J Food Sci Technol (April 2016) 53(4):2033–2039
DOI 10.1007/s13197-015-2154-z

ORIGINAL ARTICLE

# Effect of inclusion of key foods on in vitro iron bioaccessibility in composite meals

**Anamika Singh · Kiran Bains · Harpreet Kaur**

Revised: 20 September 2014 / Accepted: 1 October 2014 / Published online: 16 April 2016


**Abstract** The in vitro bioaccessibility of iron in context to fortification of key foods to cereal based diets was studied to optimize the meals for enhanced iron bioaccessibility to meet the needs of vegetarian and non-vegetarian adult women. Four individual food items and thirty six composite meals were selected to represent a wide spectrum of meal ingredients. The four individual foods: *chapati*, *r*ice, *dal and saag* were choosen on the basis of data reported on meal pattern of surveyed households of north India. The basic meals were then fortified with key food ingredients which may influence in vitro iron bioaccessibility. Eight selected key foods were salad, orange, lemonade, milk, curd, chicken, egg and tea. The results revealed that inclusion of 200 g of chicken, 135 g of salad and 120 g of orange to the basic meals of rice or *chapati* with either *dal* or *saag* enhanced iron bioaccessibility by 1.6 fold to 5.0 fold; 5.2 to 28.9 % and 4.7 to 10.7 %, respectively. The best enhancer of iron absorption for vegetarians was lemonade (250 ml) which resulted in 70.2 and 61.0 % increase of in vitro bioaccessibility of iron to the rice based meals with *dal* and *saag*, respectively. The inclusion of lemonade resulted in 1.3 fold increase in iron bioaccessibility in *chapati* based meals. The major inhibitors of iron bioaccessibility were egg and tea, the percent reduction caused by egg being 16.1 to 50.2 % while by tea, it was between 21.5 to 55.3 %. The study recommends that those vulnerable to iron deficiency should be encouraged to increase overall intake of iron from iron rich foods. The increase should be coupled with efforts to combine appropriate foods in the diet to enhance the bioaccessibility of iron and reduce inhibitory factors.

A. Singh · K. Bains (✉) · H. Kaur
Department of Food and Nutrition, Punjab Agricultural University, Ludhiana 141004,, Punjab, India
e-mail: kiranbains68@hotmail.com

**Keywords** Iron bioaccessibility · Iron inhibitors · Iron enhancers · Dialyzable iron · Total iron

## Introduction

Diets crossways the world are universally deficient in a number of micronutrients, particularly iron. In developing countries, severe iron deficiency is considered to contribute directly to cognitive impairment, decreased work productivity, and death. In India, the prevalence of anemia is high because of poor dietary intake especially iron and folic acid, poor bioaccessibility of iron in phytate and fiber rich Indian diet, chronic blood loss due to infections such as malaria and hookworm infestation (WHO 2004). Determination of bioaccessibility of dietary iron is extremely important in evaluating causes of iron deficiency anemia. Bioaccessibility is defined as the portion of ingested iron that is available for use in metabolic processes or deposition in storage compounds and is a key concept in iron nutrition (Argyri et al. 2008). Nonheme iron is poorly absorbed compared with heme iron and many nutritional factors are known to influence its absorption positively (ascorbic acid, β-carotene and protein like meat factor) or negatively (phytate, fibers, polyphenols, calcium and zinc). The total iron content of a diet, however provide little information about its content of bioavailable iron, which is considerably influenced by the foods in the diet and can vary 10 fold from different meals of similar iron content (Hallberg and Hulthen 2000). In most parts of the world, including India, wheat is the staple food. Vegetarian diets are mainly plant based. Although a vegetarian diet is likely to contain iron in amount equivalent to amount in a non vegetarian diet, the iron from vegetarian diet is likely to be substantially less available for absorption (FNB 2001) because of difference in the chemical form of iron and the accompanying constituent that enhance or inhibit iron absorption (Hunt 2003).

