Journal of Applied and Natural Science 6 (2): 701 - 706 (2014)

JANS

In vitro method for predicting the bioavailability of iron from Bathua (*Chenopodium album*) and Fenugreek (*Trigonella foenum graecum*) leaves in Indian cookies

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Received: July 19, 2014; Revised received: October 10, 2014; Accepted: November 26, 2014

Abstract: This study was considered to determine the nutritional composition and *in vitro* bioavailability of iron of the prepared food products. The *in vitro* bioavailability of iron was determined in fresh and dehydrated bathua (*Chenopodium album*) and methi (*Trigonella foenum graecum*) leaves (in the ratio of 1:1) and their based product i.e. Indian cookies (paratha and laddoo) by using thiocynate method at pH 1.35 and 7.5, using pepsin. At pH 1.35 the soluble iron and ionizable iron were higher than pH 7.5. when pH was increased from 1.35-7.5 there was decreasing trend soluble as well as ionizable iron contents. There was highly significant (F< 0.05) difference between the control and treatments of products indicating that the addition of 20% of fresh and dehydrated leaves of bathua and methi improved the overall acceptability of products. The total iron content was found 11.69mg/100g in paratha incorporated with fresh GLVs whereas *in vitro* bioavailability of iron was found 2.16mg/100g in paratha. The total iron content was found 15.16mg/100g in laddoo incorporated with dehydrated GLVs whereas *in vitro* bioavailability of iron was found 2.78mg/100g in laddoo.

Keywords: Bioavailability, *Chenopodium album*, Iron, *Trigonella foenum graecum*

INTRODUCTION

Bioaccessibility or bioavailability (or biological availability) is the key to nutrient effectiveness, which is defined as the proportion of nutrient in the food that can be absorbed and utilized. Bioavailability of dietary iron is the proportion of iron that is actually available for the absorption and utilization by the body. Bioavailability of iron is known to be influenced by various dietary components, which include both dietary inhibitors and enhancers of absorption. Among inhibitors, phytic acid, tannins, dietary fibre and calcium are the most potent, while organic acids are known to promote iron absorption (Sandberg, 2002). Many studies have shown that addition of components like tomato or tamarind juice might be also partially responsible for increase in the available iron (Sathya *et al.*, 2002 and Hemalatha *et al.*, 2007). Green leafy vegetables are the most suitable foodstuffs for enriching dietary iron of the Indians. Though the leafy vegetables contain high total iron, the bioavailability of mineral varies. Vegetarian meals have a poor bioavailability of iron in addition to poor content which is also true for zinc. *In vitro* methods offer appealing protocol, as they are simple, rapid, low cost and provide insights not achievable in whole animal studies. Dehydration also makes them a concentrated source of vitamins and minerals and thus they become a very suitable "natural fortificant" (Joshi and Mathur, 2010). Fenugreek plant contains active constituents such as alkaloids, flavonoids, steroids, Saponins etc. It is an old medicinal plant. It has been commonly used as a traditional food and medicine. Fenugreek is known to have hypoglycemic, and hypocholesterolaemic, effects, Anti-inflammatory effects (Moradi and Moradi, 2013). According to Aggarwal and Khanna (2007) children receiving fortified supplementation with leaf powder concentrates have improved anthropometric indices and vitamin- A status. Dehydrated green leafy vegetables are also rich sources of iron and β- carotene and can be used in lean season (Rao *et al.*, 2005). Gupta *et al.* (2013) also observed the influence of dehydration on nutrient composition of *Amaranthus gangeticus*, *Chenopodium album*, *Centella asiatica*, *Amaranthus tricolor* and *Trigonella foenum graecum.* Fenugreek (*T. foenum-graecum*) leaves and seeds have been used extensively for medicinal purposes. Fenugreek seed is known to exhibit anti-diabetic and anti-nociceptive properties (Acharya *et al.*, 2006). *Bathua* leaves (*C. album Linn.*) rich in micronutrients (Singh *et al.*, 2007). It contains a mucilaginous fibre (20%) and has a total fibre

ISSN : 0974-9411 (Print), 2231-5209 (Online)

content of 50%. It is a very rich source of calcium, beta carotene .The lower glycemic load after fenugreek leaves supplementation could be due to fibre which diminishes the absorption of carbohydrate to a point lower in the gut after colonic conversion (Bever *et al.*, 2008). Therefore, present study was conducted for predicting the bioavailability of iron from Bathua (*C. album*) and Fenugreek (*T. foenum graecum*) leaves *by in vitro* method.

