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Research Article

Effect of Oxate and Phytate on Iron Bioavailability from Foods Commonly Consumed in China

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Abstract: The objectives of this research were to assess the bioavailability of iron in foodstuffs found in the China diet, to provide data on the content of iron absorption inhibitors present in plant origin products and to assess the inhibitory effect of these compounds and of cooking on iron bioavailability; therefore, total content and bioavailable iron, tannins, phytic and oxalic acid were determined in vegetables, legumes and animal products, before and after cooking. Vegetables, although rich in iron, have poor iron bioavailability and a high content of inhibitory factors; cooking reduced the content of iron and inhibitory factors, whereas in animal products the treatment of cooking did not significantly reduce it. Iron bioavailability, phytate content and the phytate to iron molar ratio predicted poor iron bioavailability and, therefore, a negative impact on the nutritional status of people who rely on them as staple foods could be expected.

Keywords: Bioavailability, cooking, oxate, phytate

INTRODUCTION

Iron is an essential trace element whose biological importance arises from its involvement in vital metabolic functions by being an intrinsic component of hemoglobin, myoglobin and cytochromes (Gibson, 1994). Despite large scale intervention programmes, iron-deficiency anaemia remains the most widely prevalent nutritional problem in the world (Hemalatha *et al*., 2007; Sandberg and Andlid, 2002). 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). Iron deficiency is especially prevalent among specific population groups, such as infants (Lozoff *et al*., 1996) menstruating and pregnant women and populations with a high dietary intake of plant-derived proteins (Hallberg, 2001); it can lead to important health problems and retardation in physical and mental development (Beard and Connor, 2003; Hurrell, 2004). The bioavailability of iron from foods is defined as the proportion of the iron that can be absorbed and utilised within the body. Solubility of iron, pH of intestinal lumen, dietary factors and retention time at the digestion and absorption site influence the bioavailability of iron (Larsson *et al*., 1997).

The availability of iron for absorption is limited by the presence of Antinutritional Factors (ANF) in the legumes. Phytates, tannins and dietary fiber are the main compounds that can interact with iron and/or zinc ions (Gillooly *et al*., 1984). The presence of these antinutritional factors results in the creation of mostly insoluble complexes with divalent cations such as iron and zinc, which means that they can no longer be absorbed during intestinal digestion. These complexes can be made from two or more compounds (Reddy, 2002). Thus, there are some complexes between fibers and phytates that are able to chelate minerals and result in fiber-phytate-mineral complexes. In addition, ANF, in particular phytates (Wise, 1995) and condensed tannins (Bravo, 1998), are known for their ability to complex proteins. Phytates are complexed with proteins either directly or indirectly depending on pH. Thus, phytates can create different types of complexes depending upon the pH.

In human and animal nutrition, oxalic acid or oxalate is considered an undesirable compound. Humans and animals accidentally or intentionally eat plants high inoxalate, such as spinach; intake of high levels of oxalate can induce hyperoxaluria, potentially leading to kidney and bladder stones and, at the extreme, to renal edema and calcification (Horner *et al*., 2005). In humans or animals, oxalate is removed by excretion through the urinary system where it can precipitate calcium and other ions to form renal stones. Oxalates can be found in relatively small amounts in many plants; oxalate-rich foods are usually minor

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components in human diets but are sometimes important in seasonal diets in certain areas of the world (Savage *et al*., 2000).

Diets containing tannins are associated with decreased intake, weight gain, feed conversion efficiency and protein digestibility in animal studies (Chung *et al*., 1998; Porres *et al*., 2002). The term tannin is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups to form strong complexes with proteins and other molecules by hydrogen bonds; this characteristic provides tannins with several benefits to industry and health but it is also considered as an iron absorption inhibitor.

The objectives of this research were to assess the iron content and bioavailability of this mineral in plant and animal products normally found in the China diet, to provide basic data on the content of some iron absorption inhibitors present in plant and to assess the inhibitory effect of these compounds on the bioavailability of iron.

MATERIALS AND METHODS

Materials: The samples selected for the present study were grouped into leafy vegetables (lettuce, amaranth, spinach, leek), legumes (faba bean, azuki bean, mung bean, soybean) and animal products (beef, chicken, fish, shrimp). These samples are widely consumed in China. Commercial citric acid (dry powder) was procured from the local market. Pepsin solution, pancreatin, bile acids, hydroxylamine hydrochloride, hydrochloricacid, acetate buffer, o-phenanthroline, dimethylformamide, ferric ammonium citrate, tannic acid, ammonia, sodium phytate, iron (III) chloride hexahydrate, sulfosalicylic acid, phosphoric acid, sodium tungstate dehydrate, acetic and sulfuric acid were purchased from Sigma. All other reagents and solvents commercially obtained were of analytical grade. Acid-washed glassware were used throughout the study.

