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# Effects of vegetables on iron and zinc availability in cereals and legumes

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**Abstract:** Despite large scale intervention programmes, iron deficiency anemia and zinc deficiency remains the most widely prevalent nutritional problem in the world. Widespread deficiencies of iron and zinc, commonly found in developing country such as China dependent on plant foods, necessitate food-based strategies to maximise their bioavailability from plant foods. In this paper, β-carotene-rich vegetables were evaluated for their effects on the bioavailability of iron and zinc from cereals and legumes by employing *in vitro* digestions. Addition of carrot or spinach (2.5 g and 5 g per 10 g of grain) significantly enhanced the bioavailability of iron and zinc from the food grains, the percent increase being 9.4–73.8 in the case of carrot and 11– 179% in the case of spinach. Pure β-carotene added at an equivalent level also enhanced the bioavailability of iron (9.6–97.6% increase) and zinc (13–92.4% increase) from the cereals examined.

**Keywords: : Availability, iron, zinc, β-carotene, cereals, legumes**

## Introduction

Iron and zinc are currently receiving global attention since these minerals have become a matter of great interest, concerning the nutritional value of vegetarian diets. In China, vegetarian foods provide at least 50% of the dietary energy and nutrients. With low intake of meat and increased intake of phytate-containing legumes and cereals, absorption of both iron and zinc is lower in vegetarians than in non-vegetarians (Hunt, 2003). Cereals and legumes often have high contents of inhibitors of mineral absorption, including phytates and polyphenols, which inhibit zinc and/or iron absorption by forming insoluble complexes in the intestine (Luo *et al*., 2009; Luo *et al*., 2010b). Consequently, the availability of these micronutrients from diets based on cereals and legumes is often poor (Gibson, 1994). The availability of iron and zinc from foods is defined as the proportion of the iron and zinc that can be absorbed and utilised within the body. Solubility of iron and zinc, pH of intestinal lumen, dietary factors and retention time at the digestion and absorption site influence the availability of iron and zinc. Solubility of iron and zinc could be predicted by molar ratios of phytic acid to iron, HCl (hydrochloric acid)-extractability and *in vitro* solubility of iron and zinc. These micronutrient deficiencies are major public health problems in developing countries, including China (Kong, 1999), affecting growth, development and cognitive performance of children. Requirement of these micronutrients for vegetarian populations have to be met entirely through plant foods such as cereals, legumes and green leafy vegetables in view of the limited consumption of animal foods, which are good sources of readily available iron, zinc and preformed vitamin A.

Ascorbic acid and other organic acids are well known promoters of iron absorption from the diet (Hallberg *et al*., 1986; Hallberg *et al*., 1989). It has been reported that vitamin A and β-carotene can enhance iron absorption and thereby contribute to an increase in haemoglobin levels (Gracia-Casal *et al*., 1998). Information on such promoters of zinc availability from plant foods is lacking. In view of β-carotene- rich green leafy and yellow orange vegetables being common ingredients in our diet, the possible influence of β-carotene on zinc availability deserves to be evaluated. In this connection, β-carotene-rich sources such as carrot or spinach were evaluated for a possible effect on the availability of iron and zinc from food grains in this study. Curcumin, the principle phytochemical of turmeric, which is structurally similar to β-carotene, was also evaluated for its influence on iron and zinc availability from food grains.

## Materials and Methods

### *Materials*

Two cereals-wheat (*Triticum aestivum*) and oat (*Arrhenatherum elatius*)-and two legumes -red bean (*Vigna angularis*) and faba bean (*Vicia faba*) –were collected from local market of the same batch

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in Nanjing, Jiangsu Province, P.R. China. Pepsin (porcine), pancreatin (porcine), bile salt, iron and zinc standards, β-carotene, and curcumin were obtained from Sigma Chemical Co. (St. Louis, USA). All other chemicals used were of analytical grade.

