



Quantitative Analysis of Iron and Ascorbic acid contents in locally consumed Fruits and Vegetables

Bhuvaneswari S, Joshi M and D'Souza A

Department of Chemistry and Biochemistry, St. Aloysius' (Autonomous) College, Jabalpur, INDIA

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Abstract

Iron deficiency anemia is one of the major health problems which affect both non-industrialized and industrialized nations. Anemia affects the cognitive performance, physical capacity, immune status and reproductive performance. One way to address this problem is through a food-based approach by increasing the consumption of iron-rich foods which also have better bioavailability. Therefore the purpose of the study was to quantify the iron and ascorbic acid content from various locally consumed fruits and vegetables. The samples (Apple, common fig, ground nut, soya bean, dates, fenugreek leaves, spinach and raisins) were bought from the local market. The iron content ranged from 0.52 mg/100g to 2.4 mg/100g. The maximum content of iron was found in fenugreek leaves followed by soya bean, common fig, ground nut, apple, raisins, spinach and finally in dates. Ascorbic acid content ranged from 1.99mg/100g to 14.97 mg/100g. From the present study it can be suggested that food such as apples, common fig and ground nut can be included in our diet, because they not only contain good amount of iron but also ascorbic acid which has been reported as a powerful enhancer of non-heme iron absorption.

Keywords: Iron, ascorbic acid, bioavailability, enhancer.

Introduction

Iron is an important essential mineral which is vital for many of the biological functions of the body. Iron in the body is primarily involved in oxygen transport as well as in various biological activities like cellular proliferation, electron transfer and enzymatic reactions. Report by the World Health Organization shows that of the nutritional disorders, iron deficiency anemia is one of the most common which has major effect on both health and economy¹. Iron deficiency affects a major part of the population in almost every country in the world. Anemia is also the reason for disability as well as mortality among large group of population around the globe. The prevalence of anemia in India is very high affecting almost 58% of pregnant women, in spite of diversity in food habits². The susceptibility of anemia is more in children, adolescents and women of child-bearing age including pregnant women³. The main cause of anemia is not only due to low iron intake but also poor iron absorption. Anemia is characterized by low concentration of hemoglobin and hence their tissues get less oxygen than the required amount. Anemia results in decrease in overall physical growth of children decrease in cognitive performance as well as compromised immune status⁴. This global health problem can be addressed by improving the dietary iron bioavailability.

Iron contribution in body is mainly through diet. The two main forms of iron are heme and non-heme, the former obtained from meat, fish, poultry and the latter obtained from plant source. It has been reported that heme iron is well absorbed and is not

much affected by other components of the foods eaten in the same meal. The absorption of non-heme iron is greatly influenced by meal composition⁵. Bioavailability of iron can be altered by various components present in the food which can either enhance or inhibit iron absorption. Research work by Hallberg and his coworkers⁶ has shown that ascorbic acid prevents the formation of insoluble and un-absorbable iron compounds and the reduction of ferric to ferrous iron, which is easily absorbed. Various researches have shown that ascorbic acid is a dietary constituent which enhances the absorption of non-heme iron in humans. At acidic pH ascorbic acid forms a chelate with ferric iron and thereby enhances iron absorption⁷. When iron is present along with ascorbic acid, the absorption of iron has been shown to be increased even in the presence of inhibitors.

Various studies have also shown that the absorption of non-heme iron from diets is low due to the limited amount of foods that stimulate the absorption of non-heme iron⁸. In our country the major population has access to non-heme iron food. Moreover their diets often have a low concentration of factors such as ascorbic acid that enhance iron absorption. The population also consumes large amount of beverages such as tea and coffee which are in phytates and tannins, which has been reported to inhibit iron absorption⁹. To prevent the further increase in anemia which is increasing at an alarming rate, it is preferable to include food rich in iron that is also rich in ascorbic acid. With this background information, this present study was designed to estimate the iron and ascorbic content from various locally consumed foods.

Material and Methods

A total of eight different foodstuffs were analyzed for iron as well as ascorbic acid. The English and botanical names of the samples are listed in table-1.

