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ORIGINAL ARTICLE

Effect of soaking and sprouting on iron and zinc availability in green and white faba bean (*Vicia faba* L.)

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Abstract The changes in phytate, phytase activity and in vitro availability of iron and zinc during soaking and sprouting of green and white faba bean (*Vicia faba* L.) were investigated. Faba bean were soaked for 24 h and germinated for 72 h after soaking for 24 h to reduce phytate content and increase iron and zinc in vitro availability. The results revealed that iron and zinc content was significantly reduced from 28.2 to 39.8 % and 12.5 to 27.6 % for soaking treatment and 38.2 to 38.9 % and 24.5 to 29.2 % for sprouting treatment, respectively. Phytate content was significantly reduced from 26.9 to 32.5 % for soaking treatment and 28.0 to 34.9 % for sprouting treatment, respectively. The results proved that the main distinct point is the change of phytase activity as well as specific activity during different treatment which showed no significant differences between the green and white faba bean. The in vitro availability of iron and zinc were significantly improved as a result of soaking and sprouting treatments.

Keywords Soaking · Sprouting · Faba bean · Iron · Zinc availability

Introduction

The faba bean is one of the oldest crops that ranks sixth in production among the different legumes grown in the world. Faba beans are a good source of energy, proteins, vitamins, minerals and dietary fibers. They are relatively inexpensive compared to meat foods and they have a high carbohydrate content (50–65 %). In China, plant foods provide at least 50 % of the dietary energy and nutrients, and faba bean is one of the most important legumes (Ma et al. 2005). Faba beans are a good source of dietary minerals, such as phosphorus, potassium, calcium, sulphur, zinc and iron.

Phytic acid (myoinositol 1, 2, 3, 4, 5, 6 hexakidishydrogen phosphate) is common in faba beans and is the principal storage form of phosphorus in many dry beans. The typical phytic acid content in faba beans is 8.58 $mg\,g^{-1}$. nutritional concern about the presence of phytic acid in dry beans arises from the fact that phytic acid decreases the bioavailability of essential minerals and may possibly interfere in the utilization of proteins due to phytate-protein and phytate-mineral-protein complexes.

Iron (Fe) and zinc (Zn) are essential trace elements in human nutrition and their deficiencies are major public health threats worldwide. Among the micronutrient malnutrition situations afflicting the human population, Fe and Zn deficiencies are of major concern not only because of the serious health consequences they may have, but also because of the number of people affected worldwide particularly in China.

In view of the anti-nutritional effects of phytate, many attempts were carried to reduce it. Soaking is a domestic technological treatment that is often used by mothers to prepare complementary foods at home. Previous studies (Mubarak 2005; Vijayakumari et al. 2007) have shown that soaking significantly reduced phytate, trypsin inhibitor activity, and tannins contents. Temperature and pH value have been shown to have a significant effect on enzymatic phytate hydrolysis during soaking. If the soaking step is carried out at temperatures between 45 and 65 °C and pH values between pH=5.0 and 6.0, which are close to the optimal conditions for phytate dephosphorylation by the

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intrinsic plant phytases, a significant percentage of phytate (26–100 %) was enzymatically hydrolysed (Greiner and Konietzny 1999).

Sprouting is the practice of soaking, draining and leaving seeds until they germinate and begin to sprout. It has been identified as an inexpensive and effective technology for improving the nutritional quality of cereals and grain legumes. As water is introduced, enzyme inhibitors are disabled and the seed explodes to life (Bau et al. 1997). As germination proceeds, and enzymes trigger elaborate biochemical changes (Zielinski et al. 2005). According to Lorenz (1980) the practice of sprouting of cereal grains and legume has become popular in the western world. They can be used in many different foods including breakfast items, salads, soups, casseroles, pasta, and baked products.

Despite the importance of faba bean for human nutrition, the opportunities and limitations of soaking and sprouting for the improvement of mineral availability in faba bean have not yet been clarified. The objective of this study was to eliminate the phytate content associated with faba bean and improve iron and zinc availability by using simple methods.

Materials and methods

Materials

Faba beans Faba bean seeds with green or white hull (cultivated in Jiangsu Province and harvested in 2011) were collected from local market of the same batch in Nanjing, Jiangsu Province, P.R. China. The seeds were cleaned by hand to remove the foreign materials and then stored in polyethylene bags at room temperature (25 °C) until further use.

