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Synergistic promoting influence of citric acid, spinach and garlic on the bioavailability of iron and zinc from legumes

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ABSTRACT

Inherent phytic acid and tannins interfere with bioavailability of iron and zinc from plant-based foods. Food acidulants, β-carotene-rich vegetables and Allium spices are understood to promote mineral *in vitro* bioavailability from legumes. In this study, it has been verified whether these promoters would counter negative effects of phytate and tannin on bioavailability of iron and zinc from legumes. Combinations of promoters – citric acid, spinach and garlic with phytic acid and tannin exogenously added individually were examined for their influence on iron and zinc bioavailability from the legumes. Effect of these promoters was generally dominant in the presence of phytic acid or tannic acid. The negative effect of the inhibitor was not only annulled, but also the positive influence of the promoter was fully retained. This information helps to evolve diet-based strategy to maximize mineral bioavailability and prevent deficiency situations prevalent in population dependent on plant foods.

Key words: Bioavailability, Inhibitors, Iron, Zinc, Promoters.

INTRODUCTION

Iron deficiency anemia is highly prevalent in developing countries and afflicts predominantly children and women of childbearing age. There are several factors contributing to iron deficiency; many of these are related to the diet. Low availability of iron, rather than low iron content, is one of the most important factors (Tatala, *et al.*, 1998). The diet in developing countries is mainly cereal- based with staple foods like legumes, rice, and millets. Such a vegetable diet contains large amounts of compounds that inhibit nonheme iron absorption; for example, phytate and polyphenols (Hallberg and Rossander, 1984). The inhibitory effect of polyphenols is probably due to formation of insoluble complexes with iron (III) in the gastrointestinal tract (Brune, *et al.*, 1989a). Iron (III) is thought to bind to adjacent hydroxyls on galloyl (trihydroxybenzene) and catechol (ortho-dihydroxybenzene) groups (South and Miller, 1998) and human absorption studies indicate that galloyl groups more effectively form insoluble complexes with iron (Brune, *et al.*, 1989b).

Tannic acid is a complex polyphenolic compound, mainly with galloyl groups, that has been shown to inhibit human iron absorption in a number of studies (Brune *et al.*, 1989b; Siegenberg *et al.*, 1991). Brune *et al*. (1989) showed that the inhibitory effect of tannic acid on iron absorption was dose-dependent. Some *in vitro* studies also show that tannic acid has an inhibitory effect on iron bioavailability when added to different foods (Anand and Subadra, 1995). Furthermore, the excretion of iron in rats has been shown to increase with addition of tannic acid, with larger effects in normal rats compared with anemic rats. There are also reports on the inhibitory effect on iron absorption by polyphenol-containing beverages like tea (Hurrell, *et al.*, 1999). Hurrell *et al.* (1999) showed that both green and black tea had an inhibitory effect on human iron absorption from a bread meal, although black tea had a stronger inhibitory effect. The inhibitory effect of tea was also dose-dependent, and it was related to the total polyphenol content in the beverage. Tea polyphenols are known to have both galloyl and catechol groups in their structure (Balentine, *et al.*, 1997).

Legume seeds are important staple foods, particularly in developing countries, due to their relatively low cost, long conservation time, and high nutritional value. However, their wider use is somehow limited by the presence of antinutrient compounds in the seeds which may have adverse effects for people nutrition. In legumes, a large proportion of phosphorus is present in the form of phytate. Phytic acid is a complex salt of minerals with myo-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate and is considered as the primary storage form of phosphorus and inositol in legumes seeds (Liu, *et al.*, 1998). The complex of phytic acid and mineral elements, in the form of phytate, leads to a marked reduction in bioavailability of these nutrient elements, and thus a consequent public health problem of iron and zinc deficiency for the populations whose diets are mainly cereals and legumes (Liu, *et al.*, 2007).