Table-1
Food analyzed for iron and ascorbic acid with their Botanical names

S. No	English Name	Botanical Name
1	Apple	<i>Malus domestica</i>
2	Common Fig	<i>Ficus carica</i>
3	Ground Nut	<i>Arachis hypogaea</i>
4	Soya bean	<i>Glycine max</i>
5	Dates	<i>Phoenix dactylifera</i>
6	Fenugreek leaves	<i>Trigonella Foeniculum-graecum</i>
7	Spinach	<i>Spinacia oleracea</i>
8	Raisins	<i>Vitis vinifera</i>

Standard iron solution: Iron was estimated by the thiocyanate method¹⁰. Accurately weighed 0.48g of ferric ammonium sulphate powder and dissolved with little amount of distilled water. To this 4 ml of conc. sulphuric acid was added. The powder was left to soak in acid overnight. Then the acid/slurry powder was made up the volume to 100 ml with distilled water. The solution took few days to dissolve completely the solution thus obtained is the iron stock solution. A standard solution of ferric ammonium sulfate was prepared by pipetting 10 ml of this stock iron solution in a 100 ml volumetric flask and made up to 100 ml with distilled water. The procedure was repeated in separate 500mL volumetric flasks, pipetting in 20, 30, 40 and 50mL of stock iron solution to prepare other standard solutions.

Sample preparation for iron estimation: Food samples purchased from the market was first washed and 5g of the sample was weighed accurately. The weighed sample was taken in a crucible. The crucible was heated over a Bunsen burner and the sample was reduced completely to ash. The ash of the sample was crushed to a fine powder. The ash was soaked in 1 mol L⁻¹ hydrochloric acid. Then 5 ml of distilled water was added. The solution was filtered and transferred into a 100ml conical flask. This filtered solution was used for the estimation of iron by thiocyanate method using spectrophotometric analysis.

Preparation of ammonium thiocyanate solution: Weighed 38 g of solid ammonium thiocyanate into a 500mL volumetric flask and made up to the mark with distilled water.

Estimation of Iron by Thiocyanate method: 10 ml each of the sample solution was taken in a clean dry test tube. Simultaneously 10 ml each of the 5 standard iron solutions (containing 2, 4, 6, 8, 10 x 10⁻⁵ molL⁻¹ of iron) were also taken. Then 10 ml of ammonium thiocyanate solution was added. The solution was mixed thoroughly by swirling. A stable red color appeared by mixing the solution. The absorbance was measured

at 490 nm using UV-visible spectrophotometer. The concentration of iron from each food sample was calculated from the standard graph and expressed as mg/100g of sample.

Preparation of ascorbic acid standard: First a 500ppm ascorbic acid stock was prepared. From the stock, five different standards containing 5ppm, 10ppm, 15ppm, 20ppm and 25ppm were prepared.

Sample preparation for ascorbic acid estimation: About 10g of the samples were weighed accurately and homogenized with 50ml of 5% meta-phosphoric acid and 10% acetic acid mixture. Then it was transferred to a 100 ml volumetric flask and shaken to get a homogenous mixture. It was then made up to 100 ml with 5% meta-phosphoric acid and 10% acetic acid mixture. Then the solution was filtered and the filtrate was used for the estimation of ascorbic acid. Ascorbic acid was estimated by the method followed by Rahman and his coworkers¹¹.

Estimation of Ascorbic acid: To the sample solution few drops of bromine was added which oxidizes the ascorbic acid to dehydroascorbic acid. Then a few drops of thiourea were added to it to remove the excess bromine to obtain a clear solution. To the clear sample solution and to all the five standards 1 ml of 2,4, Dinitrophenyl hydrazine (DNPH) solution was added and incubated at 37°C for 3 hours in a water bath. After the incubation period added 5 ml of 85% sulphuric acid with constant stirring, by placing the test tubes in ice bath. Ascorbic acid reacts with 2,4, DNPH which produces an osazone which on treatment with 85% sulphuric acid forms a red colored solution. The red color formed was read at 520 nm in a spectrophotometer. The concentration of ascorbic acid obtained in the sample was expressed as mg/100g of sample.

Results and Discussion

Figure-1 shows the standard/calibration curve of iron. The concentration of iron from each food sample is calculated from the standard graph and expressed as mg/100g of sample. The iron content of the analyzed food sample is tabulated in table-2. The iron content in the analyzed samples ranged from 0.52 to 2.4 mg/100g. The highest iron content was found in fenugreek leaves followed by soya bean, common dried fig, ground nut, apple, raisins, spinach and finally in dates which is depicted in figure-2. Fenugreek leaves had the highest concentration of iron and the lowest was found to be in dates. Some of the plant-based foods are good source of iron but they may contain iron-absorption promoters or inhibitors. Depending on whether the food contains inhibitor or promoters the bioavailability of iron would differ.