*Total iron and zinc*

Iron and zinc in grain samples were analysed by atomic absorption spectrophotometry (Varian SpectrAA 200, Victoria, Australia) after dry ashing for 2 h at 530$^{o}$C. Depending on the different treatments, 2-4 g of ash was weighed in a silicon evaporating dish. Next, the ashes were wet-acid digested with nitric acid on a hot plate and solubilized with 25 ml of 0.5 N HCl.

*Iron and zinc availability*

Iron and zinc availabilities were determined using 10 g of the grain sample in the absence or presence of carrot, spinach, pure β-carotene (acetonic solution) and curcumin. Thus, various food samples evaluated for iron and zinc availability were: (1) food grain alone (10 g), (2) food grain (10 g) + carrot (2.5 g), (3) food grain (10 g) + carrot (5.0 g), (4) food grain (10 g) + spinach (2.5 g), (5) food grain (10 g) + spinach (5.0 g), (6) food grain (10 g) + 20 µg pure β-carotene, (7) food grain (10 g) + 400µg pure β-carotene, and (8) food grain (10 g) + curcumin (0.05 g).

Iron and zinc availability was defined as the relative amount of iron and zinc that becomes soluble after enzymatic treatment. Grain samples were sequentially digested with enzymes, including amylase, pepsin, pancreatin and bile, under certain conditions following the enzymatic degradation procedure described by Kiers *et al*.(2000) . Mixtures were centrifuged at 5000 g for 15 min at 4°C. The resulting supernatant was filtered (0.45 µm membrane, FP 030/3, Kaijie, Hangzhou, Zhejiang) and frozen until further analysis. Iron and zinc levels, including soluble free ionizable iron and zinc and soluble complexes of iron and zinc, were analysed by atomic absorption spectrophotometry. Each sample was enzymatically extracted in duplicate. Iron and zinc availability contents were determinated on three independent digests.

*Heat processing of food samples*

All food samples were pressure-cooked in 30-35 ml of triple distiled water for 15-20 min. The cooked samples were homogenised in a stainless steel mixer and used for the determination of mineral availability as described above.

*Statistical analysis*

Data were analysed with SPSS 15.0 for windows. The mean and standard deviation of means were calculated. The data were analysed by one-way analysis of variance (ANOVA). Duncan's multiple range test was used to separate means. Significance was accepted at probability $P < 0.05$.

## Results

*Influence of carrot on iron and zinc bioavailabilities from cereals and legumes*

Carrot and spinach were chosen as sources of β-carotene in this study to examine the influence of β-carotene, if any, on the availability of iron and zinc from food grains. The levels used here, i.e. 2.5 and 5.0 g rough provide 200 and 400 µg of β-carotene, respectively. Carrot had a significant positive effect on the availability of iron from both the tested cereals and legumes (Table 1). This positive effect was evidenced in both raw and cooked grains. The increases in availability of iron were 32.5% and 32.3% in the case of raw wheat while, in the case of cooked wheat, the percent increases were 45.6% and 45.4%, respectively, at 2.5 and 5 g of carrot per 10 g of the grain. In the case of raw oat, percent increases in bioaccessibile iron were 64.9% and 73.8%, while the same in cooked oat were 30.6% and 60.8% with the two levels of carrot. Carrot enhanced the availability of iron from raw red bean by 22.6% and 19.2% at 2.5 and 5 g, respectively. In the case of cooked red bean, the percent increases were 45.3% and 43.9%. Carrot had a similar influence on the availability of iron from faba bean in both raw and cooked form, the percent increases being 34.8 and 32.4 in raw, and 40.4% and 36.5% in the cooked grain, at the two levels of carrot. Heat treatment increased the availability of iron from food grains, and this positive effect of heat treatment was further potentiated by the presence of carrot. The higher level of carrot examined in this study generally did not produce an effect greater than the lower level.