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Zinc is the fourth important micronutrient after vitamin A, iron and iodine, and is now receiving increasing global attention. Zinc deficiency, although not completely assessed, is believed to be as widespread as that of iron and is a cause for concern, especially in the developing countries. Although many factors are responsible for iron deficiency, the most likely cause of this nutritional problem in developing countries is the poor bioavailability of dietary iron (Gibson, *et al.,* 2000; Sandberg and Andlid, 2002). Animal foods are rich sources of zinc in our diet, but this micronutrient is derived mainly through food grains by a majority of the population in developing countries. Staple foods in developing countries include cereals and legumes, which are the main sources of zinc for most of the population, but even if net zinc intake appears adequate, compromised zinc status is common(Gibson, *et al.,* 1998).

Legumes contain high levels of phytic acid and other polyphenols which inhibit zinc absorption by forming insoluble complexes in the intestine. Consequently, the bioavailability of zinc in diets based on cereals and pulses is poor (Gibson, 1994), resulting in a greater risk of zinc deficiency in persons consuming lactoovovegetarian compared with those consuming omnivorous diets.

Food acidulants such as citric acid is known to promote bioavailability of iron from a variety of cereals and legumes (Hemalatha, *et al.,* 2005). We have recently reported that bioavailability of iron from cereals and legumes is significantly enhanced by β-carotene-rich vegetables – carrot and amaranth (Luo and Xie, 2012) and also by Allium spices –garlic and onion (Luo, *et al.,* 2014). β-Carotene is shown to facilitate non-haem iron absorption by forming a soluble complex in the intestinal lumen and by preventing the inhibitory effect of phytates and polyphenols on iron absorption from food grains (Gracia-Casal, *et al.,* 1998). Similarly, there is emerging evidence that dietary sulphur amino acids influence the status of minerals including iron (Greger and Mulvaney, 1985). Hence, these three ingredients, which are known individually to enhance iron bioavailability, may have an additive/synergistic influence on the same, when used in combination.

In view of this, it would be relevant to examine the influence of normally encountered combinations of these promoters of mineral bioavailability for a possible additive or synergistic influence on iron bioavailability. Similarly, it would also be interesting to understand whether the presence of promoters such as food acidulant, β-carotene-rich source, or Allium spice would counter the negative effects of inhibitors such as phytic acid and tannin.

The objective of this study was to examine whether the presence of promoters such as food acidulant, β-carotene-rich vegetable or Allium spice would counter the negative effect of inhibitors such as phytic acid and tannin on the bioavailability of iron and zinc from legumes. For this purpose, combinations of each of the above promoters –β-carotene-rich spinach, citric acid and Allium spice garlic with the inhibitor phytic acid and tannin exogenously added individually at a level that was two times the inherent concentration in the food grains were examined for their influence on iron and zinc bioavailability from the tested legumes.

MATERIALS AND METHODS

The legumes samples included soybean, mung bean, faba bean and azuli bean were collected from local market of the same batch in Nanjing, Jiangsu Province, P.R. China. Spinach and garlic were procured from the local market, cleaned and the edible portions were ground finely and used freshly. Commercial citric acid (dry powder) was procured from the local market. Pepsin (P-7000), pancreatin (P-1750), bile extract (B-8631), phytic acid, tannic acid, iron and zinc were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and solvents commercially obtained were of analytical grade. Acid-washed glassware were used throughout the study.

Total iron and zinc content: Minerals in solid materials were analysed by atomic absorption spectrophotometry (Varian SpectrAA 200, Victoria, Australia) after dry ashing for 2 h at 530ºC. Depending on the different treatments, 2-4 g of ash were 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.

Phytate and tannin content

Phytic acid: Phytic acid contents were determined by the method of Haug and Lantzsch (Haug and Lantzsch, 1983). The sample extract (with 0.2 N HCl) was heated with an acidic iron (III) solution of known iron content (0.2 g ammonium iron (III) sulphate-12 H_2O was dissolved in 100 ml 2 N HCl and volume made up to 1000 ml with distilled water). Phytic acid content in the supernatant was measured as the decrease in absorbance of iron content using 2,2-bipyridine (Dissolve 10 g 2,2’-bipyridine and 10 ml thioglycollic acid in distilled water and make up to 1000 ml) at 419 nm.

