International Journal of Environment, Agriculture and Biotechnology
Vol-9, Issue-4; Jul-Aug, 2024
Peer-Reviewed International Journal
Journal Home Page Available: https://ijeab.com/
Journal DOI: 10.22161/ijeab

In-Vitro Bioavailability of Iron from Green Gram *(Vigna radiata)* Dhal Flour Fortified with Extrinsic Iron and its Absorption Promoter Ascorbic Acid

Rimpa Bose[1], Binata Nayak[2]

[1]Research scholar, Department of Home Science (Food and Nutrition), University of Calcutta, Kolkata, India
[2]Associate Professor, Department of Home Science (Food and Nutrition), University of Calcutta, Kolkata, India

Received: 19 Jul 2024; Received in revised form: 17 Aug 2024; Accepted: 21 Aug 2024; Available online: 31 Aug 2024

Abstract— *Iron Deficiency Anaemia (IDA) is a global health problem, especially in the developing countries and in India it is also a formidable health challenge. The World Health Organization (WHO) report already identified Iron Deficiency Anaemia as world's most serious health risk factor. The vulnerable groups are pre-school children, school going children, adolescent girls, pregnant women and partially lactating women. Fortification, as generally understood, refer to the process of addition of a nutrient to a food to improve the quality of nutrient to meet the recommended dietary allowance of the population to correctthe existing nutrient deficiency amongst them. Green Gram Dhal (Vigna radiata) was chosen as a vehicle for this study as it is the most acceptable pulse among all Indians irrespective of being vegetarian or non-vegetarian and it contains good quality of protein that helps in iron absorption, moreover pulse, considered to be the second staple food in India, has not yet being considered as a vehicle for iron or any kind of fortification. The in vitro bioavailability of iron in the green gram dhal flour fortified with extrinsic iron and ascorbic acid as iron absorption promoter was studied as compared to non fortified green gram dhal flour. It was found that green gram dhal flour fortified with both extrinsic iron and iron absorption promoter had the highest impact on enhancement of the absorption of both native and added iron, especially in acidic pH, however dhal flour fortified with extrinsic iron only had also the enhancing impact on iron absorption mainly in acidic pH. Thus it can be predicted that fortification of green gram dhal flour with extrinsic iron and iron absorption promoter increases the bioavailability of both native and added iron.*

Keywords— ***green gram dhal flour, fortification, extrinsic iron, ascorbic acid, in vitro bioavailability***

I. INTRODUCTION

Nutritional iron deficiency is a public health problem in developing countries, including India(Sheshadri,1997). Inadequate intake of iron and consumption of foods low in bio-available iron are identified as the causes of iron deficiency.The World Health Organization (WHO) report already identified Iron Deficiency Anaemia as world's most serious health risk factor. The vulnerable groups are pre-school children, school going children, adolescent girls, pregnant women and partially lactating women, even in men,in India. In the initial state of Iron Deficiency Anaemia the haemoglobin level in the blood falls below on age-sex specific standard.

Micronutrient Malnutrition (MNM) is pandemic problem and according to WHO, more than two billion people in the world are suffering from MNM amongst which 0.8 million deaths occur every year due to Iron Deficiency Anaemia.

The approach of enhancing the bioavailability of native food iron seems to be an essential strategy to combat with iron deficiency disorders among the community people, especially in developing countries like India, where poor economic status, ignorance, consumption of nutrient

deficient diet, infection, worm infestation etc. directly affect the health of common people.

According to WHO, **"Food fortification is the process whereby nutrients are added to food (in relatively smooth quantities) to maintain or improve the quality of diet of a group, a community or population.''**

Both the term **'Fortification'** and **'Enrichment'** refers to addition of nutrients to the food. The true definition does slightly vary. Enrichment is defined as 'synonymous with fortification and refers to the addition of micronutrients to the food which are lost during processing'.