MATERIALS AND METHODS

Test materials: The present investigation was carried out in the Nutrition Research Laboratory, Department of Foods and Nutrition, Ethelind School of Home Science, Sam Higginbottom Institute of Agriculture, Technology and Science (SHIATS), Allahabad. Fresh leaves of Fenugreek, Bathua and other ingredients required for the experiment were collected from the local market of Allahabad district. The leaves were carefully clean, sort to remove defective one of the lots graded according to the size and colour. The dehydration of bathua and methi leaves was done through tray drying at 60-65°C for 15 hours (Fig.1.). The fenugreek and bathua leaves was utilized in the preparation of Indian cookies (Paratha incorporated by fresh leaves of bathua and methi in the ratio of 1:1 and Laddoo incorporated by dehydrated leaf powder of bathua and methi in the ratio of 1:1) with 4 replications of each product. For each product the basic recipes (control T_0) have three variations T_1, T_2, T_3, respectively where the amount of fenugreek and bathua leaves was varied (Table 1). Sensory evaluation of the food products for their acceptability was done by a panel of judges. The score card based on the 9 point Hedonic Scale was used for sensory evaluation on the basis of evaluation of attributes like colour and appearance, texture, taste and flavour and overall acceptability (Srilakshmi, 2007).

Extraction of soluble and ionizable iron: Weighed food sample and homogenized cooked diet was first incubated with pepsin-HCL solution (0.5% pepsin in 0.1 N HCL). In case of dry foods, 2g of the food was mixed with 25 ml of pepsin- HCL, and in case of homogenized diets they were diluted with an equal volume of pepsin- HCL mixture. The pH of mixture was adjusted to 1.35 with distilled HCL and incubated in a 100 ml conical flask at 37°C in a metabolic shaker water bath for 90 minutes. At the end of this incubation, the contents of the flask were centrifuged at 3000 rpm for 45 minutes and the supernatant filtered through Whatman no. 44 filter paper, soluble and ionizable iron was determined in aliquots of the filterate at pH 1.35 as mentioned below. In another aliquots pH was adjusted to 7.5 with NaOH and incubated at 37°C for 90 minute in a metabolic shaker water bath. At the end of this incubation period the contents of the flask were centrifuged at 3000 rpm for 45 minutes, and the supernatant was filtered again. The filterate was used for the determination of soluble and ionizable iron.

Analytical methods

Total iron: The food material was dry ashed as described by the Association of Official Agriculture Chemists (AOAC, 2005) and total iron in the mineral solution was estimated by the Thiocynate method and was compared with that of a, a- dipyridyl method.

Ionizable iron: Free form of iron in the filterate, which reacts with Potassium thiocynate to yield blue color of ferric thiocynate, obtained after incubation of foods at pH 1.35 and 7.5, was determined colorimetrically as described by AOAC (2005). This form of iron corresponds to the ionizable iron.

Soluble iron: The soluble iron was determined by Tennat and Greenman (1969). The aliquot was digested with potassium permanganate to oxidize ferrous to ferric iron, decolorized with ascorbic acid and filtered. The iron content in filterate was determined by colorimetric method of thiocynate.

Bioavailability of iron: *In vitro* bioavailability of iron was estimated by using standard procedures (Narasinga and Prabhavathi, 1978).

Statistical analysis: Analysis of variance technique (ANOVA), t-test and critical difference statistical tests were used to analyze the data (Gupta and Kapoor, 2002). Values of different parameters are expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

Nutritional composition of dehydrated Bathua and Methi leaf powder in per 100g: Result shows that nutrient concentration per 100g of the product increases in dehydrated bathua and methi leaf powder as compared to the values in fresh bathua and methi leaves. The Iron in dehydrated bathua and methi leaf powder per 100 g was 20.89 (Table 2), respectively. Singh *et al.* (2007) reported that iron and carotene contents of dehydrated bathua leaves (27.48 mg/100g and 14826 µg/100g, respectively) were 6-8 times higher than fresh leaves.

Nutritional composition of value added products in per 100g: Using the fresh and dehydrated fenugreek and bathua leaves the two value added products for each leaves respectively were prepared i.e. Indian cookies (paratha and laddoo) and to analyze the total iron content. Table 2 shows that iron content was high in laddoo 15.16 mg/100g and in paratha was 11.69 mg/100g. Singh *et al.* (2007) studied that showed that iron content increased as the incorporation level of dehydrated bathua leaves increased in making paratha. T_3 i.e. (15 % incorporation level) which was also acceptable at sensory score 6.62±0.11 has got highest iron content i.e. 8.81mg/100g.