Determination of tannin content: Total tannin was determined by the method of Anonymous (2000). Flour (2 g) was extracted with 20 mL of 70% v/v acetone (analytical grade) by applying a 20-min ultrasonic treatment at 4ºC followed by overnight mechanical tumbling. Extracts were analyzed for total phenolics by spectrophotometrical methods using the Folin–Ciocalteu’s Phenol Reagent at 765 nm. Total phenolic compounds were calculated from a prepared standard curve of tannic acid under same set of conditions. Tannin was complexed with polyvinylpolypyrrolidone (Sigma) and unbound nontannin phenolics were determined as above (Anonymous, 2000). Total tannin was calculated by subtracting nontannin phenolics fromtotal phenolics.

***In vitro* iron and zinc bioavailability:** *In vitro* bioavailability of the sample was determined according to procedure described by Gil-Izquierdo *et al.* (2002), with slight modifications. Briefly, 10 g of the sample homogenate obtained from cooked beans was placed into 100 mL polystyrene tube and after the addition of distilled water (10 ml), the pH was adjusted to 2.0 with 1 M HCl and mixed with 1 mL pepsin suspension (15,750 U). The mixture was incubated at 37 ºC in a shaking water bath (Kelong, China) for 2 hr. At the end of the incubation period, a dialysis bag containing 20 ml PIPES buffer (0.15 N) was placed into the tube. Following 30 min incubation at 37 ºC in a shaking water bath, 5 ml of the pancreatin/bile mixture (0.5 g pancreatin+3 g bile extract/ 250 ml 1 N $NaHCO_3$) was added and the incubation continued for another 2 hr. At the end of the incubation, the dialysis bag was removed and rinsed by dipping in water. Measurements related to intestinal absorption were performed directly to dialysates obtained. Analysis of retentate which corresponds to unabsorbed portion through the intestinal wall was performed with both aqueous phase and methanolic extract. Methanolic extract of retentate was obtained as described above.

The levels of exogenous phytic acid and tannic acid varied in different legumes depending on phytic acid and tannin inherently present in these legumes. Inherent contents of phytic acid and tannin in legumes which were used in this study and exogenous phytic acid and tannic acid added at two levels to different legumes are given in Table 1. Experiments were carried out in independent sets for the selected legume using the ingredients or their combinations as listed in the tables of the results section with respect to the:

(1) study on the effect of two levels of phytic acid and tannic acid on the bioavailability of iron and zinc from the legumes,

(2) study on the effect of combination of citric acid and phytic acid on the bioavailability of iron and zinc from the legumes,

(3) study on the effect of combination of spinach and phytic acid on the bioavailability of iron and zinc from the legumes, and

(4) study on the effect of combination of garlic and phytic acid on the bioavailability of iron and zinc from the legumes.

Heat processing of food samples was autoclaved at 121ºC under 2MPa for 20 min with triple distilled water. The cooked samples were homogenized in a stainless steel mixer and used for the determination of mineral bioavailability as described above.

autoclaved at 121 ºC under 2MPa for 20 min

Statistical analysis: Data were analysed with SPSS 13.0 (Statistical Product and Service Solutions 13.0) 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 AND DISCUSSION

Effect of phytic acid on the bioavailability of iron and zinc from legumes: Phytic acid was exogenously added to the legumes at levels so that the final concentration corresponded to two and four times their respective inherent levels. Effect of exogenously added phytic acid on the bioavailability of iron from four selected food grains is presented in Table 2. Phytic acid had significant negative effect on iron bioavailability from faba bean and azuki bean whereas in the case of remaining two legumes – soybean and mung bean, the negative effect of phytic acid was not seen. Exogenous phytic acid added at the first level decreased the bioavailability of iron from raw faba bean by 64.1% whereas it produced an inhibition of 59.1% in the bioavailability of iron from cooked faba bean. The second level did not produce any further appreciable decrease in the bioavailability of iron. Exogenous phytic acid decreased the bioavailability of iron from raw azuki bean by 40.7 and 43.4%, respectively, at the two levels added, whereas about 40.2% decrease was observed from cooked azuki bean with either of the two levels of phytic acid.