Figure-3 shows the standard curve of ascorbic acid. Table-3 lists the ascorbic content of the selected food samples. In analyzed food samples, the highest ascorbic acid content was found to be 14.97 mg/100g. Apple had the highest content of ascorbic acid. The ascorbic acid content ranged from 1.99 mg/100g to 14.97

mg/100g that is depicted in the figure-4. In rank order of higher ascorbic acid content were apple, spinach, common fig, ground nut, raisins, fenugreek and soya bean with the lowest ascorbic content found to be in dates. Ascorbic acid is an enhancer of bioavailability of iron¹². Foods like apple, spinach and common fig would have better bioavailability of iron because the high concentration of ascorbic acid. Excessive intake of iron inhibitors, like tea, coffee and calcium rich foods in diet during mealtime will reduce the bioavailability to a very great extent.

Table-2

Iron content of food samples analyzed

Food name	Mean Iron content (mg/100g)
Apple	0.92
Common dried fig	1.32
Ground Nut	1.27
Soya bean	1.65
Dates	0.52
Fenugreek leaves	2.40
Spinach	0.58
Raisins	0.88

Iron deficiency anemia is one of the most common of the non-communicable disease which needs urgent effective corrective measures. It has been reported as the major health challenge that the world is facing¹³. It is the one of the most common nutrient deficiency which is prevalent across the globe¹⁴. Anemia has a major consequence on our health and it also causes disability and decreased performance. India is one of the countries with very high prevalence of anemia. Although anemia is a multi-factorial disease, nutritional deficiency is the main cause for it. Anemia is widely prevalent in all age group and since

nutritional deficiency is the primary cause for the anemia in India, there is an urgent need to address this situation. The contribution of iron in body is mainly through diet and the bioavailability of iron depends on the source whether heme iron or non-heme iron¹⁵. Heme iron is well absorbed whereas the absorption of non heme iron which forms the major dietary pool in India is influenced by meal composition.

Table-3

Ascorbic acid content of locally available food stuffs

Food name	Mean ascorbic acid content (mg/100g)
Apple	14.97
Common dried fig	10.53
Ground Nut	5.44
Soya bean	3.29
Dates	1.99
Fenugreek leaves	3.81
Spinach	13.46
Raisins	4.71

The cognitive performance as well as physical growth of children is greatly influenced by the iron content in the food they consume^{16,17}. People with iron deficiency have impaired gastrointestinal functions and altered metabolism and hormone production. It has been shown in experimental animals that iron plays a key role in brain function. Anemia is multi-factorial in nature and correcting anemia requires an integrated approach. One of the best strategies to prevent iron deficiency is food-based approaches to increase iron intake through consumption of food rich in iron. Food based strategies not only help prevent anemia but also bring along other long-term nutritional benefits.