Our present study is concerned with Iron Fortification in a new vehicle Pulse especially green gram dhal flour and its bioavailability after being fortified with iron and its absorption promoter ascorbic acid. Ascorbic acid is the most potent enhancer of iron absorption, both as natural component present in fruits and vegetables and also when added as the free compound(Hazell & Johnson1987). Thus fortification of green gram dhal flour with extrinsic iron salt and its absorption promoter ascorbic acid to enhance the native and added iron bioavailability appears to be an useful strategy.

OBJECTIVE:

The present study has been undertaken with the objective of evaluating the in-vitro bioavailability of iron and iron absorption promoter, ascorbic acid, when used as fortificant in green gram dhal flour.

II. LITERATURE REVIEW

The minerals present in the human body in less than 0.05%, are defined as **Microminerals or Trace elements**. Iron is one of the important micromineral that determines the good health of every human being. Iron was first recognized as a constituent of body by Lemory in 1713.

Table.1 Percentage Distribution of Iron in Human Body

SL. No.	Types of Iron	SL.no.	Body Parts	Percentage(%)
1	Functional	1.1	Haemoglobin	60-70
		1.2	Myoglobin	3-4
		1.3	Tissue Iron(enzyme)	5-15
2	Storage and Transport	2.1	Storage iron(liver, spleen, bone marrow)	15-30
		2.2	Transport Iron as Transferrin	0.10
		2.3	Serum Ferritin	<1

[Guthrie Helen A., Marry F. Picciano, Human Nutrition, McGraw-Hill,Boston,1999]

CONSEQUENCES OF IRON DEFICIENCY:

The eventual consequences of iron deficiencies are IRON DEFICIENCY ANAEMIA (IDA) where the *body's store of iron has been depleted and the body is unable to maintain levels of haemoglobin in the blood.* Children and pre-menopausal women are the most vulnerable groups, however iron deficiency anaemia is also found in men, in India.

The various symptoms of iron deficiency include, tiredness, lethargy or lack of energy, shortness of breath(dyspnoea), impaired thermoregulation, Immune dysfunction, GI disturbances, neuro-cognitive impairment, chronic kidney disease (if not treated on time),congestive cardiac failure, chronic respiratory distress (in children)

Less common symptoms include- headache, altered sense of taste, sore tongue, Pica- a desire to eat non-food items, such as ice, paper, mud etc, Tinnitus – perception of noise in one or both ears or in the head that comes from inside the body, ringing of ears, feeling itchy. (Srilakshmi B.,2008)

Table 2. Anaemia Prevalence in Children , NFHS-5(2019-2021)

Anaemia in children-

Anaemia Status	***Haemoglobin level in gm/dL***
Anaemic	*<11.0*
Mildly anaemic	*10.0-10.9*
Moderately anaemic	*7.0-9.9*

Severely anaemic	*<7.0*
Non anaemic	*11.0 or higher*

***Haemoglobin levels are adjusted for altitude in enumeration areas that are above 1000 meters.*

Sample: children 6-59 months

Table 3. Anaemia Prevalence among Women and Men, NFHS-5(2019-2021)

Haemoglobin levels below which women and men are considered anaemic-

Respondents	***Haemoglobin level in gm/Dl***
Non-pregnant women age 15-49	*<11.0*
Pregnant women age 15-49	*<12.0table 3.*
Men age 15-49	*<13.0*

**haemoglobin levels are adjusted for smoking, and for altitude in enumeration areas that are above 1000 meters.*

IMPORTANT ROLE OF IRON IN OUR BODY

I. ***Transport and storage of oxygen:*** Iron present within Hemoglobin (pigment of red blood cells) and myoglobin. It binds to the oxygen and facilitates its movement from the lungs through the arteries to the cells throughout the body. Once oxygen is delivered, the iron (as a part of haemoglobin) binds the carbon dioxide which is then transported back to the lungs from where it gets exhaled. Myoglobin is found only in the muscles. There is acts as a reservoir of oxygen which is needed to produce the energy for muscle contractions.
II. ***Cofactor for enzymes:*** The iron containing haem group is a part of several proteins involved in the release of energy during oxidation of nutrients and formation of energy rich compounds (ATP). Also iron can itself act as a co-factor for different enzymes in the body.
III. ***Formation of Red Blood Cells (RBC):*** Bone marrow produces erythroblasts. As it matures iron is required along with vitamin B6 and copper.