Inhibitory effect of phytic acid on the bioavailability of zinc from selected legumes is presented in Table 2. The negative effect of phytic acid was observed only in the case of raw and cooked azuki bean, whereas in the rest of the

Table 1: Exogenously added levels of phytic acid and tannic acid in legumes

Legume	Phytic acid (mg/100 g)		Tannic acid (mg/100 g)	
	Inherent	Exogenous	Inherent	Exogenous
Soybean	147.4	147.4	168.6	168.6
		442.2		505.8
Mung bean	216.3	216.3	71.9	71.9
		648.9		215.7
Faba bean	467.6	467.6	212.6	212.6
		1402.8		637.8
Azuki bean	289.3	289.3	178.8	178.8
		867.9		536.4

Table 2: Effect of exogenous phytic acid on the bioavailability of iron and zinc from legumes

Legume	iron bioavailability		zinc bioavailability	
	Raw	Cooked	Raw	Cooked
Soybean (10.0g)	5.87±0.23	9.36±0.34	7.69±0.56	6.26±0.44
Soybean (10.0g)+ phytic acid (0.015g)	6.14±0.14	9.87±0.53	7.21±0.58	6.38±0.34
Soybean (10.0g)+ phytic acid (0.044g)	6.23±0.34	10.24±0.45	7.40±0.49	6.41±0.52
Mung bean (10.0g)	5.26±0.25	8.36±0.32	10.21±0.63	10.54±0.57
Mung bean (10.0g)+ phytic acid (0.022g)	5.38±0.31	8.12±0.34	10.12±0.69	10.68±0.78
Mung bean (10.0g)+ phytic acid (0.065g)	5.61±0.38	7.89±0.42	10.36±0.62	10.36±0.86
Faba bean (10.0g)	6.72±0.44	7.67±0.43	32.17±0.87	21.64±0.88
Faba bean (10.0g)+ phytic acid (0.047g)	2.41±0.21*	3.14±0.23*	31.28±0.78	20.89±0.92
Faba bean (10.0g)+ phytic acid (0.140g)	2.28±0.18*	3.06±0.31*	30.96±0.98	20.76±0.94
Azuki bean (10.0g)	8.64±0.43	10.23±0.59	36.21±0.85	25.62±0.67
Azuki bean (10.0g)+ phytic acid (0.029g)	5.12±0.32*	6.12±0.38*	20.86±0.68*	12.43±0.77*
Azuki bean (10.0g)+ phytic acid (0.087g)	4.89±0.25*	6.24±0.28*	19.47±0.79*	11.65±0.67*

Values are mean±SEM of three independent determinations;
*Significantly different from corresponding grain alone ($p < 0.05$).

legumes the negative effect was not seen. Phytic acid reduced the bioavailabilityof zinc by 42.4% and 46.2% and 51.5% and 54.5% at the two levels from raw and cooked azuki bean, respectively.

Effect of tannic acid on the bioavailability of iron and zinc from legumes: Tannic acid was exogenously added to the grains at levels such that the final concentration (inclusive of inherent component) corresponded to two and four times their respective inherent levels. Table 3 presents the effect of tannic acid on iron bioavailability from legumes. Tannic acid showed a significant negative effect on iron bioavailability from soybean, mung bean and azuki bean. Tannic acid at the two levels reduced the bioavailability of iron by 25.1 and 24.7% from cooked soybean, whereas it showed no significant effect in the raw soybean. In the case of raw mung bean, tannic acid produced 58.7 and 59.1% reduction in iron bioavailability at the first and second level, respectively. The negative effect of tannic acid on iron bioavailability in the case of cooked wheat was 47 and 46% at the twolevels present. In the case of raw azuki bean, the negative effect of tannic acid was 55.4 and 52.5% with the first and second level, respectively, whereas in the case of cooked bean, bioavailability of iron was decreased by 46.7 and 46.9% with the two levels of tannic acid, respectively. Tannic acid did not produce any significant reduction at either of the levels added on the percent iron bioavailability from both raw and cooked green gram.