A survey conducted among rural families of Punjab revealed that the consumption of nutrients like energy, protein, fat, iron, folic acid, and ascorbic acid were adequate. Though the intake of iron was nearly adequate, but the blood hemoglobin level of women indicated that 47 % were anemic with an average hemoglobin level of 10.8 g/dl. The anemia prevalence was high regardless of adequate consumption of iron which is attributed to poor availability of dietary iron from traditional Punjabi diets (Bains et al. 2006). Evaluation studies of India's nationwide and long-standing supplementation program showed irregular supplies, non-compliance by the beneficiaries, poor counseling etc. As such, the supplementation strategy has proved to be inadequate (Vijayaraghvan 2002). Therefore, looking to these attempts, one slant that needs attention in future is the enhancement of bioaccessibility of iron which is highly affected by the inhibitors and enhancers present in Indian traditional meals. Several experimental studies on the availability of food iron and related aspects have shown the possibility of improving the bioaccessibility of iron in plant foods (Allen and Ahluwalia 1997). An in vitro method that stimulates human digestion and absorption of dietary iron from complex meals has been described by Miller et al. (1981). Both enhancing and inhibiting factors of iron absorption have been shown to respond in vitro in the same way as they affect iron absorption in vivo. A significant correlation was found between iron absorption in vivo and the in vitro method of iron availability assessment. Today, solving of this micronutrient malnutrition 'anemia' is an important goal of international community in order to improve nutritional and health status by giving a right direction to their meal pattern. This can be achieved through standardization of meals while observing the multiple factors such as availability, purchasing power, food habits and century old practices which affect the meal pattern. In the study, the in vitro bioaccessibility of iron in context to fortification of key foods such as green leafy vegetables, fruits, meat, poultry, legumes, milk, curd and tea to cereal based diets was studied.

## Materials and Methods

Four individual food items and thirty six composite meals were selected to represent a wide spectrum of meal ingredients. In India, two major cereals that have been consumed as staple are wheat and rice. In Punjab, the staple is wheat, however, considering southern states, the other staple i.e. rice was also been chosen for the analysis. *Saag* prepared from commonly grown green leafy vegetables and *dal* (a legume preparation) was chosen as accomplice of staple in a meal. The four individual foods chosen were:

a) *Chapati* prepared from whole wheat flour
b) Rice prepared from milled rice
c) *Dal* prepared from dehusked and split chickpea.
d) *Saag* prepared from mustard, spinach and fenugreek leaves in the weight ratio of 75:15:10.

All the four food items were cooked in traditional manner using additional ingredients. The cooked weight of individual foods was assessed. The quantity of individual foods taken for a meal meant for adult women was according to a prior survey observations.

The basic meals were then fortified with key food ingredients which may influence in vitro iron bioaccessibility. The key foods were commonly consumed in surveyed households. Eight selected key foods were salad composed of cucumber, onion, tomato and lemon; orange, lemonade, salad, milk (1.5 % fat), curd (1.5 % fat) chicken, egg and tea. Out of eight key foods, lemonade chicken, boiled egg and tea were prepared in the traditional manner as reported by surveyed households. The cooked weights of individual foods, basic meals and basic meals fortified with key foods have been shown in Tables 1, 2, 3, 4 and 5.

Three replications each of 4 individual foods, 4 basic meals and 32 meal combinations were analyzed for total iron, and dialyzable iron. In vitro iron bioaccessibility was calculated for each food/meal.

Iron was estimated by Atomic Absorption Spectrometer after wet digestion. In vitro iron bioaccessibility was assessed by the method given by Miller et al. (1981). The samples were digested in vitro and the proportion of iron which was diffused across semi permeable dialyzable membrane was used as an index of iron bioaccessibility. All the parameters were calculated per mg of iron present in each meal sample. An in vitro method for estimating food iron involves simulated gastrointestinal digestion followed by measurement of soluble, low molecular weight iron. Meals were homogenized and exposed to pepsin at pH 2. Dialysis was used to adjust the pH to intestinal levels and digestion was continued after the addition pancreatin and bile salts. Iron from the digestion mixture which diffused across a 6000 to 8000 molecular weight cutoff

**Table 1** Dialyzable iron, total iron and in vitro iron bioaccessibility of individual foods

| Meal no. | Meal weight, g | Total iron, mg | Dialyzable iron, mg | In vitro bioaccessibility, % |
|---|---|---|---|---|
| *Chapati* | 120 | 1.95 ± 0.01 | 0.10 ± 0.01 | 5.13 ± 0.40 |
| Rice | 200 | 0.36 ± 0.01 | 0.05 ± 0.01 | 13.43 ± 2.04 |
| *Dal* | 200 | 2.62 ± 0.01 | 0.22 ± 0.01 | 8.40 ± 0.29 |
| *Saag* | 160 | 17.62 ± 0.01 | 0.28 ± 0.01 | 1.59 ± 0.05 |
| *P* Value | | ≤0.01 | ≤0.01 | ≤0.01 |
| CD at 5 % | | 0.02 | 0.01 | 8.83 |