Samples were washed with distilled water and each batch was treated as follows. One part was boiled using tap water in a ratio of 2:1 (v/v) for legumes and 1:1(v/v) for leafy vegetables and animal products; boiling treatment was kept until water was evaporated. The second part remained uncooked and stored at 4°C.

Total iron content: Total Fe contents were determined by atomic absorption spectrophotometry (Varian SpectrAA 200, Victoria, Australia) after dry mineralization for 2 h at 530°C. 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.

***In vitro* 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°C in a shaking water bath (Kelong, China) for 2 h. 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°C 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 h. 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.

Determination of tannin content: Total tannin was determined by the method of Anonymous (2000). Flour (2.0 g) was extracted with 20 mL of 70% v/v acetone (analytical grade) by applying a 20 min ultrasonic treatment at 4°C followed by overnight mechanical tumbling. Extracts were analysed for total phenolics by spectrophoto metrical 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 non-tannin phenolics were determined as above (Anonymous, 2000). Total tannin was calculated by subtracting non-tannin phenolics from total phenolics.

Phytic acid: Phytic acid contents were determined by the method of 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 H2O was dissolved in 100 mL 2 N HCl and volume made up to 1000 mL with distilled water). The phytic acid was precipitated with an acidic iron-III-solution of known iron content. 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.

Oxalate analysis: Oxalate was extracted by a method based on that described by Savage *et al*. (2000).

Samples (1 g) were extracted with 50 mL of 1.0 MH_2SO_4 at 21°C for 15 min in a shaking water bath. The extracts were transferred into a 100 mL volumetric flask and made to volume with 1.0 M H_2SO_4 for total oxalate and with distilled water for soluble oxalate. The dissolved oxalate solution was separated by centrifugation at 3000 rpm for 15 min and passed through a 0.45 mL nylon syringe filter. The oxalate concentration in each sample was determined by HPLC, using an Agilent 1100 series chromatograph with autosampler, isocratic pump and UV-Vis detector set at 210 nm. Data capture and analysis were done by using Chemstation software Version A-7.1. A 5 ll injection volume was used with an Aminex Ion exclusion HPX-87H 300×7.8 mm analytical column fitted with an AminexCation-H guard column. Isocratic elution was used with 0.0125 M H_2SO_4 (Sigma Aldrich, UK) as mobile phase and a flow of 0.5 mL/min. The analytical column was held at 65°C and the column was equilibrated with a flow rate of 0.2 mL/min prior to use.

Statistical analysis: Data were analysed with SPSS (Statistical Package for the Social Sciences) 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 a probability $p<0.05$.

RESULTS AND DISCUSSION

Leafy vegetables: The content of iron (total and bioavailable) of raw and cooked food samples is presented in Table 1. As can be observed, green leafy vegetables presented the highest total iron content against other food groups; within this group, the iron content ranged from 29.18 mg/100 g Dried Weight (DW) for lettuce to 65.88 mg/100 g DW for spinach, which had the highest content of iron whether raw or cooked.

No significant difference was found in the total iron content after boiling lettuce and spinach; however, cooking seems to significantly influence the iron content in amaranth and leek-the decreased content of iron in these samples may be due to losses by boiling, since iron might be in its soluble form. In spinach, a decrease of 1% in total iron content was obtained; on the other hand, boiling did not affect the bioavailability of iron in this food group. The content of bioavailable iron ranged from 3.83 mg/100 g DW for amaranth to 5.25 mg/100 g DW for spinach, which represents no more than 10.0% of the total iron content.

Green leafy vegetables form an important part of the diet. Although rich in iron, green leafy vegetables have poor iron bioavailability and a high content of inhibitory factors, such as fiber, phytates and oxalates, which bind with iron, rendering it insoluble and therefore unavailable to the body.