Results of exogenously added tannic acid on the bioavailability of zinc from the four food legumes examined are presented in Table 3. Tannic acid had a significant negative effect on zinc bioavailability only from azuki bean. It produced 41.6 to 43.5% reduction in the bioavailability of zinc from raw samples at the first and second level of tannic acid, respectively, whereas in the case of cooked bean, there was 40.1 to 42.3% decrease in zinc bioavailability at the first and second level of tannic acid, respectively.

Further studies were carried out to examine the possibility of countering the negative effect of phytic acid

Table 3: Effect of exogenous tannic acid on the bioavailability of iron and zinc from legumes

Legumes	iron bioavailability		zinc bioavailability	
	Raw	Cooked	Raw	Cooked
Soybean (10.0g)	6.23±0.23	12.37±0.58	7.86±0.43	6.84±0.75
Soybean (10.0g)+ tannic acid (0.017g)	6.17±0.34	9.27±0.53*	7.72±0.49	6.72±0.69
Soybean (10.0g)+ tannic acid (0.051g)	5.95±0.35	9.31±0.48*	7.74±0.38	6.76±0.46
Mung bean (10.0g)	5.23±0.29	6.36±0.52	9.26±0.56	8.84±0.69
Mung bean (10.0g)+ tannic acid (0.007g)	2.16±0.29*	4.16±0.49*	9.14±0.65	8.67±0.76
Mung bean (10.0g)+ tannic acid (0.021g)	2.14±0.28*	4.11±0.47*	9.23±0.49	8.54±0.59
Faba bean (10.0g)	3.67±0.33	4.28±0.38	25.1±0.87	18.7±0.78
Faba bean (10.0g)+ tannic acid (0.021g)	3.24±0.27	3.84±0.38	23.2±0.88	19.2±0.62
Faba bean (10.0g)+ tannic acid (0.063g)	3.16±0.43	3.89±0.46	23.6±0.65	18.9±0.62
Azuki bean (10.0g)	7.20±0.48	9.68±0.68	36.8±0.96	31.4±0.91
Azuki bean (10.0g)+ tannic acid (0.018g)	3.21±0.35*	5.16±0.67*	21.5±0.65*	18.6±0.53*
Azuki bean (10.0g)+ tannic acid (0.054g)	3.42±0.32*	5.14±0.49*	20.8±0.69*	18.1±0.49*

Values are mean±SEM of three independent determinations;
*Significantly different from corresponding grain alone ($p < 0.05$).

or tannic acid on iron and zinc bioavailability from the selected food grain, by the simultaneous presence of promoters.

Effect of combination of phytic acid and promoters (citric acid, spinach, garlic) on the bioavailability of iron from legumes: Among the four legumes tested, phytic acid had a negative effect on the bioavailability of iron only from faba bean and azuki bean. Hence, the combination of phytic acid with different known promoters of iron bioavailability was examined for countering the effect of promoters on the negative effect of phytic acid in these two grains. The effect of the combination of phytic acid and promoters –citric acid, spinach and garlic, on iron bioavailability from faba bean is presented in Table 4.

Although phytic acid decreased the bioavailability of iron from raw faba bean by 33.6%, citric acid independently enhanced it by 22.1%. The combination of citric acid and phytic acid also resulted in as much increase in iron bioavailability, which was 23.2%. Similarly, although phytic acid produced an inhibition of 39.8% in the bioavailability of iron from cooked faba bean, citric acid enhanced the bioavailability by 14.3%, when added at the level indicated. This enhancing effect of citric acid remained almost the same (23.2% increase) even in the presence of phytic acid. Thus, the positive effect of citric acid has dominated the negative influence of phytic acid on iron bioavailability from both raw and cooked faba bean, and the negative influence is completely countered in either of the instances.