Values are Mean ± SD

**Table 2** Total iron, dialyzable iron and in vitro iron bioaccessibility of *dal* and *chapati* as basic meal fortified with key foods

| Meal combination | Meal Weight, g | Total iron, mg | Dialyzable Iron, mg | In vitro bioaccessibility, % |
|---|---|---|---|---|
| Basic meal | | | | |
| Dal + chapati | 200 + 120 | 4.56 ± 0.01 | 0.24 ± 0.01 | 5.20 ± 0.15 |
| Basic meal with key foods | | | | |
| Dal + chapati + salad | 200 + 120 + 135 | 5.60 ± 0.01 | 0.32 ± 0.01 | 5.71 ± 0.09 |
| Dal + chapati + orange | 200 + 120 + 120 | 4.95 ± 0.01 | 0.28 ± 0.01 | 5.73 ± 0.23 |
| Dal + chapati + lemonade | 200 + 120 + 250 | 4.78 ± 0.01 | 0.58 ± 0.01 | 12.16 ± 0.19 |
| Dal + chapati + chicken | 200 + 120 + 200 | 6.45 ± 0.01 | 1.66 ± 0.02 | 25.74 ± 0.28 |
| Dal + chapati + milk | 200 + 120 + 250 | 4.57 ± 0.01 | 0.23 ± 0.02 | 5.00 ± 0.34 |
| Dal + chapati + curd | 200 + 120 + 200 | 4.57 ± 0.01 | 0.23 ± 0.01 | 5.12 ± 0.15 |
| Dal + chapati + egg | 200 + 120 + 50 | 5.02 ± 0.01 | 0.13 ± 0.01 | 2.59 ± 0.14 |
| Dal + chapati + tea | 200 + 120 + 200 | 4.55 ± 0.01 | 0.13 ± 0.01 | 2.88 ± 0.33 |
| *P* value | | ≤0.01 | ≤0.01 | ≤0.01 |
| CD at 5 % | | 0.02 | 0.02 | 0.36 |

semi-permeable dialyzable membrane was used as an indicator of available iron. Iron present in the dialysate at the end of simulated gastrointestinal digestion represented the dialyzable iron fraction. Dialysates were mixed with 5 ml of nitric acid and analyzed the dialyzable iron content at Atomic Absorption Spectrophotometer. Bioavailable iron was calculated as: In vitro iron bioaccessibility (%) = Dialyzable iron/ Total iron x 100.

Mean and standard deviation for various parameters were computed. Analysis of variance was employed to assess the difference of meal combinations and Critical Difference (C.D.) was calculated using Microsoft excel (2007) statistical analysis tool pack where results of analysis of variance were significant.

## Result and Discussion

### Dialyzable iron, total iron and in vitro iron bioaccessibility of individual foods

Two cereal foods namely *chapati* and rice have been consumed as staple foods in Indian diets. These staple foods are most commonly consumed with either a legume or vegetable preparation. Hence, based on the survey observations in the present study, two commonly consumed foods i.e. *dal* (prepared from split and dehusked chickpea) and *saag* (prepared from mustard, spinach and fenugreek leaves) were chosen as accomplice of the two staples.

**Table 3** Total iron, dialyzable iron and in vitro iron bioaccessibility of *dal* and rice as basic meal fortified with key foods

| Meal combination | Meal Weight, g/ml | Total iron, mg | Dialyzable Iron, mg | In vitro bioaccessibility, % |
|---|---|---|---|---|
| Basic meal | | | | |
| Dal + rice | 200 + 200 | 2.99 ± 0.01 | 0.28 ± 0.01 | 9.52 ± 0.16 |
| Basic meal with key foods | | | | |
| Dal + rice + salad | 200 + 200 + 135 | 4.00 ± 0.01 | 0.39 ± 0.01 | 9.75 ± 0.19 |
| Dal + rice + orange | 200 + 200 + 120 | 3.37 ± 0.00 | 0.37 ± 0.01 | 10.11 ± 0.40 |
| Dal + rice + lemonade | 200 + 200 + 250 | 3.18 ± 0.01 | 0.52 ± 0.02 | 16.21 ± 0.67 |
| Dal + rice + chicken | 200 + 200 + 200 | 4.79 ± 0.00 | 1.19 ± 0.01 | 24.84 ± 0.16 |
| Dal + rice + milk | 200 + 200 + 250 | 2.99 ± 0.00 | 0.27 ± 0.02 | 9.02 ± 0.58 |
| Dal + rice + curd | 200 + 200 + 200 | 2.98 ± 0.01 | 0.27 ± 0.01 | 9.02 ± 0.27 |
| Dal + rice + egg | 200 + 200 + 50 | 3.48 ± 0.05 | 0.16 ± 0.01 | 4.59 ± 0.20 |
| Dal + rice + tea | 200 + 200 + 200 | 2.99 ± 0.01 | 0.13 ± 0.01 | 4.26 ± 0.33 |
| *P* value | | ≤0.01 | ≤0.01 | ≤0.01 |
| CD at 5 % | | 0.03 | 0.02 | 0.60 |