Haem iron absorption- it consists primarily of haemoglobin and myoglobin. It represents small fraction of the iron in the diet but with high biological value and absorption. Most of the haem iron seems to enter the intestinal absorptive cell as an intact metalloporphyrin. Subsequently iron is released from the porphyrin in the intestinal mucosa by mucosal haem oxygenase and enters the circulation as metallic iron.

Non-haem iron absorption- It is found typically in cereal pulse based diet and also available from green leafy vegetables. Non-haem iron absorption occurs mostly in the proximal small intestine. (UNICEF report2011, Srilakshmi B.,2008)

In both the cases, ascorbic acid plays a key role in accelerating iron absorption in human body, specially from non-haem iron, converting the ferric form to absorbable ferrous form.

Bioavailability can be defined as the proportion of total mineral in a food, meal or diet that is available for normal body functions. This involves various stages, each of which is affected by different dietary and physiological factors. The amount of mineral that is available for absorption is dependent upon dietary composition, gastrointestinal secretion and luminal interactions. The proportions that is taken by the mucosal cells depends upon a number of host – related factors and the degree of utilizations in the body, depends again upon physiological factors as modified by the chemical form of the mineral.

Bioavailability of trace elements can be broadly classified under three categories i.e. high, medium and low bioavailable elements depending upon how much the human body is able to absorb them from the diet. Much of the research into trace elements bioavalability has been focussed on iron.(Narasingha Rao B.S.,1994)

In vitro methods are relatively simple, rapid, inexpressive methods were developed an alternatives to human absorption studies and usually involve a simultaneous gastric digestion followed by measurement or soluble or dialyzable iron available for absorption. It helps in the measurement of the amount of iron that is soluble and potentially bio-available.(NarsinghaRao, Prabhavathi,1978)

The addition of ascorbic acid causes substantial increase in the amount of iron absorbed from most of the iron compounds, in most of the studies, addition of ascorbic

acid as an absorption promoter enhanced the iron absorption. (WHO report on Guidelines on food fortification with micronutrients,2006)

III. METHODOLOGY

Green gram dhal flour, Ferrous sulphate heptahydrate(FeSO4,7H2O), Ascorbic acid were used for the study. The dhal flour wasprocured locally and after ensuring the fact that it was free from contaminant, it was processed for fortification by washing, drying, milling respectively. Ferrous sulphate salt, a cost effective source of iron, L-ascorbic acid were of analytical grade and procured locally.

According to various studies, it was found that Indian adolescents girls are one of the vulnerable group suffering from Iron Deficiency Anaemia (IDA), hence the present study considered the Recommended Dietary Allowance (RDA) of adolescents girls of 16-18 years i.e. 32mg/day (ICMR2020) to derive the level of fortification. Usually, fortification should 1/3rd of the RDA, hence it was almost equal to*10 mg/day.* Green gram dhal flour has 4mg intrinsic iron/100gm. Considering the acceptable edible quantity as 50gm/day by any human being, the available intrinsic iron would be 2mg/100gm and rest part can be fortified with extrinsic iron.

With the calculated amount of green gram dhal flour, extrinsic iron as FeSO4,7H2O and ascorbic acid as iron absorption promoters, the following combinations were prepared for the study:

1. *Green Gram dhal flour +no fortificant (control)*
2. *Green Gram dhal flour + $FeSO_4, 7H_2O$*
3. *Green Gram dhal flour + $FeSO_4, 7H_2O$ + Ascorbic acid*

A five time concentrated premix of each of the fortificants was prepared in green gram dhal flour base. A two-stage dry mixing (hand procedure)was adopted to obtain a homogenous preparation and then it was diluted by mixing the vehicle in the desired amount.