Bioavailability of iron from raw faba bean was decreased from 6.72 to 4.23% in the presence of exogenous phytic acid. Spinach on the other hand, enhanced the bioavailability of iron to 8.67%, when added at the level indicated. The effect of added spinach remained the same, namely 30% increase even in the presence of phytic acid. A similar trend was observed in cooked faba bean, in which phytic acid produced a 28.6% decrease in percent iron bioavailability, and spinach produced 34.4% increase in the same. Even in the presence of phytic acid, the same level of spinach completely countered the negative effect of the former.

Although phytic acid decreased the bioavailability of iron from raw faba bean by 35.1%, garlic increased it by 37.3%. The positive effect of garlic was predominant even in the presence of phytic acid, and this combination produced a net increase in iron bioavailability by 37.2%. Similarly, the combination of garlic and phytic acid had a net positive influence of 34.3% in iron bioavailability from cooked faba bean, whereas phytic acid independently had a negative influence by 35.1%, and garlic had a positive influence by 31%. Thus, garlic completely overcame the negative influence of phytic acid on iron bioavailability from faba bean, and also retained the extent of its positive influence.

The effect of the combination of phytic acid and promoters – citric acid, spinach and garlic on iron bioavailability from azuki bean is presented in Table 5. Phytic acid decreased the bioavailability of iron from raw and cooked azuki bean by 39 and 39.5%, respectively. Citric acid increased iron bioavailabilityby 84.3 and 53.8% from raw and cooked azuki bean, respectively. In presence of phytic acid, citric acid promoted iron bioavailability by 76.8 and 46.6%, thus completely countering the negative effect of phytic acid. Thus, the promoting effect of citric acid was such that it could counter the negative effect of phytic acid on iron bioavailability from azuki bean. Spinach also brought about a significant positive effect on iron bioavailability even in the presence of phytic acid in cooked azuki bean. The percent increase in iron bioavailability from cooked azuki bean was 25.3% in the case of spinach and phytic acid

Table 4: Effect of combination of phytic acid and promoters (citric acid, spinach and garlic) on the bioavailability of iron from faba bean

Legume	iron bioavailability	
	Raw	Cooked
Faba bean (10.0g)	6.75±0.34	7.68±0.45
Faba bean (10.0g)+ phytic acid (0.047g)	4.48±0.36*	4.62±0.39*
Faba bean (10.0g)+ citric acid (0.75g)	8.24±0.51*	8.78±0.48*
Faba bean + citric acid + phytic acid	8.32±0.53**	8.02±0.76**
Faba bean (10.0g)	6.72±0.58	7.82±0.48
Faba bean (10.0g)+ phytic acid (0.047g)	4.23±0.38*	5.58±0.44*
Faba bean (10.0g)+ spinach (2.50g)	8.67±0.62*	10.51±0.48*
Faba bean + spinach + phytic acid	8.72±0.59**	10.45±0.65**
Faba bean (10.0g)	6.79±0.49	7.52±0.38
Faba bean (10.0g)+ phytic acid (0.047g)	4.41±0.34*	5.53±0.33*
Faba bean (10.0g)+ garlic (1.50g)	9.32±0.47*	9.86±0.58*
Faba bean + garlic + phytic acid	9.12±0.52**	9.92±0.64**

Values are mean±SEM of three independent determinations;
* Significantly different from grain alone ($p < 0.05$);
** Significantly different from grain+Phytic acid ($p < 0.05$).

combination. Phytic acid and garlic together brought about 28.6 and 25.3% increase in iron bioavailability after countering the negative effect in raw and cooked azuki bean, respectively.

Thus, the three promoters of iron bioavailability examined in this study not only completely countered the negative influence of exogenous phytic acid, but also retained the extent of their positive influence on the same. This situation is at least true when phytic acid level was double the amount inherently present in the bean. This study also suggests that promoters have a dominating influence than the inhibitors of mineral bioavailability, whether it is food acidulant, β-carotene-rich vegetable or an *Allium* spice.