**Table 4** Total iron, dialyzable iron and in vitro iron bioaccessibility of saag and chapati as basic meal fortified with key foods

| Meal combination | MealWeight, g | Total iron, mg | Dialyzable Iron, mg | In vitro bioaccessibility, % |
|---|---|---|---|---|
| Basic meal | | | | |
| Saag + chapatti | 160 + 120 | 19.56 ± 0.01 | 0.29 ± 0.01 | 1.49 ± 0.04 |
| Basic meal with key foods | | | | |
| Saag + chapati + salad | 160 + 120 + 135 | 20.77 ± 0.01 | 0.40 ± 0.01 | 1.92 ± 0.04 |
| Saag + chapati + orange | 160 + 120 + 120 | 19.94 ± 0.00 | 0.33 ± 0.01 | 1.65 ± 0.04 |
| Saag + chapati + lemonade | 160 + 120 + 250 | 19.76 ± 0.01 | 0.67 ± 0.01 | 3.37 ± 0.05 |
| Saag + chapati + chicken | 160 + 120 + 200 | 20.76 ± 0.00 | 1.87 ± 0.01 | 8.99 ± 0.05 |
| Saag + chapati + milk | 160 + 120 + 250 | 19.56 ± 0.01 | 0.27 ± 0.01 | 1.38 ± 0.03 |
| Saag + chapati + curd | 160 + 120 + 200 | 19.57 ± 0.01 | 0.29 ± 0.01 | 1.48 ± 0.04 |
| Saag + chapati + egg | 160 + 120 + 50 | 20.02 ± 0.01 | 0.25 ± 0.01 | 1.25 ± 0.05 |
| Saag + chapati + tea | 160 + 120 + 200 | 19.56 ± 0.01 | 0.23 ± 0.01 | 1.17 ± 0.04 |
| *P* value | | ≤0.01 | ≤0.01 | ≤0.01 |
| CD at 5 % | | 0.05 | 0.02 | 0.07 |

The total iron, dialyzable iron and in vitro iron bioaccessibility of the individual foods have been shown in Table 1. The total iron was highest in *saag* as expected, the value being 17.62 mg in a serving of 160 g of *saag*. Gopalan et al. (2004) reported the iron content of some common green leafy vegetables, the values for mustard leaves, fenugreek leaves and spinach being 16.3, 4.2 and 1.14 mg per 100 g of fresh vegetable. In comparison to *saag*, *dal* had lower iron content i.e. 2.62 mg in a serving of 200 g.

The two cereals foods namely *chapati* and boiled rice had quite a low content iron when compared to *saag* and *dal*. A significantly ($p \leq 0.01$) higher content of total iron was observed in *chapati* (1.95 mg per serving of 120 g) as compared to boiled rice (0.36 mg per serving of 200 g). As per the survey results, it was observed that the studied population used whole wheat flour for preparing *chapaties* while polished rice was used for preparing boiled rice. The lower iron content of boiled rice was because polished rice was used in the present study. A significant amount of iron is lost during milling as bran of all the cereals and especially rice is rich in iron and other minerals. On the other hand, *chapaties* were prepared from whole wheat flour which included bran too.

The dialyzable iron of an individual serving of *chapati*, rice, *dal* and *saag* was 0.10, 0.05, 0.22 and 0.28 mg, respectively. The results highlighted that though *saag* had total iron content 6.7 times higher than *dal* but dialyzable iron was only 1.3 times higher. This shows that though the iron in *saag* is not highly bioavailable, still dialyzable iron is higher to that present in *dal*. Similarly, *chapaties* had 5.3 times higher iron content but dialyzable content was two times higher. The

**Table 5** Total iron, dialyzable iron and in vitro iron bioaccessibility of saag and chapati as basic meal fortified with key foods