***In vitro* bioavailability of iron:** The *in vitro* bioavailability

Table 1. Details of control and treatment combinations (Each time four replicates were taken).

S. N.	Ingredients in Indian cookies	Treatments			
		T_0	T_1	T_2	T_3
1.	**Paratha**				
	Potato	40	30	20	10
	Wheat flour	40	40	40	40
	Cauliflower	10	10	10	10
	Turnip greens	10	10	10	10
	Fresh bathua and methi leaves 1:1	-	10	20	30
2.	**Laddoo**				
	Gingelly seeds	50	60	50	40
	Jaggery	50	30	35	40
	Dehydrated bathua and methi leaf powder 1:1	-	10	15	20

Paratha : Control (T_0): prepared from potato, wheat flour, cauliflower and turnip greens; Treatments: (T_1): prepared from potato, wheat flour, cauliflower, turnip greens and mixture of fresh leaves (bathua and methi 1:1) in a ratio of 30:40:10:10:10; (T_2): prepared from potato, wheat flour, cauliflower, turnip greens and mixture of fresh leaves (bathua and methi 1:1) in a ratio of 20:40:10:10:20; (T_3): prepared from potato, wheat flour, cauliflower, turnip greens and mixture of fresh leaves (bathua and methi 1:1) in a ratio of 10:40:10:10:30. Laddoo: Control (T_0): prepared from gingelly seeds and jaggery;TreatmentsT_1: prepared from gingelly seed, jaggery and mixture of dehydrated leaf powder (bathua and methi 1:1) in a ratio of 60:30:10;(T_2): prepared from gingelly seed, jaggery and mixture of dehydrated leaf powder (bathua and methi 1:1) in a ratio of 50:35:15; (T_3): prepared from gingelly seed, jaggery and mixture of dehydrated leaf powder (bathua and methi 1:1) in a ratio of 40:40:20.

Table 2. Iron content in dehydrated powder and value added food products incorporated with fresh and dehydrated green leafy vegetables bathua (*C. album*) and fenugreek (*T. foenum graecum*).

Indian products	Iron (mg/100g)
Dehydrated bathua and methi leaf powder 1:1	20.89± 0.75
Paratha (Fresh bathua and methi leaves 1:1)	11.69± 0.57
Laddoo (Dehydrated bathua and methi leaf powder 1:1)	15.16± 0.97

Mean ± SD of 4 replicates sampled products of GLVs

of iron was determined in fresh bathua and methi leaves, fresh bathua and methi leaves based product paratha, dehydrated bathua and methi leaf powder and dehydrated bathua and methi leaf powder based product laddoo by using thiocynate method at variable pH 1.35 to 7.5 using pepsin at different concentration. Iron may become soluble or ionized when it comes in contact with HCl in stomach but when it enters the duodenum the pH becomes alkaline compared to gastric pH, where much of the iron which is ionized at the acid pH becomes insoluble depending upon the factors present in the diet.

The total iron, soluble iron and ionizable iron was studied (Table 3). The soluble iron and ionizable iron was calculated at the pH 1.35 and 7.5, when the pH was increased from 1.35 to 7.5, both the ionizable iron decreases in all the foods. The results revealed that at pH 1.35 the soluble iron and ionizable iron contents were higher than pH 7.5. When pH was increased

Table 3. Effect of incorporation of fresh leaves and dehydrated bathua (*C. album*) and fenugreek (*T. foenum graecum*) at different levels on the bioavailability of iron at pH 1.35 and pH 7.5.

S. N.	Products	Total iron (mg/100g)	pH=1.35		pH=7.5	
			Soluble iron	Ionizable iron	Soluble iron	Ionizable iron
1.	Fresh bathua and methi leaves	3.06	1.84 (60.13%)	0.94 (30.71%)	1.77 (57.84%)	0.87 (28.43%)
	Fresh bathua and methi leaves based product Paratha	11.69	6.56 (56.11%)	3.63 (31.05%)	5.98 (51.15%)	3.56 (30.45%)
2.	Dehydrated bathua and methi leaf powder (1:1)	20.59	10.25 (61.78%)	6.78 (40.86%)	8.24 (40.01)	4.65 (22.58%)
	Dehydrated bathua and methi leaf powder based product Laddoo	15.16	7.98 (58.85%)	5.12 (33.77%)	7.07 (49.90%)	4.89 (32.25%)

Table 4. Bioavailability of iron of the products prepared by incorporating fresh and dehydrated Bathua (*C. album*) and Fenugreek (*T. foenum graecum*) leaves (per 100g).

