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# Effects of Phytase, Cellulase, and Dehulling Treatments on Iron and Zinc *in Vitro* Solubility in Faba Bean (*Vicia faba* L.) Flour and Legume Fractions

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Simulations of gastrointestinal digestion were used to try to identify the nature of the complexes between antinutritional factors and iron and zinc in faba bean and legume fractions. In digestible residue of raw faba bean flour, simultaneous action of cellulase and phytases made it possible to release about 28% units more iron than that released with the treatment without enzymes. About 49.8% of iron in raw faba bean flour was solubilized after *in vitro* digestion and simultaneous action of cellulase and phytase. In the hull fraction, the action of phytases and the simultaneous action of cellulase and phytase allowed about 7 and 35% units of additional zinc to be solubilized, respectively. Single enzymatic degradation of phytates from dehulled faba bean allowed solubilization from 65 to 93% of zinc, depending upon the treatment. In dehulled faba bean, iron was chelated by phytates and by fibers, whereas zinc was almost exclusively chelated by phytates. In the hull of faba bean, a high proportion of iron was chelated by iron-tannins, while the rest of iron as well as the majority of zinc were chelated in complexes between phytates and fibers.

**KEYWORDS: Solubility; iron; zinc; faba bean; fractions**

## INTRODUCTION

The bioavailability of iron and zinc from foods is defined as the proportion of the iron and zinc that can be absorbed and utilized within the body. The solubility of iron and zinc, the pH of the intestinal lumen, dietary factors, and the retention time at the digestion and absorption site influence the bioavailability of iron and zinc (*1*). Furthermore, mineral absorption is also influenced by the level of mineral content and by factors that enhance their absorption in the diet, as well as by the physiological status of the subjects, such as age, disease, or stores of mineral (*2*). The solubility of iron and zinc could be predicted by molar ratios of phytate to iron and zinc, HCl (hydrochloric acid)-extractability, and *in vitro* solubility of iron and zinc. In legume-based foods, the availability of iron and zinc 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 (*3*). 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 (*4*). 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 (*5*) and condensed tannins (*6*), 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. However, this effect of pH on the nature of complexes has not been highlighted for tannin−protein or tannin−mineral−protein complexes.

Because of the low iron and zinc bioavailability in legume-based foods and the importance of faba bean consumption in China (*7*), in the first step, we studied the iron *in vitro* availability in faba bean by different processing methods and found that soaking, germination, and fermentation can decrease phytic acid content in faba bean. The most effective approach, which was accelerated fermentation (*3*), could reduce 7.02 mg $g^{-1}$ of total phytic acid and could decrease the molar ratio of phytic acid to iron below 5. However, results from *in vitro* solubility measurement of iron showed little improvement in fermented faba beans over untreated raw faba bean. This could result from the presence of components such as dietary fiber leading to the formation of insoluble iron complexes (*8*).

The aim of the present paper was to try to identify the nature of the complexes between antinutritional factors and iron and zinc in faba bean. To this aim, we evaluated the effect of the action of fiber- and/or phytate-degrading enzymes on the solubilization of iron and zinc from insoluble residues obtained after *in vitro* digestion (a) of faba bean flour with decreased phytate contents and (b) of two faba bean fractions (dehulled faba beans and hulls) with low or high tannin and fiber contents, after total phytate degradation or not.