| Meal combination | Meal weight, g | Total iron, mg | Dialyzable Iron, mg | In vitro bioaccessibility, % |
|---|---|---|---|---|
| Basic meal | | | | |
| Saag + rice | 160 + 200 | 17.98 ± 0.01 | 0.38 ± 0.01 | 2.13 ± 0.03 |
| Basic meal with key foods | | | | |
| Saag + rice + salad | 160 + 200 + 135 | 19.20 ± 0.08 | 0.43 ± 0.01 | 2.24 ± 0.05 |
| Saag + rice + orange | 160 + 200 + 120 | 18.37 ± 0.01 | 0.41 ± 0.01 | 2.23 ± 0.03 |
| Saag + rice + lemonade | 160 + 200 + 250 | 18.08 ± 0.00 | 0.62 ± 0.01 | 3.43 ± 0.06 |
| Saag + rice + chicken | 160 + 200 + 200 | 19.19 ± 0.01 | 1.88 ± 0.01 | 9.80 ± 0.04 |
| Saag + rice + milk | 160 + 200 + 250 | 17.98 ± 0.00 | 0.34 ± 0.01 | 1.87 ± 0.06 |
| Saag + rice + curd | 160 + 200 + 200 | 17.98 ± 0.01 | 0.36 ± 0.01 | 2.01 ± 0.05 |
| Saag + rice + egg | 160 + 200 + 50 | 18.56 ± 0.01 | 0.23 ± 0.01 | 1.24 ± 0.06 |
| Saag + rice + tea | 160 + 200 + 200 | 17.98 ± 0.01 | 0.21 ± 0.01 | 1.15 ± 0.06 |
| *P* value | | ≤0.01 | ≤0.01 | ≤0.01 |
| CD at 5 % | | 0.05 | 0.02 | 0.08 |

results highlighted that *saag* and *chapati* had lesser bioavailable iron, still they might be better contributors of dialyzable iron to the meals as compared to *dal* and rice because they had significantly higher total iron. D'Souza *et al*. (1987) reported higher intakes of dietary fiber and phytate with mineral binding characteristics among the West Indian and Asian children attributed to frequent consumption of pulses and unleavened *chapaties* and rare consumption of red meats due to which iron status was compromised.

The bioaccessibility of individual foods calculated from total and dialyzable iron showed that out of individual foods analyzed, maximum bioaccessibility was from boiled rice (13.43 %) followed by *dal* (8.40 %) and *chapati* (5.13 %). Extremely low bioaccessibility of iron was found in *saag* (1.59 %). The low bioaccessibility of iron in *saag* was due to presence of large amounts of inhibitory factors such as oxalates and dietary fiber in green leafy vegetables. Spinach contains iron absorption-inhibiting substances, including high levels of oxalate, which can bind to the iron to form ferrous oxalate and render much of the iron in spinach unusable by the body as reported by Noonan (1999)).

## Dialyzable iron, total iron and in vitro iron bioaccessibility of basic meals fortified with key foods

Four basic meal combinations i.e. *dal* with *chapati*, *dal* with rice, *saag* with *chapati* and *saag* with rice were analyzed for total iron and dialyzable iron. The iron bioaccessibility was then calculated from total and dialyzable iron. The results have been shown in Tables 2, 3, 4 and 5.

Basic meal of *dal* and *chapati* was fortified with 8 key foods reported to improve or depress iron absorption. These foods were part of the regular diets of the surveyed group. The key foods were salad, orange, lemonade, chicken, milk, curd, egg and tea. The results revealed that the total iron content of basic meal was 5.20 mg. A significant ($p \leq 0.01$) difference was observed in total iron content among meal combinations. The total iron in meals with chicken > salad > egg > orange > lemonade. No significant difference was observed in basic meal and the basic meal fortified with milk, curd or tea.

The dialyzable iron was highest in the meal fortified with chicken (1.66 mg), the reason being the presence of heme iron with low molecular weight. Addition of lemonade to basic meal increased the dialyzable iron by 2.4 folds, whereas addition of chicken increased dialyzable iron by 6.9 folds. A small but significant ($p \leq 0.01$) increase in dialyzable content was also observed when salad and orange were added to the basic meal. The dialyzable iron with chicken > lemonade > salad > orange. No significant difference was found in dialyzable iron content of basic meal when it was fortified with either milk or curd. A significant ($p \leq 0.01$) reduction in dialyzable iron was observed when egg and tea was included in the basic meal. The findings revealed that egg and tea reduced dialyzable iron while addition of salad, orange, lemonade and chicken had positive effect on dialyzable iron when fortified to basic meal of *dal* and *chapati*.

The addition of chicken to the basic meal showed marked increase in iron absorption (25.74 %). Next to the flesh food, it was lemonade, which showed positive effect on iron absorption, the value of iron bioaccessibility being 12.16 %. A significant ($p \leq 0.01$) increase was observed when orange was included in the main meal. A non significant reduction was observed in the in vitro iron bioavailablity of meals fortified with either milk or curd when compared to the basic meal, however, the bioaccessibility decreased significantly ($p \leq 0.01$) when egg and tea were included in the meal, the values of bioaccessibility iron being 2.59 and 2.88 %, respectively. Egg contains phosvitin a protein compound that binds iron molecules together and prevents the body from absorption iron from foods. One boiled egg can reduce iron absorption by as much as 28 % as reported by Weinberg (1978).