Similar to the effect of iron, carrot generally had a significant positive effect on the availability of zinc from all the food grains examined, but of a lesser magnitude (Table 1). In the case of wheat, this effect was evident only in the cooked grain, the percent increases in zinc availability being 26.7% and 25.1% at the two levels of carrot examined. Zinc availability was enhanced by 78.9% and 73.2% in raw and 25.8% and 23.8% in cooked oat, respectively. In red bean, zinc availability increased by 16.5% and 15.3% in raw whilst in cooked red bean, the percent increases were 29.3% and 27.2%. Carrot also increased zinc

**Table 1.** Effect of carrot on the bioavailability of iron and zinc from cereals and legumes

| | Iron bioavailability (%) | | | | Zinc bioavailability (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | **Wheat** | **Oat** | **Red bean** | **Faba bean** | **Wheat** | **Oat** | **Red bean** | **Faba bean** |
| Raw grain | 8.56±0.34 | 7.36±0.55 | 3.85±0.12 | 10.89±0.54 | 27.90±0.98 | 8.23±0.39 | 34.12±0.96 | 42.3±2.01 |
| Raw grain + 2.5 g carrot | 11.34±0.56* | 12.58±0.86* | 4.72±0.23* | 14.68±0.89* | 27.21±1.02 | 14.72±0.65* | 39.60±1.06* | 48.4±2.23* |
| Raw grain + 5.0 g carrot | 11.58±0.87* | 13.26±1.02* | 4.59±0.34* | 14.42±0.95* | 28.31±1.35 | 14.26±0.58* | 39.21±1.12* | 50.2±2.54* |
| Cooked grain | 12.61±0.26 | 12.54±1.05 | 3.62±0.35 | 13.00±1.02 | 13.20±0.56 | 6.23±0.35 | 24.21±0.68 | 34.8±1.52 |
| Cooked grain + 2.5 g carrot | 18.36±0.68* | 16.38±1.21* | 5.26±0.45* | 18.25±1.56* | 16.72±0.68* | 7.84±0.41* | 31.30±0.69* | 34.2±1.35 |
| Cooked grain + 5.0 g carrot | 18.34±0.74* | 20.16±1.32* | 5.21±0.51* | 17.74±1.68* | 16.52±0.87* | 7.71±.043* | 30.81±0.74* | 38.1±1.38* |

Values are mean ± standard deviation of three determinations.
*: Significant increase compared to the corresponding grain alone.

availability from faba bean, the percent increase being 14.4% and 18.7% at 2.5 and 5 g, respectively, in the raw grain, whilst this positive effect was evident in the cooked grain only at the higher level of carrot (9.4% increase). Cooking itself reduced the availability of zinc from wheat, oat and red bean, and this negative effect of cooking was partially countered by the addition of the β-carotene provider, namely, carrot.

*Influence of spinach on iron and zinc bioavailabilities from cereals and legumes*

Spinach, another rich source of β-carotene, representing green leafy vegetables, was also examined here for its influence on the bioavailabilities of iron and zinc from wheat, oat, red bean and faba bean (Table 2). Similar to carrot, spinach also had a significant positive effect on the availability of iron from all the food grains evaluated in both raw and cooked conditions. Iron availability was increased by about 23% at both the levels of spinach tested, from raw wheat whilst, in cooked wheat, the percent increases were 10% and 13.5% at 2.5 and 5 g of spinach, respectively. The positive effect of spinach was enormous in the case of iron availability from raw oat, the percent increases being 92.6% and 179%, at 2.5 and 5 g, respectively. In the case of cooked oat, the increases in availability of iron were 32.1% and 32.2%, at the two levels, respectively. Spinach increased the availability of iron from raw red bean by 16.2% and 12.1% at the two levels, respectively whilst, in cooked red bean, the percent increases were 30.1% and 62.5% at 2.5 and 5 g, respectively. Iron bioavailabilities were enhanced by 42.6% and 30.1% in raw faba bean whilst the same in cooked faba bean were 6.3% and 31.3% at the two levels of spinach examined. The magnitude of positive effect of amaranth on iron availability was greater at the higher level of this green leafy vegetable tested.