Pepsin, bile extracts, pancreatin, lipase were purchased from Sigma– Aldrich Chemical Co. (Sigma Chem, Co, St Louis, MO). All chemicals used were of analytical grade. Acid-washed glassware were used throughout the study.

Soaking of faba bean

Faba bean seeds were soaked in distilled water for 24 h with a ratio 1:5 w/v and the soaked water changed twice. At the end of soaking period, the soaked water was discarded. The seeds were rinsed twice in distilled water and the grains were dried at 45 °C. Flours of faba bean was prepared in a hammer-mill type grinder (HY-04B, Beijing Xinhuanya, China) and sieved through a 1 mm screen. All samples were analyzed in triplicate. The beans were kept at −18 °C until analysis.

Sprouting of faba bean

Soaked seeds were germinated for 72 h at room temperature 25 °C. The seeds were germinated in trays on moist filter papers with the water solution, which, as needed, was added during the course of germination. And then germinated seed samples were freeze-dried and stored at room temperature in airtight containers prior to chemical analysis. The root portions were manually removed. Flours of faba bean was prepared in a hammer-mill type grinder (HY-04B, Beijing Xinhuanya, China) and sieved through a 1 mm screen. The beans were kept at −18 °C until analysis.

Chemical analysis

Iron and zinc determination

Iron and zinc contents in materials were analysed by atomic absorption spectrophotometry (Varian SpectrAA 200, Victoria, Australia) after dry ashing 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.

Phosphorus and phytate determination

The colorimetric method AOAC 995.11 (Horwitz 2000) was used to determine total phosphorus levels after ashing of the sample with 1 N HCL. Acid soluble phosphate forms a blue complex with sodium molybdate in the presence of ascorbic acid as reducing agent. The intensity of blue colour was measured spectrophotometrically at 823±1 nm (7200, Unico, Shanghai, China).

Phytate 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 H_2O was dissolved in 100 ml 2 N HCl and volume made up to 1,000 ml with distilled water). The phytate was precipitated with an acidic iron-III-solution of known iron content. Phytate 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 1,000 ml) at 419 nm.

Phytase activity assay

Extraction of phytase

Phytase activity assayed according to the procedure described by Barrientos et al. (1994) and modified. Sample (2 g) was added to ice cold Buffer (16 ml of 10 mM Tris–HCl, pH7.0, containing reduced glutathione, 0.5 mM). The suspension was stirred with a glass rod. Solid cetylpyridinium bromide (80 mg, finalconcentration 0.5 %w/v) was added to the suspension. The suspension was homogenized

with homogenizer at 27,000 rpm for 261 min. with a 1 min delay in-between. The resulting crude homogenate was centrifuged at 10,000 g for 30 min. The supernatant containing phytase activity was collected.

Alkaline phytase assay

Alkaline phytase activity was assayed by measuring the inorganic phosphate (Pi) released by the enzyme. The assay mixture contained Tris–HCl buffer (100 mM, pH 8.0), NaCl (0.5 M), $CaCl_2$ (1 mM), sodium phytate (1 mM), NaF (10 mM), and an aliquot of enzyme solution in a total volume of 250 ml. The assay mixture was incubated at 37 °C for 1 h and the reaction was stopped by the addition of 50 ml of 50 % TCA. In brief, ammonium molybdate solution (700 ml of a 1:6 solution of 10 %w/v ascorbic acid and 0.42 % ammonium molybdate (w/v) in 0.5 M H_2SO_4) was added and the solution was incubated at 37 °C for 1 h. Absorbance at 820 nm was measured and the inorganic phosphate concentration was determined from a calibration curve using KH_2PO_4 as the standard. One unit of enzyme is defined as the amount of enzyme that releases 1 mmol of Pi from sodium phytate per minute under these conditions.

Acid phytase assay

Acid phytase activity was assayed in a solution containing sodium acetate buffer (100 mM, pH5.0), sodium phytate (1 mM), and $CaCl_2$ (1 mM). NaF was not added to this assay mixture. The assay mixture was incubated at 37 °C for 1 h and the reaction was stopped by the addition of 50 ml of 50 % TCA. Pi released in the reaction was quantified as described above. Soluble protein was determined according to Lowry et al. (1951) and specific activity was defined as unit per milligram protein.