The findings revealed that egg and tea were the major inhibitors of iron absorption as they reduced the iron bioaccessibility by 50.2 and 44.4 % while chicken and lemonade were the noteworthy promoters of iron absorption as they increased the iron absorption by 4.95 and 2.34 folds. Cook and Monsen (1977)) reported an addition of animal tissue to semi-synthetic meal resulted in a fold to 4 fold increase in iron absorption. Bach (2005) observed that inclusion of meat to a vegetarian diet increased iron absorption by 50 %.

The addition of citrus fruit can increase iron availability markedly as reported by Seshadri (1993). García et al. (1998)) reported that the addition of 25 mg of ascorbic acid as lemonade consumed at two meals and improved the iron status of women.

*Dal* and rice was also analyzed as basic meal. Though this meal combination is not prevalent in Punjab state, but in other parts of the country, this meal combination is found in the regular diets. The basic meal of *dal* and rice was fortified with all the 8 key foods which were earlier fortified in *dal* and *chapati* meal. The total iron content of basic meal was 2.99 mg. A significant ($p \leq 0.01$) increase in total iron content was found when this meal was fortified with salad (4.00 mg), orange (3.37 mg), lemonade (3.18 mg), chicken (4.79 mg) and egg (3.48 mg), however no significant difference was found between basic meals when it was fortified with either milk, curd or tea.

Dialyzable iron was found to be maximum when chicken was added to basic meal (1.19 mg) followed by lemonade (0.52 mg), the dialyzable iron was increased by 4.25 and 1.86 times when chicken and lemonade was added to the basic meals, respectively. A significant ($p \leq 0.01$) increase was also observed when salad (0.39 mg) and orange (0.37 mg) were added to the basic meal. Contrary to this, milk, curd, egg and

tea added to the basic meal reduced the dialyzable iron significantly ($p \leq 0.01$), the range of dialyzable iron in the meals with above mentioned key foods being 0.13 to 0.18 mg, the lowest being for the meals with tea.

The in vitro bioaccessibility of iron for the basic meal of *dal* and rice was 9.52 % and was 1.83 times higher than *dal* and *chapati* meal which was attributed to significantly ($p \leq 0.01$) higher bioavailable iron in rice as compared to *chapati*. The in vitro bioaccessibility of iron was highest for the basic meal of *dal* and rice in combination with chicken (24.84 mg) followed by lemonade (16.21 %). Salad and orange increased in vitro iron bioaccessibility, however, it was non-significant as compared to the basic meal. Inclusion of egg and tea to the basic meal resulted in a significant ($p \leq 0.01$) reduction in iron bioaccessibility, the percent reduction being 4.59 to 4.26 %, respectively.

The findings highlighted that addition of flesh food to the basic meal is the most effective way to enhance iron bioaccessibility as addition of chicken enhanced iron bioaccessibility by 2.61 folds. Inclusion of lemonade is the second best way to improve iron absorption as it increased iron bioaccessibility by 70.27 %. Other useful means are the inclusion of a either citrus fruits or use of ascorbic acid rich vegetables like lemon and tomatoes as part of green salad as done in the present study. Lycopene, lutine and zeaxanthene pigments present in green vegetables increased iron absorption in human beings from corn and wheat meals as reported by Garcia-Casal (2006).

Though inclusion of animal tissue is the most effective but keeping in view that majority of Indian population is vegetarian due to cultural cum religion reasons or due to unaffordability of animal foods, the best option for them is increasing the ascorbic acid level of their meals through ascorbic acid rich fruits and vegetables. Milk and curd reduced the iron bioaccessibility of basic meal by 36.8 %. The maximum inhibitory effect was however observed for tea as it reduced bioaccessibility by 55.3 % while egg reduced it by 51.8 %. Hallberg (1998) reported that about 30 to 50 % more iron was absorbed when no milk or cheese was served with the main meal, which provided most of the dietary iron.

*Saag* is a common leafy vegetable preparation which is most frequently consumed in Punjab and in other states of India as well. *Saag* can be prepared from many kinds of leafy vegetables e.g. in winters, the mustard, fenugreek and spinach leaves are being used to prepare *saag*. In Punjab, summers are the lean period, when leafy vegetables are not much available. Total iron of *saag* and *chapati* meal was 19.56 mg which was significantly ($p \leq 0.01$) higher than the meal of *dal* and *chapati*. A significantly ($p \leq 0.01$) higher iron was found in meal fortified with salad, orange, lemonade, chicken and egg when compared to the basic meal.