Spinach had a significant positive effect on the availability of zinc from all the food grains examined (Table 2). Spinach increased zinc bioavailabilities from raw wheat by 26.3% and 24.8% at the two levels, respectively, whilst this effect on cooked wheat was evident only with the higher level and the increase was as high as 66%. The positive effect of spinach on the availability of zinc was also enormous in the case of both raw and cooked oat, and the percent increase ranged from 68% to 99%. In the case of red bean, the positive effect of spinach on zinc spinach was evident only at the higher level, the percent increase in the same being 31.2% and 13.1% from raw and cooked red bean, respectively. In the case of faba bean, the positive effect of spinach was limited to only the cooked grain (8.7% and 11.9% increases at the two levels of spinach). The higher level of spinach (5 g) produced a greater enhancing effect on zinc availability from cooked wheat and raw red bean.

*Influence of pure β-carotene on iron and zinc bioavailabilities from cereals and legumes*

To confirm that the positive effect of carrot and spinach on the availability of minerals is attributable to the β-carotene present in them, the effects of pure β-carotene, included at 200 and 400 µg/10 g of the grain, on the bioavailabilities of iron and zinc from the same food grains was also examined here (Table 3). β-carotene had a significant positive effect on iron availability from wheat, oat, and cooked red bean. In the case of raw and cooked wheat and raw oat, β-carotene, added at the 200 µg level, produced increases of 20.4% (raw wheat), 35.7% (cooked wheat) and 63.9% (raw oat). This positive effect of pure β-carotene was greater at the higher level (400 µg), the increase in iron availability being as high as 97.6% in raw oat and 9.6% in cooked oat. As in the case of carrot and spinach, the magnitude of the positive effect of β-carotene was greatest in the case of oat.

Pure β-carotene had a positive effect on the availability of zinc only from wheat and oat (Table 3). Zinc availability from raw wheat was enhanced by 13% and 14.5%, respectively, at 200 and 400 µg levels whilst, in cooked wheat, this effect was more pronounced, the percent increase being 63.1 and 92.4, at the two levels of β-carotene. In the case of oat, 400 µg of β-carotene produced 16.1% and 12.3% increases in the raw and cooked grains, respectively.

*Influence of curcumin on iron and zinc bioavailabilities from cereals and legumes*

In view of the desirable effects observed with β-carotene and its vegetable sources, another

**Table 2.** Effect of spinach on the bioavailability of iron and zinc from cereals and legumes

| | Iron bioavailability (%) | | | | Zinc bioavailability (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Wheat | Oat | Red bean | Faba bean | Wheat | Oat | Red bean | Faba bean |
| Raw grain | 8.92±0.45 | 8.68±0.56 | 3.46±0.23 | 8.67±0.56 | 24.72±0.68 | 9.43±0.35 | 32.10±0.68 | 42.3±1.34 |
| Raw grain + 2.5 g spinach | 10.97±0.56* | 16.72±0.85* | 4.02±0.35* | 12.36±0.67* | 31.23±0.98* | 16.12±0.57* | 33.70±0.97 | 42.9±1.21 |
| Raw grain + 5.0 g spinach | 10.98±0.57* | 24.23±1.23* | 3.88±0.27* | 11.28±0.74* | 30.86±1.02* | 15.85±0.69* | 42.1±1.05* | 42.7±1.13 |
| Cooked grain | 12.14±0.64 | 15.38±1.21 | 2.72±0.14 | 10.46±0.65 | 10.62±0.23 | 7.52±0.55 | 21.3±0.65 | 34.5±0.96 |
| Cooked grain + 2.5 g spinach | 13.36±0.61* | 20.32±1.35* | 3.54±0.32* | 11.12±0.52* | 10.89±0.34 | 14.6±0.21* | 22.7±0.34 | 37.5±0.85 |
| Cooked grain + 5.0 g spinach | 13.78±0.65* | 20.34±1.11* | 4.42±0.25* | 13.74±0.87* | 17.62±0.68* | 14.96±0.25* | 24.1±0.54* | 38.6±0.74* |

Values are mean ± standard deviation of three determinations.
*: Significant increase compared to the corresponding grain alone.