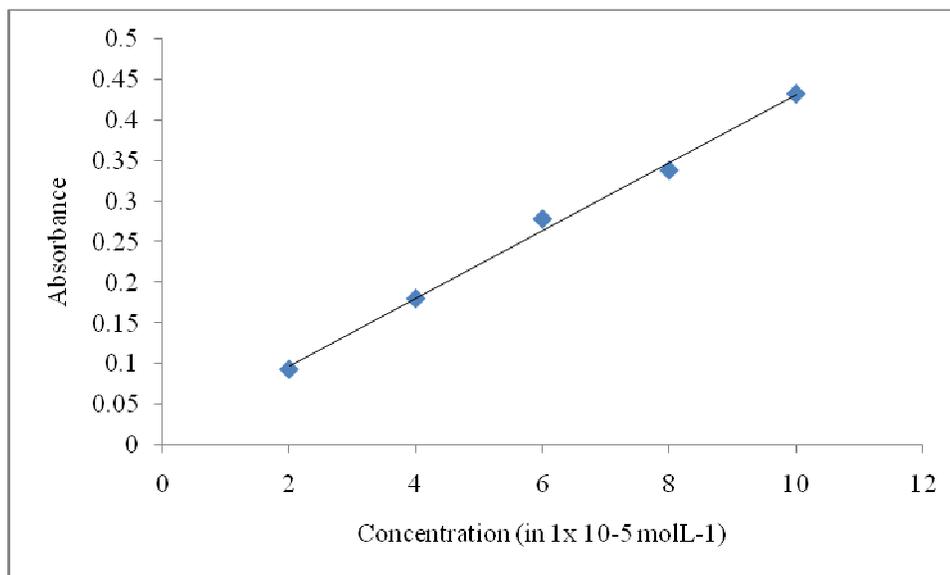


Figure-1

Standard graph of iron concentration against absorbance

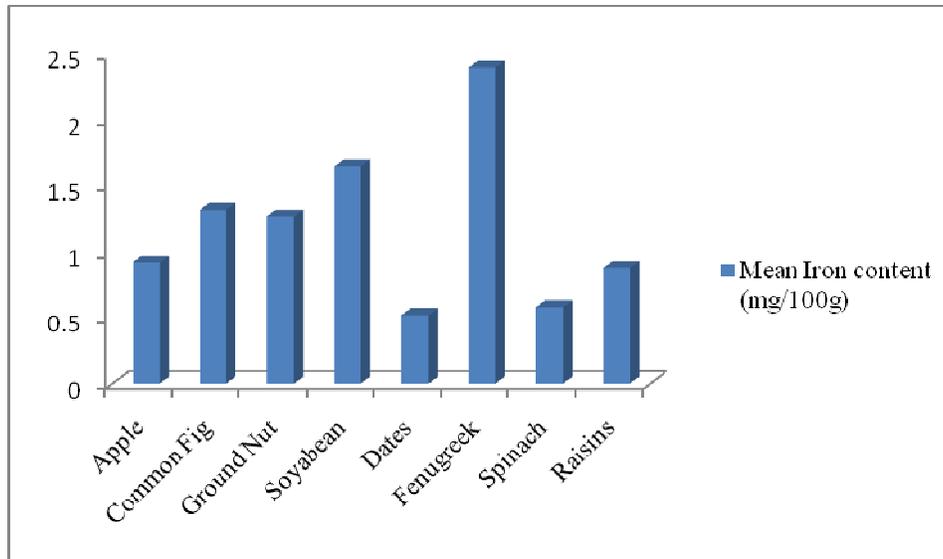


Figure-2
Iron content of food samples analyzed

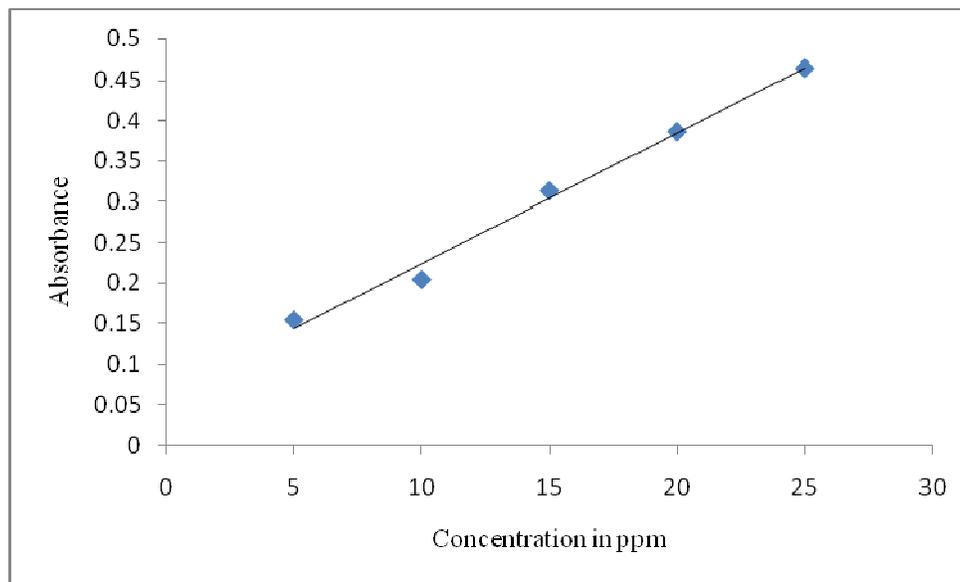


Figure-3
Standard graph of ascorbic acid concentration against absorbance

As shown in the figure-5 fenugreek which has got the highest content of iron has a comparatively less amount of ascorbic acid. So the absorption of iron from fenugreek will be less. Apple is one food sample which has good concentration of iron as well as ascorbic acid, so the iron absorption from apple would be very good. Though spinach contains good amount of iron as well as ascorbic acid it has been reported to contain inhibitors like polyphenols which will affect the bioavailability of iron in spite of the presence of the enhancer, ascorbic acid¹⁸.

Iron absorption from both common fig and ground nut would be good because of the presence of the enhancer, ascorbic acid.