The dialyzable iron was 0.29 mg in *saag* and *chapati* meal while it was 0.24 mg for *dal* and *chapati* meal. The results highlighted a wide difference in total iron while small difference in dialyzable iron in the two meals indicating that though *saag* is very high in total iron but dialyzable iron values were closer but still higher than *dal*. Jonnalagadda and Seshadri (1994)) reported that the addition of 100 g fenugreek leaves to a meal increased the total iron content of meal significantly, however the available iron was observed to decrease at a significant level The maximum dialyzable iron was in the meal fortified with chicken (1.87 mg) followed by with lemonade (0.67 mg), salad (0.40 mg) and orange (0.33). A significant ($p \leq 0.01$) reduction in dialyzable iron was observed when egg and tea were including in the basic meal, the values for dialyzable iron being 0.25 and 0.23 mg, respectively.

All the meal combinations of *saag* and *chapati* showed significantly ($p \leq 0.01$) poor iron bioaccessibility as compared to meal combination of *dal* and *chapati*. Excluding the *saag* and *chapati* in combination with chicken, other combination had poor iron bioaccessibility, the range being 1.17 to 3.37 mg. Out of all meal combinations, the meals with chicken, lemonade, salad and orange enhanced in vitro iron bioaccessibility significantly ($p \leq 0.01$), the values for bioaccessibility being 8.99, 3.37, 1.92 and 1.65 %, respectively. Inclusion of chicken and lemonade to the basic meal increased bioaccessibility by 6.0 and 2.3 folds, respectively. On the other hand, two key foods namely egg and tea reduced bioaccessibility of the basic meal by 16.1 and 21.5 %, respectively.

The meals where rice is the part of basic meal had significantly ($p \leq 0.01$) higher iron bioaccessibility as compared to the basic meals comprising of *chapati* as one of the two foods, however the total iron was less attributing to low total iron content but dialyzable iron was higher in saag and rice meal as compared to *saag* and *chapati* meal. A significantly ($p \leq 0.01$) higher total iron was found in the meals fortified with salad, orange, lemonade, chicken and egg when compared to the basic meal. The maximum dialyzable iron was found in the basic meal supplemented with chicken (1.88 mg) followed by lemonade (0.62 mg) and orange (0.41 mg). The dialyzable iron was significantly ($p \leq 0.01$) lower in the meals fortified with milk (0.34 mg) and curd (0.36) but a sharp reduction in dialyzable iron was observed when egg or tea was included in the basic meal, the values for dialyzable iron were 0.23 and 0.21 mg, respectively.

A significant ($p \leq 0.01$) improvement in iron bioaccessibility was observed in *saag* and rice meal when salad, orange, lemonade, chicken and curd were included, the values for iron bioaccessibility being 2.24, 2.23, 3.43, 9.80 and 2.27 %, respectively. Milk, egg and curd reduced in vitro iron bioaccessibility significantly ($p \leq 0.01$), however, the maximum reduction was observed when tea was supplemented in basic meal (1.24 %) closely followed by egg (1.24 %).

Based on the experiments carried out by inclusion of selected eight key foods namely chicken, salad, orange, lemonade, milk, curd, egg and tea to the basic meals, the meals of people vulnerable to iron deficiency can be optimized. The findings revealed that non vegetarians can enhance iron bioaccessibility by 1.6 fold to 5.0 fold by inclusion of 200 g of chicken to then basic meals of rice or *chapati* with either *dal* or *saag*. However, vegetarian women can improve iron bioaccessibility by 5.2 to 28.9 % with the inclusion of 135 g salad and by 4.7 to 10.7 % with the inclusion of 120 g of orange. The best enhancer of iron absorption for vegetarians is lemonade (250 ml) which resulted in 70.2 and 61.0 % increase in in vitro bioaccessibility of iron to the rice based meals with *dal* and *saag*, respectively. However, in *chapati* based meals, the inclusion of lemonade resulted in 1.3 fold increase in iron bioaccessibility. The major inhibitors of iron bioaccessibility were egg and tea, the percent reduction caused by egg being 16.1 to 50.2 % while by tea, it was between 21.5 to 55.3 %, therefore, consumption of these must be avoided with the meals.

## Conclusion

The results highlighted that *saag* and *chapati* had lesser bioavailable iron, still they might be better contributors of dialyzable iron to the meals as compared to *dal* and rice because they had significantly higher total iron. The bioaccessibility of individual foods calculated from total and dialyzable iron showed that out of individual foods analyzed, maximum bioaccessibility was from boiled rice followed by *dal* and *chapati*. Extremely low bioaccessibility of iron was found in *saag*.