Iron content of all the preparations were in the mineral solution of the dry digested samples according to Wongs method. About 5-10gm of control and fortified green gram dhal flour were made ash at 600degree Centigrade in a muffle furnace for 12 hours. The residues were treated with concentrated nitric acid and hydrochloric acid and evaporated to dryness. The residue thus obtained was dissolved in 5ml 6N HCL and filtered and process was repeated for 2-3 times with glass distilled water. The combined filtrate was made upto 100ml and further steps were followed as described in Wongs method. (Nayak B., Nair K.M.2003)

In case of in-vitro bioavailability analysis of iron, the method described by Narsigha Rao and Prabhavathi (1978)was used. The method involves incubation of duplication of 8% (g/v) homogenate (25ml) of the fortified green gram dhal flour in pepsin-HCL solution (0.5% pepsin in 0.1 N HCL solution, pH 1.35 adjusted with distilled water at 37 degree centigrade for 90 minutes after which the contents from each set were centrifused at 3000rpm for 45 minutes and the supernatant was filtered and saved for iron estimation. The pH of the other set was adjusted to 7.5 with 5N Sodium hydroxide and incubated and processed as mentioned above to obtain the filtrate. Ionizable iron in the filtrate was estimated by α-α' dipyridylmethod (AOAC,1965).

To working standard iron solution (1-15µg) and blank were added 1ml of hydroxylamine hydrochloride solution, 5ml of acetate buffer solution and 2 ml of αα' – dipyridyl solution in that order. Test tubes were shaken after the addition of each reagent. The solution was made upto 15 ml with water and mixed. Duplicate aliquots (3ml & 5ml) of the acid – pepsin digested extract of pH 1.35 and alkaline extract of pH 7.5 were taken. To the other aliquot, all the reagents described above were added and the intensity of colour measured at 510 nm against reagent blank.

After adjusting the instruments with reagent blank, standard, extract blank and tests were read. From the difference between test and extract blank the ionisable iron present in the green gram dhal flour combinations were calculated, substracting the optical density of extract blank from that of the sample.

IV. RESULTS

The t-test, also known as Student's T test were used for statistical analysis. The Null Hypothesis was considered asthere were no differences between the groups. T-Test provide p-value based on t-distribution and if the p-value is less than the chosen significance level (0.05), the null hypothesis is rejected and the groups are statistically significantly different as per the alternative hypothesis.

The study also represents the data in a Box and Whisker Plots and other diagrams to get a snapshot of the data and analysis at a glance. Box plots are used to show distributions of numeric data values, especially when we want to compare them between multiple groups. They are built to provide high-level information at a glance, offering general information about a group of data's symmetry, skew, variance, and outliers.

The iron content of each of these combinations were estimated and the were as follows:

