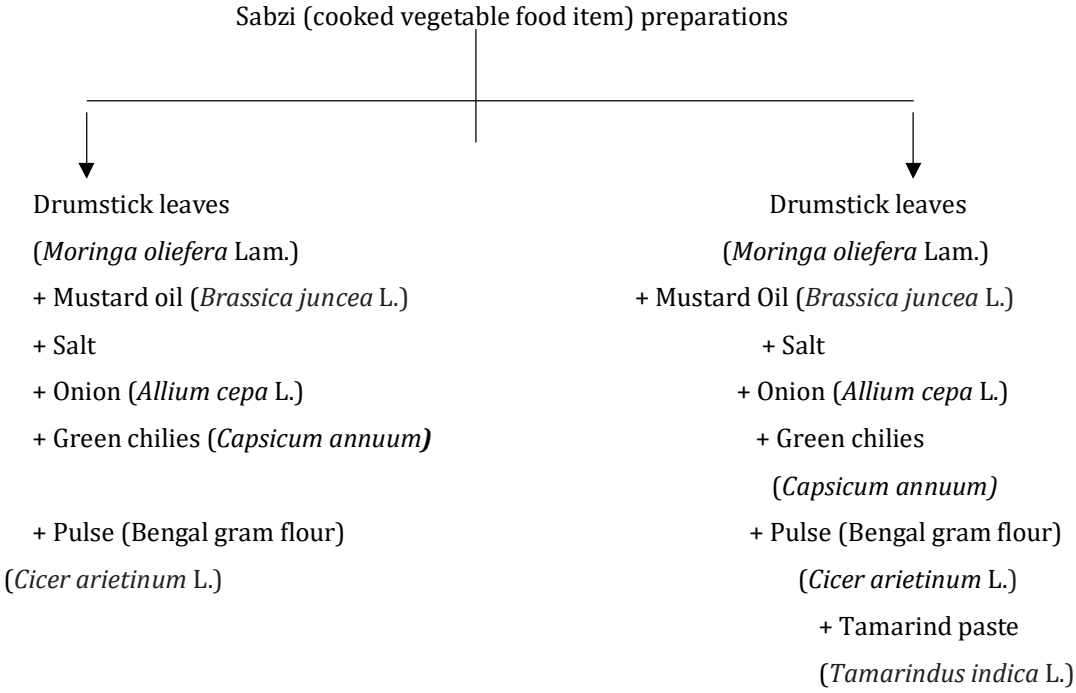

Fig. 1 Ingredients used in the preparation of sabzis (cooked vegetable food item) made from *Moringa oliefera* Lam. leaves.

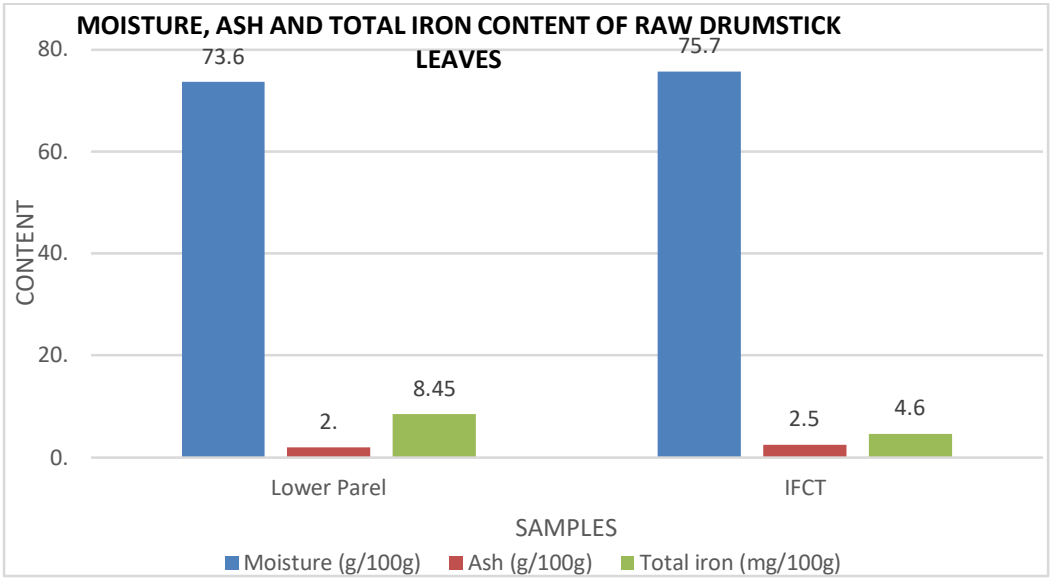

Fig. 2 Moisture, ash, and total iron content of raw leaves collected from Lower Parel along with their values given by IFCT (2017).

CONCLUSION

Drumstick being a cheap, fast-growing, drought-resistant, perennial plant; can be incorporated in everyday meals of Indians. Since Indians are largely vegetarian, the incorporation of drumstick leaves in various forms in our daily diet may be beneficial for combatting the prevalence of iron deficiency anaemia to a great extent. However, the ionizable iron availability of these green leafy vegetables might be less when compared to the total iron content, which may be due to certain compounds such as phytates, tannins, polyphenols, calcium, etc. In the present study, it is seen that the addition of tamarind paste to sabzi preparations has helped slightly increase the ionizable iron bioavailability of non-haem iron in the human body. Thus, it can be concluded that the inclusion of drumstick leaves sabzi prepared by adding besan and tamarind paste in our daily diet may be considered beneficial for increasing the iron content of a vegetarian diet and thereby may act beneficial towards tackling iron deficiency anaemia. Further studies can be done to test the use of tamarind and its iron bioavailability with other foods/recipes.

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DECLARATION OF CONFLICTING INTERESTS

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ETHICAL STATEMENT

This research did not require ethical approval, as there was no direct involvement of human or animal subjects.

AUTHOR'S CONTRIBUTIONS

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the Bulletin of Environment, Pharmacology and Life Sciences Journal.

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