**Table 3.** Effect of pure β-carotene on the bioavailabilities of iron and zinc from food grains

| Food grain | Wheat | Oat | Red bean | Faba bean |
|---|---|---|---|---|
| **Iron bioavailability %** | | | | |
| Raw grain | 9.72±0.71 | 7.65±0.57 | 3.54±0.21 | 10.34±0.65 |
| Raw grain + 200 µg β-carotene | 11.71±0.85* | 12.54±0.85* | 3.12±0.24 | 9.82±0.74 |
| Raw grain + 400 µg β-carotene | 13.71±0.86* | 15.12±0.86* | 3.86±0.31 | 8.74±0.65 |
| Cooked grain | 12.68±0.68 | 12.83±0.75 | 4.12±0.34 | 12.21±0.87 |
| Cooked grain + 200 µg β-carotene | 17.21±1.20* | 11.56±0.68 | 3.52±0.54 | 11.51±0.68 |
| Cooked grain + 400 µg β-carotene | 16.15±1.12* | 14.06±0.92* | 5.52±0.51* | 13.26±0.90 |
| **Zinc bioavailability %** | | | | |
| Raw grain | 27.6±1.12 | 8.12±0.51 | 34.2±0.98 | 42.7±1.34 |
| Raw grain + 200 µg β-carotene | 31.2±2.14* | 8.86±0.64 | 35.9±1.05 | 46.8±1.36 |
| Raw grain + 400 µg β-carotene | 31.6±1.68* | 9.43±0.54* | 36.1±0.24 | 45.6±1.48 |
| Cooked grain | 15.7±1.21 | 6.12±0.35 | 23.8±0.68 | 34.8±0.75 |
| Cooked grain + 200 µg β-carotene | 25.6±2.10* | 5.90±0.41 | 23.5±0.64 | 34.2±0.67 |
| *Cooked grain + 400 µg β-carotene* | 30.2±2.23* | 6.87±0.46* | 27.2±0.69* | 35.2±0.64 |

Values are mean ± standard deviation of three determinations.
*: Significant increase compared to the corresponding grain alone.

antioxidant phytochemical, namely curcumin of turmeric (a widely used spice), was also examined here for a possible beneficial influence on the bioavailabilities of iron and zinc from food grains. Curcumin, included at 0.05/ 10 g grain, significantly enhanced the availability of iron from oat, red bean and faba bean, in both raw and cooked condition (Table 4). The extent of increase in the availability of iron ranged from 16.4% in raw faba bean to 173% in raw oat. Curcumin, on the other hand, did not have a similar positive effect on the availability of zinc from any of the grains tested (Table 4).

## Discussion

β-carotene is reported to have a facilitating influence on nonheme iron absorption by forming a complex with iron, keeping it soluble in the intestinal lumen and preventing the inhibitory effect of phytates and polyphenols on iron absorption (Gracia-Casal *et al*., 1998). The cited researchers observed that β-carotene (0.58-2.06 µmol/100 g cereal) increased the absorption of iron in human subjects more than threefold from rice and 1.8-fold from wheat and corn. Increasing the doses of β-carotene did not further increase iron absorption (Gracia-Casal *et al*., 1998). Garcia-Casal *et al*. also conducted experiments to characterise ferrous fumarate uptake by Caco-2 cells in the presence of increasing amounts of vitamin A, β-carotene, phytate, and tannic acid added to the incubation mixtures (García-Casal *et al*.,2000). Added β-carotene (6 µM) significantly increased iron uptake compared to the control. Moreover, in the presence of phytate or tannic acid, β-carotene generally overcame the inhibitory effects of both these compounds, depending on their concentrations. The cited authors conclude that β-carotene improves iron uptake and overcomes the inhibition by potent inhibitors of iron absorption (García-Casal *et al*., 2000).