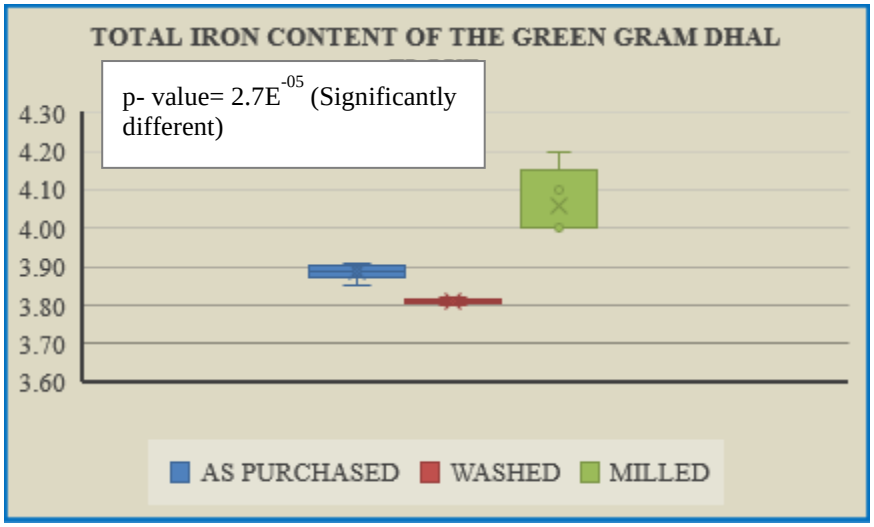

Fig.1. Total Iron Content of the Green Gram Dhal Flour as Purchased, after Washing and after Milling

Table 4. Total Iron content of Premixes, Fortified Green Gram Dhal Flour

Combinations of Research sample	**Iron content of Premix (observed)**	**Iron content of Fortified Dhal flour(observed)**
	Mean±S.D.	Mean±S.D.
Green Gram dhal flour (control) – No fortificant	4.04±0.049	4.01±0.007
Green Gram dhal flour + $FeSO_4$, $7H_2O$	35.06±0.040	9.40±0.005
Green Gram dhal flour + $FeSO_4$, H_2O + Ascorbic acid	33.19±0.024	10.12±0.075

Table 5. In Vitro bioavailability of Iron(Ionizable Iron) from Green Gram Dhal Flour Fortified with Iron and Iron-Absorption Promoter

	Average total iron (mg/100gm)	**Ionizable Iron (mg/100gm)**		**% of Total Iron**	
Combinations of Research		**PH 1.35**	**PH 7.5**	**PH 1.35**	**PH 7.5**
		Mean±S.D.	Mean±S.D.	Mean±S.D.	Mean±S.D.
Green Gram dhal flour (control) – No fortificant	4.0	1.5±0.012	0.6±0.005	38.7±0.170	16.1±0.028
Green Gram dhal flour + $FeSO_4$, $7H_2O$	9.41	4.5±0.005	1.4±0.012	50.3±0.094	15.8±0.082
Green Gram dhal flour + $FeSO_4$, H_2O + Ascorbic acid	9.60	6.4±0.005	2.4±0.012	63.8±0.047	23.6±0.125