The content of phytates, oxalates and tannins in leafy vegetables is presented in Table 2; as can be observed, boiling significantly decreased the content of these anti-nutritional compounds in almost all green leafy vegetables with the exception of the content of phytates in spinach and tannins in lettuce. Since phytate is heat-stable, significant reduction during cooking is not expected unless the cooking water is discarded, given that some nutrients will be dissolved in the cooking water. Discarding cooking water will also result in a certain loss of nutrients at the same time; therefore, this method may not be effective in maintaining their nutritional value and in improving the bioavailability of minerals. Almana (2000) reported that discarding excessive water in cooking rice may result in 37-65% of phytate degradation, while retaining the water only results in 12% of degradation. Our results are in agreement with this statement; as can be seen in

Table 1: Total and bioavailable iron contents in China food samples

	Total iron content (mg/100 g)		Iron bioavailable (mg/100 g)	
	Raw	Cooked	Raw	Cooked
Leafy vegetables				
Lettuce	29.18±0.64[a]	28.67±0.54[a]	4.36±0.21[a]	4.32±0.24[a]
Amaranth	33.27±0.71[a]	30.19±0.68[b]	3.81±0.18[a]	3.83±0.16[a]
Spinach	65.88±1.26[a]	65.21±1.12[a]	5.26±0.31[a]	5.25±0.27[a]
Leek	41.29±1.14[a]	38.81±0.96[b]	4.18±0.26[a]	4.15±0.21[a]
Legumes				
Faba bean	13.86±0.56[a]	12.75±0.65[b]	3.47±0.54[b]	3.68±0.47[a]
Azuki bean	11.67±0.48[a]	11.01±0.72[b]	2.85±0.36[a]	2.84±0.34[a]
Mung bean	9.26±0.62[a]	8.64±0.68[b]	2.16±0.28[a]	2.14±0.26[a]
Soybean	9.38±0.67[a]	8.86±0.54[b]	2.67±0.27[a]	2.65±0.25[a]
Animal products				
Beef	22.85±1.21[a]	22.17±1.17[b]	7.67±0.68[a]	7.61±0.54[a]
Chicken	34.68±1.67[a]	34.26±1.64[b]	9.26±0.82[a]	9.24±0.76[a]
Fish	8.67±0.84[b]	18.51±0.62[a]	3.68±0.41[a]	3.12±0.36[a]
Shrimp	18.62±0.74[a]	18.01±0.71[b]	6.17±0.38[a]	6.15±0.41[a]

Analysis performed in triplicate; Results presented as mean±standard deviation; Different superscript letters between columns indicate significant differences between treatments $p<0.05$

Table 2: Tannin, oxalate and phytate contents of green leafy vegetables

	Tannins (g/100 g)		Oxalate (g/100 g)		Phytate (g/100 g)	
	Raw	Cooked	Raw	Cooked	Raw	Cooked
Lettuce	0.32±0.03[a]	0.31±0.01[a]	2.17±0.21[a]	1.58±0.18[b]	0.62±0.04[a]	0.54±0.03[b]
Amaranth	1.18±0.03[a]	0.02±0.05[b]	4.26±0.23[a]	2.16±0.21[b]	0.38±0.03[a]	0.34±0.02[b]
Spinach	0.46±0.04[a]	0.35±0.03[b]	9.87±0.47[a]	6.37±0.38[b]	0.56±0.04[a]	0.55±0.05[a]
Leek	0.59±0.05[a]	0.54±0.02[b]	4.72±0.36[a]	2.21±0.29[b]	0.71±0.12[a]	0.62±0.14[b]

Analysis performed in triplicate. Results presented as mean±standard deviation; Different superscript letters between columns indicate significant differences between treatments p<0.05

Table 3: Tannin and phytate contents of cereals and legumes

	Tannins (g/100 g)		Phytate (g/100 g)	
	Raw	Cooked	Raw	Cooked
Legumes				
Faba bean	0.62±0.12[a]	0.58±0.17[a]	0.84±0.17[a]	0.82±0.42[a]
Azuki bean	0.38±0.08[a]	0.35±0.05[a]	0.92±0.27[a]	0.87±0.35[a]
Mung bean	0.43±0.06[a]	0.41±0.06[a]	0.68±0.21[a]	0.65±0.31[a]
Soybean	0.36±0.03[a]	0.34±0.03[a]	0.76±0.19[a]	0.68±0.24[a]

Analysis performed in triplicate. Results presented as mean±standard deviation; Different uppercase superscript letters between columns indicate significant differences between treatments p<0.05

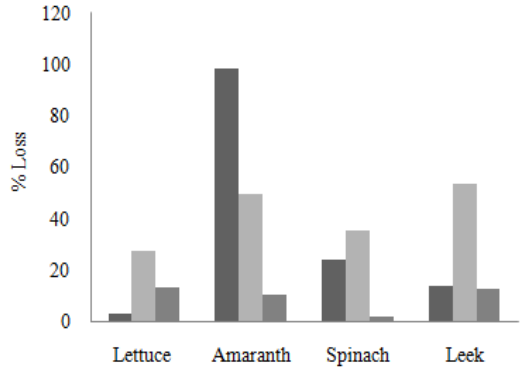

Fig. 1: Average loss of oxalate, phytates and tannins on boiling of green leafy vegetables

Fig. 1, phytates losses were below 12% but the loss of oxalates and tannins was up to 49.3 and 98.3% in amaranth, respectively.