In vitro availability of iron and zinc

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 5,000*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.

Statistic analysis

The experiments were conducted in triplicates. 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

Changes in iron, zinc and phytate content, phosphorus during soaking and sprouting of green and white faba bean

From Table 1, it could be noticed that the Fe content ranged between 3.5 and 4.7 mg/100 g raw faba bean, while the Zn

Table 1 Changes in iron and zinc during soaking and sprouting of green and white faba bean

Treatments	Fe (mg/100 g DW)	Phy/Fe molar ratio	In vitro iron availability %	Zn (mg/100 g DW)	Phy/Zn molar ratio	In vitro zinc availability %
Raw						
Green	4.7±0.27^{a}	15.2	32.2±1.22^{c}	3.3±0.23^{b}	25.3	31.6±1.22^{d}
White	3.5±0.18^{b}	20.7	28.6±1.83^{c}	3.9±0.21^{a}	21.6	33.4±1.44^{cd}
Soaking						
Green	3.4±0.21^{b}	15.4	50.5±2.11^{b}	2.9±0.18^{c}	21.1	38.4±2.13^{c}
White	2.2±0.17^{d}	22.2	58.8±2.62^{a}	2.8±0.16^{c}	20.1	44.2±1.72^{b}
Sprouting						
Green	2.9±0.19^{c}	17.7	51.2±2.32^{b}	2.5±0.17^{d}	24.1	49.3±1.67^{b}
White	2.2±0.15^{d}	22.0	58.9±2.54^{a}	2.8±0.23^{c}	19.8	58.7±1.55^{a}

Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at $P<0.05$

DW dry matter

contentranged between 3.3 and 3.9 mg/100 g raw faba bean. The same result was observed by Ma et al. (2005) who reported that Fe concentration of the faba bean grains ranged from 3.0 to 11.3 mg/100 g. The Zn concentration ranged from 1.1 to 4.4 mg/100 g.

After soaking, the Fe content of the faba bean was significantly lower than raw faba bean ($P<0.05$). After soaking, the losses of Fe contents were 28.2 and 39.8 %. These finding are incontrast with the findings of Lestienne et al. (2005) who reported that up to 40 % of Fe content of sorghum grain may be lost as a result of soaking. As for sprouting, the Fe content of the faba bean was significantly reduced by 38.2 and 38.9 % ($P<0.05$).

Lestienne et al. (2005) found that the zinc content also decreased significantly, but the reduction did not exceed 30 % except on Zn content of Shandweel-6. Reduction after soaking may be attributed to leaching of iron and zinc ions into the soaking medium. The leaching of zinc was lower than iron and this phenomenon may be due to the fact that zinc and iron are not located in the same place in the seeds nor are they linked with the same molecules. Indeed, zinc is found in a large number of enzymes and other proteins, where it plays an important structural role (Lestienne et al. 2005).

The phytate contents before and after treatments are shown in Table 2. Phytate content varied from 836.2 to 857.6 mg/100 g DW of raw faba bean. These values are close to those reviewed by Greiner and Konietzny (2006) whom found that faba bean phytate ranged from 590 to 1,180 mg/100 g dwt. Depending on the amount of plant derived foods in the diet and the grade of food processing, the daily intake of phytate can be as high as 4,500 mg. On average, daily intake of phytate was estimated to be 2,000–2,600 mg for vegetarian diets (Reddy and Sathe 2002).

After soaking and sprouting there was a 26.9–32.5 % and 28.0–34.9 % decrease in phyate content, respectively. These findings are in range of the findings in previous studies found that soaking, germination, mashing, boiling and fermentation strongly reduced the phytate content and is more effective if whole grains are used (Luo et al. 2009). The magnitude of reduction induced by soakingin this study can be explained by the leaching in soaking medium or by partial hydrolysis by endogenous phytase. The reduction in phytate caused by soaking may be due to water solubilization of some phytic acid salts. Also, phytate content in the sorghum flour was significantly ($P<0.05$) reduced in all processed samples, eg soaking, boiling and fermentation (Towo et al. 2006). In addition, germination activates endogenous grain phytase which can degrade phytate (Kayode et al. 2007). During germination, phytins are broken down by endogenous phytase enzymes, releasing their P, myo-inositol (hereafter referred to as 'inositol') and mineral contents for use by the growing seedling.