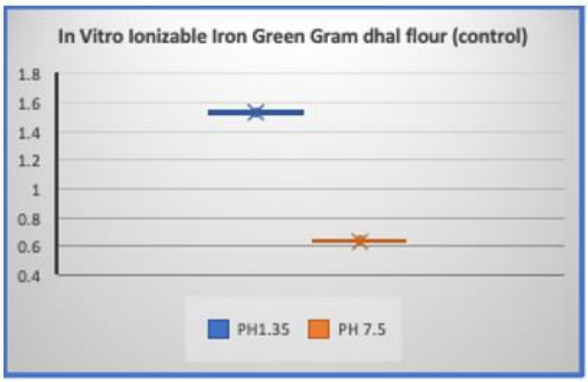

Fig.2

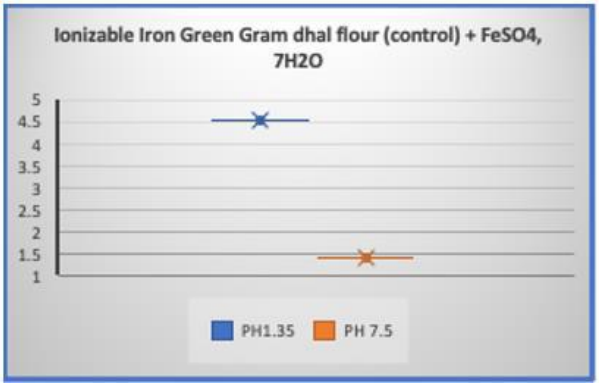

Fig.3

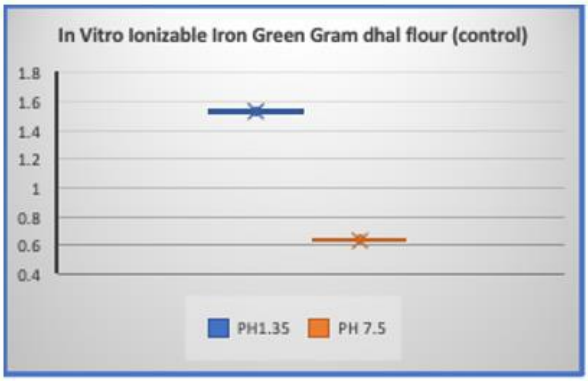

Fig.4

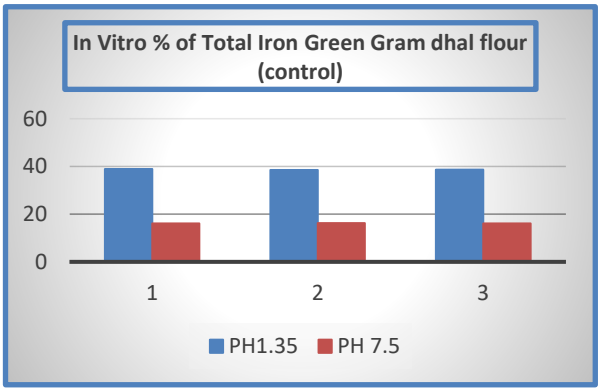

Fig.5

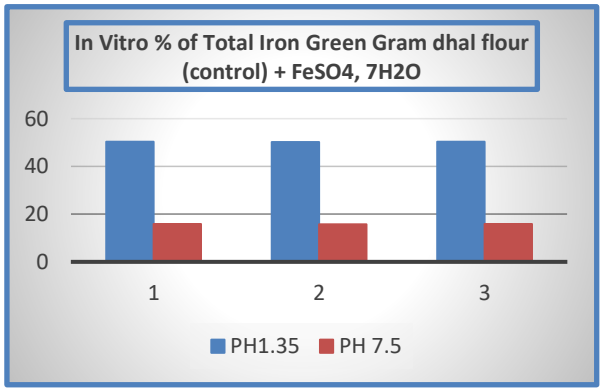

Fig.6

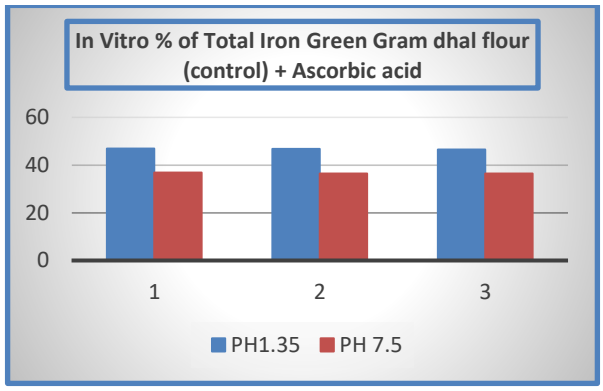

Fig.7

It can be interpreted from the result that the bioavailability of ionisable iron was much higher in presence of extrinsic iron as compared to unfortified green gram dhal flour and gave best result in presence of ascorbic acid as iron absorption promoter. The percentage of availability of total ionizable iron was highest in presence of ascorbic acid in both acidic and alkaline pH. It was also observed that the bioavailability of ionisable iron is much higher in acidic pH as compared to alkaline pH in all combination.

V. DISCUSSION

The statistical analysis showed that there was no change in the intrinsic iron content of green gram dhal flour as purchased and after washing but a significant change was found after milling of the flour, probably because of the use of iron body mortar.