From the present study it can be suggested that food like apple, common fig and ground nut can be included in our diet, because they not only contain good amount of iron but also ascorbic acid. Ascorbic acid has been reported as a powerful enhancer of non heme iron absorption and it has been shown to reverse the inhibiting effect of substances such as tea and calcium¹⁹. Study done by Reddy and Cook²⁰ shows that, the presence of ascorbic acid increases the iron absorption from vegetable meals. Reported work by few researchers suggests that ascorbic acid helps in increasing the iron bioavailability than other organic acids²¹. In a country like India where the maximum population is poor it is best to recommend the intake of natural sources of ascorbic acid present in fruits and vegetables.

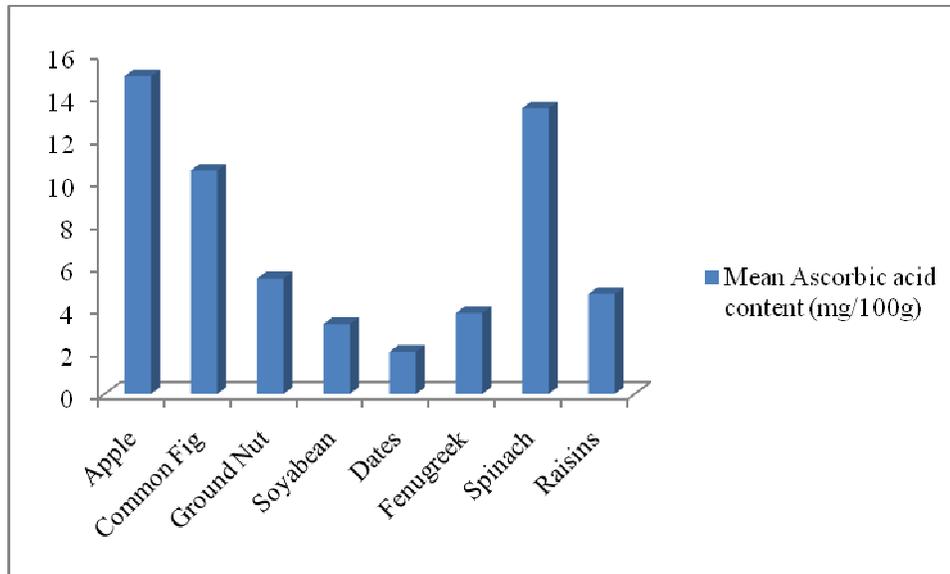


Figure-4
 Ascorbic acid content of food samples analyzed

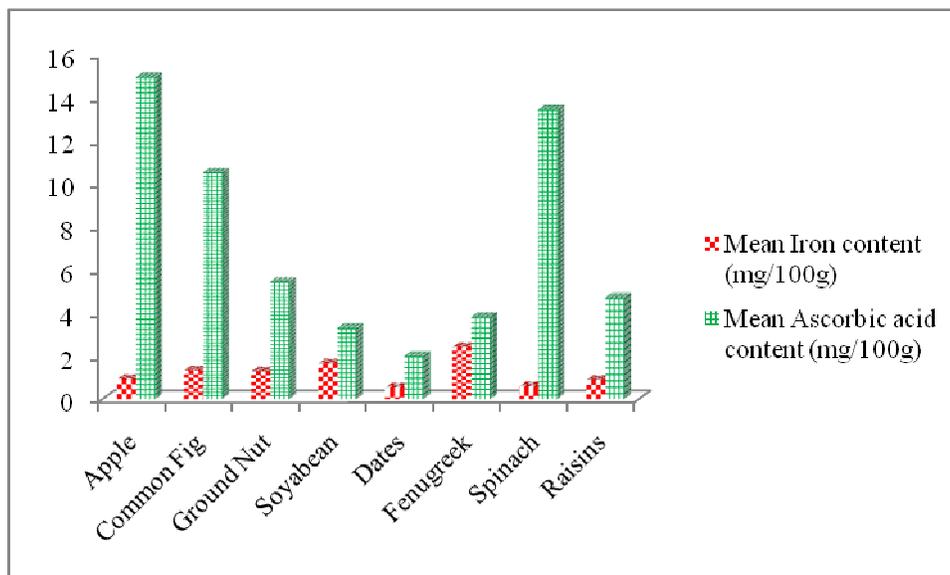


Figure-5
 Comparative chart showing the iron and ascorbic acid content in various food samples

Conclusion

Few commonly used fruits and vegetables were analyzed for iron and ascorbic acid content. The values are tabulated and a comparative graph is also shown in the result. Fenugreek leaves had the highest iron content and apple had the highest content of ascorbic acid. Among the sample analyzed apple, common fig and ground nut had good amount of iron as well as ascorbic acid content. Good knowledge about the iron content and the factors which increase its bioavailability will help to select proper food to be included in the diet. Proper selection of food will in turn help to reduce iron deficiency anemia in our country.