The higher percentage of oxalate reduction during boiling may be due to its solubility in boiling water. Boiling may cause considerable skin rupture and facilitate the leakage of soluble oxalate into the cooking water; this may be why a high reduction in oxalate level upon boiling was observed.

Savage *et al*. (2000) found a similar oxalate content in raw spinach. The moderate effect of blanching on oxalate content indicates that blanching, as the sole treatment and if not applied excessively, is not sufficient to obtain a spinach product compatible with a low-oxalate diet (Betsche and Fretzdorff, 2005). Judprasong *et al*. (2006) found that there was a significant reduction in total oxalate in vegetables due to cooking by boiling (18-76%); our results are in agreement with this research.

Legumes: In general, legumes had lower contents than green leafy vegetables. Boiling also decreased the total iron content in legumes but it did not affect iron bioavailability in most of the samples from this group. Iron bioavailability was positively affected by boiling faba bean (Table 1), Martínez *et al*. (1998) reported that the *in vitro* availability of iron in cooked Phaseolus vulgaris averaged 4.1-9.0%; in faba bean, the content of bioavailable iron found in this study was 3.47 and 3.68% for raw and cooked beans, respectively.

Table 3 presents the content of tannins and phytates in legumes; as may be observed, the content of legumes was reduced by boiling. The reduction of tannins content ranged from 2.4% in mung bean to 7.9% in azuki bean; in the case of phytates, the reduction was up to 10.5% in soybean. The apparent decrease in phytate content during cooking may be partially due to the formation of insoluble complexes between phytate and other components, such as phytate_protein and phytate_protein_mineral complexes, or to the inositol hexaphosphate hydrolyzed to pentaphosphates and tetraphosphates (Bhandari and Kawabata, 2006; Siddhuraju and Becker, 2001). Soaking mayremove 6-28% of phytate and the longer the periods of soaking, the greater losses in the phytate content (Luo and Xie, 2014). Boiling or pressured boiling may also cause a certain loss of phytate. According to Hur *et al*. (2011), cooking and autoclaving do not affect the phytate content in beans.

Loss or reduction of phytates by these procedures seems to be by solubility in the cooking water, although the loss or reduction in tannins and phytates contents was significant; in traditional cooking procedures of legumes in China, although including soaking, maceration and boiling, the cooking water is normally consumed as part of the dish.

Animal products: As is observed in Table 1, the total content of iron in animal products was higher than legumes but lower than leafy vegetables; but, as

Table 4: Phytate/iron molar ratio of leafy vegetables and legumes

	Phytate/iron molar ratio	
	Raw	Cooked
Leafy vegetables		
Lettuce	5.46	4.65
Amaranth	8.89	7.83
Spinach	12.34	11.87
Leek	8.39	7.46
Legumes		
Faba bean	8.43	7.32
Azuki bean	6.55	5.69
Mung bean	9.42	8.73
Soybean	6.42	5.21

expected, iron bioavailability was two to four times higher than any of the other food groups. The total iron content ranged from 8.67 mg/100 g DW for fish to 34.68 mg/100 g DW for chicken. Cooking or boiling of animal products did not affect the total iron content; iron bioavailability was also not affected.

Phytate/iron molar ratio: The phytate/iron molar ratios are used to predict the inhibitory effect on the bioavailability of minerals. A phytate/iron molar ratio >1 is regarded as indicative of poor iron bioavailability (Ma *et al*., 2005). Table 4 summarizes the molar ratio of phytate/iron of the selected food items for this study; all ratios were >1, which indicates that the bioavailability of this mineral would be impaired by phytates present in these foods.

CONCLUSION

Vegetable and animal products normally found in the China diet had significant total iron content; however, boiling decreased the total iron content in vegetables, whereas in animal products the treatment of cooking did not significantly reduce it. Phytate contents of leafy vegetables and legumes and calculated phytate to iron molar ratios predict poor iron bioavailability and therefore a negative impact on the nutritional status of people who rely on them as staple foods.

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