In case of iron content of fortified green gram dhal flour premix and diluted fortified flour, there were no change in the control portion but iron content of premix was desirably high as compared to the diluted fortified dhal flour.

After the statistical analysis it was found that with reference to the average total iron content of non fortified

dhal flour, 38.7% of ionisable iron was obtained at the acidic pH and 16.1% of ionisable iron was obtained at the alkaline pH. The ionisable iron obtained from extrinsic iron fortified green gram dhal flour was 50.3% at the acidic pH and that of alkaline pH was 15.8%. When the green gram dhal flour was fortified with both iron salt and iron absorption promoter ascorbic acid, the ionisable iron obtained at the acidic pH was 63.8%, by far the highest among all combinations and ionisable iron obtained at alkaline pH was 23.6% which was also high among all the three combinations. Hence it was indicative that the fortification of green gram dhal flour with extrinsic iron and absorption promoter elevates the in vitro bioavailability of iron as compared to non fortified dhal flour. It was also found from the study that bioavailability of iron in all combinations wassignificantly higher in the acidic pH as compared to the alkaline pH.

Thus it can be predicted from the in vitro bioavailability study that green gram dhal flour fortified with both extrinsic iron and ascorbic acid as absorption promoter has the highest enhancing impact on the absorption of iron where as the dhal flour fortified with extrinsic iron also increases the iron absorption as compared to the non fortified dhal flour having only native iron and with the lowest absorption capacity.

REFERENCES

[1] Seshadri, S., Gopaldas, T. (1989). Impact of iron supplementation cognitive functions in preschool and school –aged children: the Indian experience. *The American Journal of Clinical Nutrition*, 50, 675-686.

[2] Anand, K., Kant, S., Kapoor, S. K.(1999).*Nutritional Status of Adolescent school children in rural North India*: Comprehensive Rural Health Service Project report. New Delhi : AIIMS.

[3] Hellert, W. S., Kirsting, M., Alexy, U., Manz, F.,(2000).Ten years trends in Vitamin and Mineral intake from Fortified food in German children and adolescents. *European Journal of Clinical Nutrition*, Germany,54, 81-86.

[4] UNICEF, WHO,(2001). *Iron Deficiency Anaemia, Assessment, Prevention and control: A guide for Programme Manager*. WHO,United Nations University. Geneva, Switzerland, 1-114.

[5] All India Institute of Hygiene and Public health, (2001). *Iron Deficiency Anaemia and Control. National pilot programme on Control of Micronutrient Malnutrition*. AIIHPH, Directorate General of Health Services, Ministry of Health and Family Welfare, Govt. Of India.

[6] Berger, J., Dillon, J.C., (Jan-mar 2002). Control of Iron deficiency in Developing countries. *Santé, Nutrition Alimentation Societies, France*, 12(1), 22-30, PMID: 11943635. Raghuramula, N., Nair, K. M., Kalyanasundaram, S.,(2003)*A Manual of Laboratory Techniques*. National Institute of Nutrition, Hyderabad.

[7] Raghuramula, N., Nair, K. M., Kalyanasundaram, S.,(2003)*A Manual of Laboratory Techniques*. National Institute of Nutrition, Hyderabad.

[8] Nayak, B., Nair, K. M.,(2003). In vitro bioavailability of iron from Wheat flour fortified with ascorbic acid, EDTA and sodium hexametaphosphate, with or without iron. *Food Chemistry*, 80(4), 545-550.

[9] Global Alliance for Improved Nutrition (GAIN) report (12th march 2008). *Initiative for Ending Malnutrition, Discussion Session on Food Fortification*. GAIN, YWCA complex New Delhi. Retrieved from www.gainhealth.org.

[10] AOAC,(1995) Official Methods Analysis of the Association Of the Agricultural Chemists, Washington DC:AOAC(1995).

[11] Srilakshmi B., (2008), chapter 10: Micro minerals- Iron, Nutrition Science, New Age International Publisher pvt. Ltd.