Effect of combination of phytic acid and promoters (citric acid, spinach and garlic) on the bioavailability of zinc from azuki bean: Table 5 presents the effect of combination of phytic acid and promoters (citric acid, spinach and garlic) on the bioavailability of zinc from azuki bean. The negative effect of phytic acid on the bioavailability of zinc from azuki bean was also independently countered by the promoters – citric acid, spinach and garlic. Phytic acid reduced the bioavailability of zinc by 42.4 and 51.4% in raw and cooked azuki bean, whereas the combination of this inhibitor with promoters –citric acid, spinach and garlic, had overcome the negative effect of phytic acid. Citric acid in the presence of phytic acid brought about 35.2% increase in zinc bioavailability in raw azuki bean and 104.3% increase in cooked azuki bean. Spinach increased the bioavailability of zinc by 64.4% in raw azuki bean and 119.8% in cooked azuki bean in the presence of phytic acid. Similarly, the percent increase in the bioavailability of zinc with the combination of garlic and phytic acid was 32.7% and 85.5% in raw and cooked azuki bean, respectively.

Effect of combination of tannic acid and promoters (citric acid, spinach and garlic) on the bioavailability of iron from azuki bean: The effect of combination of tannic acid and promoters –citric acid, spinach and garlic on the bioavailability of iron from azuki bean is presented in Table 6. Tannic acid inhibited the bioavailability of iron by 55.4 and 46.7% in raw and cooked azuki bean, respectively. Citric acid increased iron bioavailability by 71.7 and 57.3% from raw and cooked azuki bean, respectively. Citric acid also increased the bioavailability of iron by 51.1 and 52.7% after countering the negative effect of tannic acid. Garlic brought a significant positive effect on the bioavailability of iron in the presence of tannic acid. Garlic increased the bioavailability of iron from raw and cooked azuki bean by 101 and 101.6%, respectively, in the presence of tannic acid. Garlic increased the bioavailability of iron from raw and cooked azuki bean by 50 and 57.6%, respectively. Garlic also completely countered the negative effect of tannic acid in both raw and cooked food bean.

Effect of combination of tannic acid and promoters (citric acid, spinach and garlic) on the bioavailability of zinc from azuki bean: Table 6 presents the effect of combination of tannic acid and promoters –citric acid, spinach and garlic, on the bioavailability of zinc from azuki bean. Individually, tannic acid decreased the bioavailability of zinc by 41.6 and 40.8% in raw and cooked food grain, respectively. Citric acid promoted zinc bioavailability by 31.0 and 79.3% in raw and cooked azuki bean. Citric acid countered the negative effect of tannic acid whereas increasing the zinc bioavailability by 24.2 and 49.0% in raw and cooked azuki bean in the presence of the inhibitor. Garlic increased the bioavailability of zinc in the presence of tannic acid by 77.5 and 87.2% in raw and cooked azuki bean, respectively. Garlic acid increased the bioavailability of zinc by 36.2 and 40.4%

Table 5: Effect of combination of phytic acid and promoters (citric acid, spinach and garlic) on the bioavailability of iron and zinc from azuki bean

Legume	iron bioavailability		zinc bioavailability	
	Raw	Cooked	Raw	Cooked
Azuki bean (10.0g)	8.59±0.45	10.34±0.69	36.21±0.34	25.62±0.24
Azuki bean (10.0g)+ phytic acid (0.029g)	5.24±0.49*	6.26±0.59*	20.86±0.54*	12.43±0.26*
Azuki bean (10.0g)+ citric acid (0.75g)	11.45±0.58*	14.86±0.78*	48.95±0.64*	52.34±0.56*
Azuki bean + phytic acid +citric acid	11.38±0.53**	12.32±0.66**	48.56±0.34**	50.33±0.67**
Azuki bean (10.0g)	8.55±0.49	10.11±0.54	35.88±0.54	25.68±0.35
Azuki bean (10.0g)+ phytic acid (0.029g)	5.22±0.43*	6.38±0.59	21.86±0.26*	12.65±0.27*
Azuki bean (10.0g)+ spinach (2.5g)	15.76±0.67*	15.55±0.67*	58.98±0.46*	56.32±0.63*
Azuki bean + phytic acid +spinach	15.12±0.66**	14.82±0.77**	56.62±0.55	48.17±0.46**
Azuki bean (10.0g)	8.47±0.59	10.32±0.65	35.67±0.39	25.45±0.39
Azuki bean (10.0g)+ phytic acid (0.029g)	5.23±0.52	6.31±0.82	21.78±0.34*	12.86±0.23*
Azuki bean (10.0g)+ garlic (1.50g)	10.56±0.67*	13.23±0.59*	45.65±0.49*	46.88±0.32*
Azuki bean + phytic acid +garlic	10.89±0.39**	12.93±0.71**	47.32±0.51**	47.21±0.33**