Inclusion of 200 g of chicken, 135 g of salad and 120 g of orange to the basic meals of rice or *chapati* with either *dal* or *saag* enhanced iron bioaccessibility by 1.6 fold to 5.0 fold; 5.2 to 28.9 % and 4.7 to 10.7 %, respectively. The major inhibitors of iron bioaccessibility were egg and tea, the percent reduction caused by egg being 16.1 to 50.2 % while by tea; it was between 21.5 to 55.3 %. The inclusion of animal tissue is the most effective but keeping in view that majority of Indian population is vegetarian due to cultural cum religion reasons or due to un affordability of animal foods, the best option for them is increasing the ascorbic acid level of their meals through ascorbic acid rich fruits and vegetables. Non vegetarians can enhance iron absorption by including chicken or other flesh foods to their meals for enhanced iron bioaccessibility. The strong inhibitors of iron bioaccessibility namely egg and tea should be restricted in meals.

## References


Allen L H and Ahluwalia N (1997) Improving iron status through diet. The application of knowledge concerning dietary iron bioaccessibility in human populations. OMNI - Opportunities for Micronutrient Interventions. Washington, DC, John Snow Inc. /OMNI Project, United States Agency for International Development (USAID)

Argyri K, Birba A, Miller DD, Komaitis M, Kapsokefalou (2008) Predicting relative concentrations of bioavailable iron in foods using in vitro digestion: New development. J Food Chemistry 113:602–607

Bains K, Aggarwal R and Barakoti L (2006) Development and impact of iron-rich mungbean recipes. Proceedings of Final Workshop of project 'Improving income and nutrition by incorporating mungbean in cereal fallows in the Indo-Gangetic plains of South Asia'. AVRDC- World Vegetable Center Publication, Shanhua, Taiwan

Cook JD, Monsen ER (1977) Vitamin C, the common cold and iron absorption. Am J Clin Nutr 30:235–241

D'Souza SW, Lakhani P, Waters HM, Boardman KM, Cinkotai KI (1987) Iron deficiency in ethnic minorities: association with dietary fibre and phytate. Early Hum Dev 15:103–111

FNB (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press, Food and Nutrition Board, Institute of Medicine, Washington, DC

García O P, Díaz M, Rosado J L and Allen L H (1998) Community trial of the efficacy of lime juice for improving iron status of iron deficient Mexican women. FASEN J 12(5) A 647 Abst 3762. Cited in L.H. Allen & N. Ahluwalia, *Improving iron status through diet. The application of knowledge concerning dietary iron bioaccessibility in human populations.* OMNI Opportunities for Micronutrient Interventions. Washington, DC, John Snow Inc./OMNI Project, USAID.

Garcia-Casal MN (2006) Carotenoids increase iron absorption from cereal based food in the humans. Nutr Res 26:340–344

Gopalan C, Ramasastri BV, Balasubra-manium (2004) Nutritive value of Indian Foods. National Institute of Nutrition, ICMR, Hyderabad

Hallberg L (1998) Does calcium interferes with iron absorption? Am J Clin Nutr 68:96–102

Hallberg L, Hulthen L (2000) Prediction of dietary iron absorption: an algorithm for calculating absorption and bioaccessibility of dietary iron. Am J Clin Nutr 71:1147–1160

Hunt RJ (2003) Bioaccessibility of iron, zinc and other trace minerals from vegetarian diet. Am J Clin Nutr 78:633–639

Jonnalagadda SS, Seshadri S (1994) In vitro availability of iron from cereal meal with the addition of protein isolates and fenugreek leaves (*Trigonella foenum-graecum*). J Food Hum Nutr 45:119–125

Miller D, Schricker BR, Rasmussen RR, Campen DV (1981) An in vitro method for estimation of iron availability from meals. Am J Clin Nutr 34:2248–2256

Noonan S (1999) C and Savage G P oxalate content of foods and its effect on humans. Asia Pacific Journal Clinical Nutrition 1:64–74

Seshadri S (1993) Bioaccessibility of microelements with special reference to iron: advantages and limitations of the in vitro method. Proc Nutr Soc India 40:103–112

Vijayaraghvan K (2002) Control of micronutrient deficiencies in India: Obstacles and Strategies. Nutr Rev 60:73–76

Weinberg ED (1978) Iron and infection. Microbiol Rev 42:45–66

WHO (2004) Micronutrient deficiency anaemia: the challenge. Available from: http://www.who.int/nut/ ida.htm, accessed on April 24, 2010