S. N.	Products	Total Iron (mg/100g)	Ionizable Iron (mg/100g)	Ionizable Iron (%)	*In vitro* available Iron
1.	Fresh Bathua and Methi leaves (3)	*3.06±0.69	0.87±0.19	28.43±2.21	0.89±0.22
	Fresh Bathua and Methi leaves based product Paratha (3)	11.69±0.89	3.56±0.27	30.45±2.40	2.16±0.31
2.	Dehydrated Bathua and Methi leaf powder (3)	20.59±0.43	4.65±0.30	22.58±1.06	2.67±0.34
	Dehydrated Bathua and Methi leaf powder based product Laddoo (3)	15.16±0.65	4.89±0.34	32.25±2.10	2.78±0.38

Values in parentheses indicate the number of samples; Values are means, ± S.E. of three independent replicates. *In vitro* available iron based on prediction equation for iron absorption using percent ionizable iron at pH 7.5 was calculated: Y= 0.4827+0.4707X, where X is percent ionizable iron at pH 7.5. * Value of total iron of fresh bathua and methi leaves (3.06) referred from Gopalan *et al.*, 2007; Nutritive value of Indian foods.

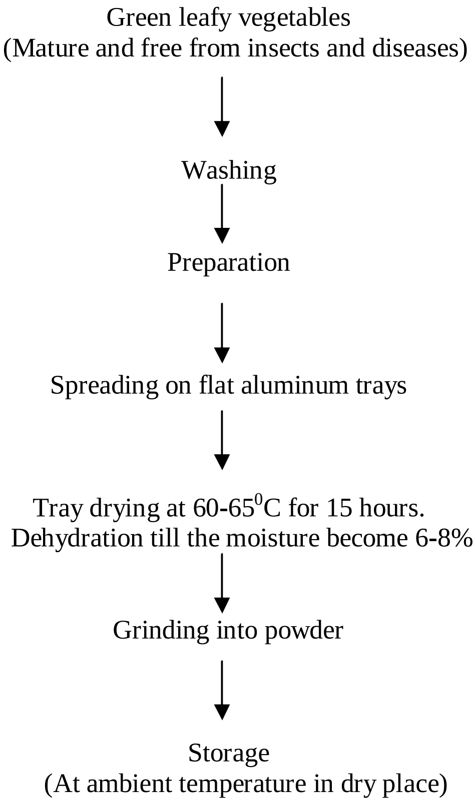

Fig. 1. *Flow diagram of green leafy vegetable dehydration (Source: Srivastava and Kumar, 2002).*

from 1.35-7.5 there was decreasing trend soluble as well as ionizable iron contents samples. The total iron content of fresh bathua and methi leaves, fresh bathua and methi leaves based product paratha, dehydrated bathua and methi leaf powder and dehydrated bathua and methi leaf powder based product laddoo were 3.06±0.69 mg/100g, 11.69±0.89 mg/100g,20.59±0.43mg/100g and 15.16±0.65 mg/100g, respectively. It was found that the availability of iron increases significantly at pH 1.35 and pH 7.5. Dehydrated bathua and methi leaf powder and dehydrated bathua and methi leaf powder based product laddoo contained more iron as compared with fresh bathua and methi leaves and fresh bathua and methi leaves based product paratha (Table 4) indicates that the range of total iron in the prepared products was 3.06-20.59 mg/100g. The dehydrated bathua and methi leaf powder has the highest score of total iron i.e. 20.59±0.43mg/100g.

The soluble and ionizable iron was calculated at pH 1.35 (Table 3). The percent of soluble iron for prepared food items was different. The soluble iron at the pH 1.35 was ranged from 1.84-10.25 mg/100g. Dehydrated bathua and methi leaf powder has the highest score of soluble iron i.e. 10.25mg/100g. The ionizable iron at the pH 1.35 ranged from 0.94-6.78 mg/100g. Here also, the ionizable iron content was rich in dehydrated bathua and methi leaf powder.