Information on the influence of β-carotene needs to be verified with respect to availability of this mineral (i.e., release of iron from the food matrix, rendering it available for absorption) in different food systems employing β-carotene-rich natural sources, such as yellow-orange and green leafy vegetables. The possibility of a similar influence of β-carotene on zinc availability from food systems also merits investigation. In view of this, the present investigation examined carrot and spinach as abundant sources of β-carotene for their influence on iron and zinc availability from representative cereals and pulses commonly consumed in China. Incidentally, these two vegetables are also normally consumed in combination with the cereals and pulses examined here, in the China diet.

The present study has evidenced that both carrot and spinach had positive effects on the availability of iron from the food grains tested, the effect being observed in both raw and cooked forms. The magnitude of this positive effect was maximal with the level of vegetable sources corresponding to 200 µg β-carotene per 10 g of grain. The positive effect of β-carotene-rich sources on the availability of either iron or zinc was generally greater in the cooked grains than the raw grains. The beneficial effect of carrot and spinach on iron and zinc bioavailabilities was highest in oat amongst the four grains evaluated. To

**Table 4.** Effect of curcumin on the bioavailabilities of iron and zinc from food grains

| Food grain | Wheat | Oat | Red bean | Faba bean |
|---|---|---|---|---|
| **Iron bioaccessibility %** | | | | |
| Raw grain | 8.17±0.56 | 7.82±0.56 | 4.14±0.24 | 10.41±0.58 |
| Raw grain +curcumin | 7.56±0.41 | 21.34±1.23* | 5.62±0.35* | 12.12±0.69* |
| Cooked grain | 10.45±0.68 | 15.64±0.84 | 4.1±0.41 | 12.41±0.74 |
| Cooked grain + curcumin | 9.12±0.55 | 20.7±0.95* | 4.5±0.40 | 16.47±0.85* |
| **Zinc bioaccessibility %** | | | | |
| Raw grain | 27.5±1.23 | 8.57±0.45 | 32.8±1.24 | 41.5±1.35 |
| Raw grain +curcumin | 26.1±1.24 | 8.62±0.51 | 32.9±1.34 | 41.4±1.54 |
| Cooked grain | 12.5±0.68 | 4.82±0.34 | 21.8±0.85 | 24.2±0.69 |
| *Cooked grain + curcumin* | 12.8±0.66 | 5.27±0.36 | 23.6±0.81 | 25.5±0.58 |

Values are mean ± standard deviation of three determinations.
*: Significant increase compared to the corresponding grain alone.

test whether the positive effect of carrot and spinach on the availability of minerals is solely attributable to the β-carotene present in them, the effect of pure β-carotene, included at 200 and 400 μg/10 g of the grain, was also examined here. The beneficial effect of pure β-carotene on iron and zinc availability was, however, restricted to only the two cereals examined. The absence of an effect of pure β-carotene similar to that of carrot and spinach on iron and zinc availabilities in the case of the other grains may be attributable to the probable loss of this isolated provitamin under the conditions of the experimental procedure employed in this determination.

Curcumin, the antioxidant phytochemical of turmeric, which has a structure partially similar to that of β-carotene, was also examined for a possible promoting influence on mineral availability. Curcumin was examined here at a level which corresponds to roughly five times the normal intake of turmeric, its parent spice. At the level examined, curcumin significantly enhanced the availability of iron from oat, red bean and faba bean, but did not similarly influence zinc availability. Thus, the effect of curcumin appears to be restricted to only iron availability.

Domestic food processing methods of grains, such as cooking, germination, fermentation, malting and dehulling are also recently reported to favourably influence the availability of iron and, in some cases, zinc also (Luo *et al*., 2009; Luo *et al*., 2010a). The present observation of the beneficial influence of vegetable sources of β-carotene is novel information. Knowledge of the availability of iron and zinc from food grains, as influenced by these beneficial modifiers, by virtue of their β-carotene content, will have an application in evolving strategies to achieve maximum availability of these trace minerals.

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