Values are mean±SEM of three independent determinations;
* Significantly different from grain alone ($p < 0.05$);
** Significantly different from grain+Phytic acid ($p < 0.05$).

Table 6: Effect of combination of tannic acid and promoters (citric acid, spinach and garlic) on the bioavailability of iron and zinc from azuki bean

Legume	iron bioavailability		zinc bioavailability	
	Raw	Cooked	Raw	Cooked
Azuki bean (10.0g)	7.20±0.31	9.68±0.33	36.8±0.87	31.4±0.48
Azuki bean (10.0g)+ tannic acid (0.018g)	3.21±0.22*	5.16±0.32*	21.5±0.66*	18.6±0.23*
Azuki bean (10.0g)+ citric acid (0.75g)	12.36±0.48*	15.23±0.65*	48.2±0.59*	56.3±0.43*
Azuki bean + citric acid + tannic acid	10.88±0.56**	14.78±0.48**	45.7±0.69**	46.8±0.39**
Azuki bean (10.0g)	7.24±0.37	9.54±0.33	36.5±0.55	31.3±0.41
Azuki bean (10.0g)+ tannic acid (0.018g)	3.28±0.24*	5.28±0.23*	21.2±0.36*	18.5±0.38*
Azuki bean (10.0g)+ spinach (2.50g)	14.56±0.33*	19.23±0.59*	65.4±0.54*	61.2±0.48*
Azuki bean + spinach + tannic acid	14.23±0.42**	17.46±0.76**	64.8±0.59**	58.6±0.38**
Azuki bean (10.0g)	7.18±0.28	9.66±0.58	35.9±0.38	32.4±0.59
Azuki bean (10.0g)+ tannic acid (0.018g)	3.27±0.22*	5.31±0.35*	23.5±0.54*	19.6±0.53*
Azuki bean (10.0g)+ garlic (1.50g)	10.77±0.33*	15.22±0.76*	48.9±0.49*	45.5±0.44*
Azuki bean + garlic + tannic acid	8.97±0.41**	13.41±0.63**	50.2±0.58**	42.4±0.59**

Values are mean±SEM of three independent determinations;
* Significantly different from grain alone ($p < 0.05$);
** Significantly different from grain+Phytic acid ($p < 0.05$).

in raw and cooked azuki bean. Garlic countered the negative effect of tannic acid by increasing the zinc bioavailability by 39.8 and 30.9% in raw and cooked azuki bean, respectively.

Although the values from *in-vitro* methods are relative rather than absolute estimates of mineral absorbability, being not subjected to the physiological factors that can affect bioavailability, such relative estimates on the comparative zinc/iron bioavailability values for food grains are still valid and suffice to form a strategy to derive maximum mineral availability. The observation that the promoters of mineral bioavailability, viz. the food acidulant, β-carotene-rich vegetable and the Allium spice, generally are dominant in their positive influence and that they can counter the negative influence of inhibitors of mineral bioavailability, *viz*. phytic acid and tannic acid are interesting and also assume significance, although the information is obtained from only one or two representative food grains. The basic information generated in this study employing an *in-vitro* method for comparing the bioavailability of iron from food samples as influenced by various modifiers of iron bioavailability will be useful to evolve diet-based strategies to maximize iron bioavailability from the available sources, and thus could address iron deficiency situations prevalent in the society. The observations of this study also suggest the need for extension of the evaluation of the influence of promoters of zinc bioavailability to other food grains in the presence of exogenous phytic acid and tannic acid.

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