References

1. Iron deficiency anemia: Assessment, prevention and control, A guide for programme managers, Geneva, World Health Organization (2001)
2. NNMB, Technical Report No.22, National Nutrition Monitoring Bureau (NNMB), Prevalence of micronutrient deficiencies, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad (2003)
3. Gomber S, Kumar S, Rusia U, Gupta P, Agarwal K.N.

- and Sharma S., Prevalence and etiology of nutritional anemias in early childhood in an urban slum, *Indian. J. Med. Res.*, **107**, 269-73 (1998)
4. Raman L, Pawashe A.B. and Ramalakshmi B.A., Iron nutritional status of preschool children, *Indian. J. Pediatr.*, **59**, 209-12 (1992)
 5. Hallberg L and Rossander L, Improvement of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid and ferrous sulphate on iron absorption from a simple Latin American-type of meal, *Am. J. Clin. Nutr.*, **39**, 577-583 (1983)
 6. Hallberg L, Brune M and Rossander L., The role of Vitamin C in iron absorption, *Int. J. Vitam. Res. Suppl.*, **30**, 103-8 (1989)
 7. Cook J D and Reddy M B, Effect of ascorbic acid intake on non-heme iron absorption from a complete diet, *Am J Clin Nutr*, **73(1)**, 93-98 (2001)
 8. Lynch S.R. and Cook J.D., Interaction of vitamin C and iron, *Ann.N.Y.Acad.Sci.*, **355**, 32-44 (1980)
 9. Gilooly M, Bothwell T.H, Torrance J.D, MacPhil A.P., Derman D.P. and Bezwoda W.R et al., The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables, *Br. J. Nutr.*, **49**, 331-42 (1983)
 10. www.outreach.canterbury.ac.nz, (2015)
 11. Rahman MM, Khan MMR and Hosain MM, Analysis of vitamin C (ascorbic acid) contents in various fruits and vegetables by UV-spectrophotometry, *Bangladesh J Sci Ind Res*, **42(4)**, 417-424 (2007)
 12. Lynch S.R. and Cook J.D., Interaction of vitamin C and iron, *Ann.N.Y.Acad.Sci.*, **355**, 32-44 (1980)
 13. World Health Organization, Worldwide prevalence of Anemia 1993-2005- WHO Global Database on Anemia, World Health Organization (2008)
 14. De Maeyer E, Adiels-Tegman M, The prevalence of anemia in the world, *World Health Statistics Quarterly*, **38**, 302-316 (1985)
 15. Hurrell R and Egli L, Iron bioavailability and dietary reference values, *Am. J. Clin. Nutr.*, **91**, 1461S-7S (2010)
 16. Sivakumar B, Nair K.M, Sreeramulu D, Suryanarayana P, Ravinder P and Shatrungna V et al., Effect of micronutrient supplement on health and nutritional status of school children: Biochemical Status, *Nutrition*, **22**, S15-S25 (2006)
 17. Srikantia S.G, Sivaprasad J, Bhaskaram C and Krishnamchari KAVR, Anaemia and immune response, *Lancet*, **7973**, 1307-09 (1976)
 18. Rutzke C.J, Glahn R.P, Rutzke M.A, Welch R.M, Langhans R.W. and Albright L.D. et al., Bioavailability of iron from spinach using an in-vitro/human Caco-2 cell bioassay model, *Habitation*, **10**, 7-14 (2004)
 19. Hallberg L., Iron requirements and bioavailability of dietary iron, *Experientia Suppl.*, **44**, 223-44 (1983)
 20. Reddy M.B. and Cook J.D., Assessment of dietary determinants of non-heme-iron absorption in humans and rats, *Am. J. Clin. Nutr.*, **54**, 723-728 (1991)
 21. Teucher B, Olivares M and Cori H., Enhancers of iron absorption: ascorbic acid and other organic acids, *Int. J. Vitam. Nutr.Res.*, **74(6)**, 403-19 (2004)