As shown in Table 2, revealed that the values of total phosphorus of raw faba bean were 386.8 and 357.6 mg/100 g DW. After soaking and germination the total phosphorus content was decreased from 298.4 to 361.5 and 201.6 to 227.2 mg/100 g dwt, respectively. Phytate phosporus were 173.7 and 182.1 mg/100 g DW. These findings are in range of the findings by Radhakrishnan and Sivaprasad (1980) and Godoy et al. (2005).

Effect of soaking and sprouting of green and white faba bean on phytate (iron and zinc) molar ratios and phytases (acid and alkaline) activities

The effect of soaking and sprouting of raw faba bean on phy/ Fe and phy/Zn molar ratios were determined (Table 1). Phy/Fe and phy/Zn molar ratios were associated with iron and zinc absorbtion capacity. It could be noticed that the phy/Fe molar ratios were 15.2 and 20.7 for raw green and

Table 2 Changes in phytate content, total phosphorus and phytate phosphorus during soaking and sprouting of green and white faba bean

Treatments	Phytate content mg/100 g DW	Total phosphorus mg/100 g DW	Phytate phosphorus mg/100 g DW	Percentage PP/TP %
Raw				
Green	836.2±15.62^{b}	386.8±13.54^{a}	173.7±12.22^{a}	44.9
White	857.6±14.24^{a}	357.6±12.82^{b}	182.1±11.63^{a}	50.9
Soaking				
Green	611.4±12.31^{c}	361.5±13.12^{b}	123.1±11.34^{b}	34.1
White	578.9±13.13^{d}	298.4±12.65^{c}	126.8±12.38^{b}	42.5
Sprouting				
Green	602.3±16.24^{c}	227.2±11.64^{d}	115.9±11.41^{c}	51.0
White	558.4±14.87^{d}	201.6±12.13^{e}	110.9±13.15^{c}	55.0

Values are mean of three replicates ±SD, number in the same column followed by the same letter are not significantly different at $P<0.05$

DW dry matter

TP total phosphorus

PP phytate phosphorus

white faba bean. While the phy/ Zn ratio were 25.3 and 21.6 in raw green and white faba bean. Our conclusion proved that soaking and sprouting increased the phy/Fe molar ratio (20.7–22.2 and 15.2–17.7) while the phy/Zn molar ratio decreased (25.3–21.1 and 21.6–19.8), respectively. In fact there was an increase in Phy/Fe molar ratio after soaking, because of the decrease in the iron content. Our results reinforce previous results that showed that the bioavailability of zinc in cereals and legumes would be lower than that in vegetables and in some roots and tubers whose Phy/ Zn molar ratios are generally less than 20 (Adeyeye et al. 2000). Kayode et al. (2006) calculated the Phy /Fe and Phy /Zn molar ratios as an index for the potential mineral bioavailability. Also, faba bean phytate was hydrolyzed during sprouting, so that iron solubility under simulated physiological conditions was greatly increased. It is somewhat difficult to predict the overall impact of soaking or sprouting on iron solubility. Soaking or sprouting might be effective in reducing the phytate content of faba bean.

The activities of phytases (acid and alkaline) before and after treatments are shown in Table 3. The data showed significant differences between activity of acid and alkaline phytase and no significant increase in acid and alkaline phytase activities after soaking and sprouting. Phytase enzymes will be activated during drying in equal form in green and white faba bean. Therefore the main distinct point is the change of phytase activity as will as specific activity during different treatment which showed no significant differences between the green and white faba bean. These finding are in agreement with the findings of Marero et al. (1991) who reported that phytate has been degraded in cereal foods by adding phytases or by activating endogenous phytase by a combination of soaking, germination and fermentation which is of a similar order of magnitude as observed by us. Also, humans have negligible intestinal phytase activity Kumar et al. (2010), even if they usually consume high phytate diets. Legumes, however, contain an endogenous phytase. Because the endogenous legume phytase has a pH optimum of 5.15, it is probably inactivated in the low pH of the stomach. Thus, there has been some interest in reducing the phytate content of legumes by soaking or germination (which activate endogenous phytase), or by adding a commercial phytase enzyme (Luo et al. 2010). Soaking under optimal conditions activates naturally occurring phytases in cereals and results in varying degrees of phytate hydrolysis depending on the kind of legumes.