Our results show values for iron concentration in prepared products similar to those found in the study performed by Singh *et al.* (2007) who have observed that iron content increased as the incorporation level of dehydrated bathua leaves increased in making *paratha*. These values were similar those reported by Das *et al.* (2005) who determined *in vitro* iron from six GLV's and amount of ionizable iron which was found to range from 30.22±1.10% in bachali (*Basella alba)* to 52.13±1.90 percent of total iron in fenugreek leaves. However, the ionizable iron content varied from 0.55 mg/100 g in bacchali to 1.75 mg/100 g in agathi.

Yadav and Sehgal (2002) reported that the iron content of bathua and spinach leaves was 20.63 and 26.54 mg/100 g, respectively on dry weight basis and also studied, *in vitro* and *in vivo* availability of iron from bathua and spinach leaves and found that iron contents of these green leafy vegetables varied from 20 percent to 26 percent (on dry weight basis) and in vitro availabilities of iron ranged from 2.79 to 3.03 percent. These leaves helped in increasing the haemoglobin level also. This study also supported the work of Bhavyashree, *et al.*, 2009 who studied on *In vitro* accessibility of iron, and zinc from cereal based composite meals and ready to eat foods. Hemalatha *et al.* (2007) investigated bioaccessibility of iron and zinc from cereals and pulses consumed in India. The bioaccessibility of zinc and iron was lowest in sorghum (5.51% and 4.13%, respectively) and highest in rice (21.4% and 8.05%, respectively). Whereas in pulses it ranged from 1.77 to 10.2 per cent lowest in cowpea and highest in french beans. Gupta *et al.* (2006) investigated the relative influence of oxalic acid, phytic acid, tannin and dietary fibre on *in vitro* availability of iron from green leafy vegetables. Bioavailability of iron in 13 GLVs was estimated by equilibrium dialysis method. Singh and Kawatra (2006) studied on ionizable iron content of products viz., pakora, vada, namakpara, kurmura biscuit and cake prepared with addition of fresh and dried powder of amaranthus leaves. Ionizable iron content of products ranged from 1.3 in kurmura to 2.9 mg/100 g in biscuit prepared from dried leaves. Ionizable iron expressed as percent of total iron was highest in biscuit (57.4%) followed by cake (27.5%) and namakpara prepared with dried and fresh amaranthus leaves (25% and 23.7%, respectively), pakora with fresh leaves (19.3%), kurmura with dried leaves (16.1%), vada (16.2%) and kurmura with fresh leaves (22.4%). This study also supported with Sahai and Gupta (2012) who studied on *in vitro* bioavailability of iron of the products prepared from the leaves of the weed, Indian Sorrel (*Oxalis Corniculata).* Indian sorrel leaves incorporated 10%, 20% and 30% into three recipes, peanut chutney, lemon drink and idli" using their standard ingredients. The iron content ranged between 27.05 to 60.55 mg/100g. The soluble and ionizable iron both were also calculated at the two different pH i.e.1.35 and 7.5, that ranged between 9.35-13.26 mg/100g, 4.48-6.89 mg/100g, 8.02-12.03 mg/100g, 3.38-5.58 mg/100g, respectively. The highest iron content was found in peanut chutney. i.e. 60.55±20.58.

Conclusion

It is concluded that incorporation of fresh leaves and dehydrated bathua and methi leaf powder in Indian cookies like paratha and laddoo are well acceptable based on sensory evaluation and nutrient concentration. Paratha and laddoo were rich in iron content. Bioavailability of iron was also tested and the dehydrated bathua and methi leaf powder has the highest amount of total iron, soluble iron and the ionizable iron (pH 1.35). Dehydrated bathua and methi leaf powder based product laddoo has the highest score in ionizable iron and *in vitro* availability iron at pH 7.5 i.e. 4.89±0.34mg/100g and 2.78±0.38, respectively. This method can be used to determine the extent to which availability of iron can be improved by manipulation of diets and used to predict iron absorption from diets and iron sources that are intended for use in prevention of iron deficiency anemia. Therefore, it is recommended that these green leafy vegetables should be included in the diets of general masses especially adolescent girls, who are prone to iron deficiency anaemia. Nutrition education programme should also emphasize the use of green leafy vegetables, which are inexpensive and can be used easily by community.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. S. Sheikh, Dean of Ethelind school of Home-science and Prof. P. J. George, Department of Soil Science, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad for the support during this course of study and their excellence technical assistance is gratefully acknowledged.

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