Most plant grains and seeds exhibit phytate-degrading activity over a wide pH range (pH=3–10) (Greiner and Konietzny 2006) with maximal activity at pH values from pH=5–5.5. Compared to cereals, legumes exhibit, a significantly higher phytate-degrading activity in the pH range from pH=5–5.5 (Egli et al. 2002; Steiner et al. 2007), whereas phytate-degrading activity at pH=8.0 was slightly lower in legumes compared to cereals (Greiner and Konietzny 2006). To understand phytate hydrolysis it is important to recognize and account not only for phytase activity, but also for activities of further phosphatases present in the plant material. Per definition all enzymes capable of dephosphorylating phytate are classified as phytases. However, myo-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates, the products of phytase action on phytate, might be further dephosphorylated during food processing by phytases as well as phosphatases which do not accept phytate as a substrate. Regarding the specific activity (unite/mg protein), data showed significant differences between activity of acid and alkaline phytase and non significant decrease in acid and alkaline phytase activities after soaking and germination.

Effect of soaking and sprouting of green and white faba bean on in vitro iron and zinc bioavailability

In vitro iron and zinc bioavailability before and after soaking and sprouting are shown in Table 1. It could be noticed

Table 3 Effect of soaking and sprouting of green and white faba bean on acid and alkaline phytase activity and specific activity

Treatments	Acid Phytase activity (unite/g DW)	Alkaline Phytase activity (unite/g DW)	Acid Phytase specific activity unit/mg protein	Alkaline Phytase specific activity unit/mg protein
Raw				
Green	2.1±0.07[a]	1.6±0.02[b]	0.26±0.005[a]	0.19±0.002[b]
White	2.0±0.05[a]	1.7±0.02[b]	0.24±0.007[a]	0.17±0.004[b]
Soaking				
Green	2.1±0.05[a]	1.4±0.01[b]	0.19±0.002[a]	0.17±0.007[b]
White	2.1±0.05[a]	1.5±0.01[b]	0.22±0.003[a]	0.15±0.003[b]
Sprouting				
Green	2.1±0.04[a]	1.7±0.02[b]	0.23±0.004[a]	0.16±0.002[b]
White	2.1±0.04[a]	1.7±0.02[b]	0.22±0.003[a]	0.15±0.004[b]

Values are mean of three replicates ±SD, number in the same column or row followed by the same letter are not significantly different at $P<0.05$

DW dry matter

that the in vitro iron and zinc availability ranged between 28.6–32.3 and 31.6–33.4 % for green and white faba bean. The in vitro iron and zinc bioavailability after soaking and sprouting increased (50.5–58.8 and 38.4–44.2 for soaking treatment and 51.2–58.9 and 49.3–58.7 for germination treatment). The bioavailability of iron and zinc were significantly improved as a result of soaking and sprouting treatments especially for white bean. Also, phytase enzymes break down inositol hexa and penta phosphates, which inhibit iron absorption to smaller inositol phosphates and inorganic phosphate, which do not affect iron absorption. Soaking of wheat bran increased the soluble iron content from less than 5 % to over 50 % by destroying practically all their phytate thereby enhancing in vitro iron availability (Sandberg and Svanberg 1991). Two common inhibitors of Fe absorption are tannins and phytate. These components form complexes with Fe within the intestinal lumen, reducing Fe bioavailability (Elkhalil et al. 2001). Some antinutritional factors chelate dietary mineral in the gastrointestinal tract reducing their bioaccessibility and bioavailability. Processing techniques such as soaking, cooking, germination and fermentation have been found to reduce significantly the levels of phytates and tannins by exogenous and endogenous enzymes formed during processing. Iron bioavailability is low due to high levels of dietary phytates and fibers in vegetarian diets (Troost et al. 2003). Vegetarian meals have a poor bioavailability of zinc, and these diets may or may not have low zinc content.

Conclusions

Soaking and sprouting decreased phytate content in green and white faba bean. The in vitro availability of iron and zinc were significantly improved as a result of soaking and sprouting treatments.

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