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**Table 1.** Preparation of Faba Bean Flours with Decreased Phytate Content and Faba Bean Fractions Flour with Low and High Fiber and Tannin Contents

| nomenclature | treatment |
|---|---|
| endogenous phytases (1 h) | phytate degradation by endogenous phytases for 1 h |
| endogenous phytases (3 h) | phytate degradation by endogenous phytases for 3 h |
| exogenous phytases (1 h) | phytate degradation by endogenous and exogenous phytases for 1 h |
| exogenous phytases (3 h) | phytate degradation by endogenous and exogenous phytases for 3 h |
| dehulled faba bean | no |
| dephytinized dehulled faba bean | total phytate degradation by endogenous and exogenous phytases |
| hull | no |
| dephytinized hull | total phytate degradation by endogenous and exogenous phytases |

**Table 2.** Phytate, Tannin, Insoluble Dietary Fiber (IDF) and Soluble Dietary Fiber (SDF) Contents of Faba Bean Flours and Legume Fractions[a]

| faba bean flours and legume fractions | phytate[b] | tannin[b] | IDF[b] | SDF[b] |
|---|---|---|---|---|
| raw | 0.84 ± 0.02 b | 0.64 ± 0.02 b | 9.65 ± 0.25 b | 4.63 ± 0.21 b |
| endogenous phytases (1 h) | 0.43 ± 0.02 d | 0.52 ± 0.01 c | 10.52 ± 0.36 b | 4.12 ± 0.23 b |
| endogenous phytases (3 h) | 0.27 ± 0.03 e | 0.45 ± 0.03 c | 10.95 ± 0.24 b | 3.85 ± 0.24 b |
| exogenous phytases (1 h) | 0.28 ± 0.01 e | 0.36 ± 0.01 d | 10.25 ± 0.31 b | 4.24 ± 0.20 b |
| exogenous phytases (3 h) | 0.02 ± 0.02 f | 0.24 ± 0.01 e | 10.87 ± 0.24 b | 3.54 ± 0.19 b |
| dehulled faba bean | 0.93 ± 0.01 a | 0.26 ± 0.02 e | 3.24 ± 0.18 c | 2.38 ± 0.24 c |
| dephytinized dehulled faba bean | 0.02 ± 0.00 f | 0.14 ± 0.01 f | 3.25 ± 0.20 c | 2.41 ± 0.12 c |
| hull | 0.63 ± 0.03 c | 1.28 ± 0.03 a | 18.11 ± 0.57 a | 6.24 ± 0.32 a |
| dephytinized hull | 0.02 ± 0.00 f | 0.51 ± 0.03 c | 19.39 ± 0.61 a | 6.78 ± 0.34 a |

[a] Values are mean ± standard deviation, three determinations on a dry matter basis, mg/100 g, DM. [b] Values within a column without common superscripts are significantly different ($P < 0.05$).

## MATERIALS AND METHODS

**Materials.** Two kinds of samples were prepared: (1) samples with different levels of phytates and (2) samples with high and low fiber and tannin contents. **Table 1** summarizes their treatments as well as their nomenclatures.

**Faba Bean Flours with Decreased Phytate Content.** faba beans of the same batch (Qidou 2, cultivated in Jiangsu Province and harvested in 2008) were collected from a local market in Nanjing, Jiangsu Province, P. R. China. The mean moisture, protein, carbohydrate, fat, and ash content were 12.6%, 21.2%, 55.4%, 0.4%, and 3.2%, respectively. Flours of faba bean were prepared in a hammer-mill type grinder (HY-04B, Beijing Xinhuanya, China) and sieved through a 1 mm screen.

Samples with decreased phytate contents were obtained by incubating whole faba bean flour in 0.1 M acetate buffer pH 5.0 at the ratio 1:3 (w/v), with or without exogenous phytases, for a short or a long period, at 55 °C under low shaking (60 rpm) in an incubator. Incubation in acetate buffer allowed moderate phytate degradation by endogenous phytases, while addition of exogenous phytases allowed a greater degree of degradation. A phytase from wheat (Sunson, Beijing, China) was used, with an activity of 6000 U $g^{-1}$, optimum temperature 55 °C, and optimum pH 5.5. Phytase was mixed in 0.1 M acetate buffer, pH 5.0 at 500 U $L^{-1}$. The same conditions were used for incubations with both endogenous and exogenous phytase because pH and temperature may influence leaching phenomena. The pH and temperature conditions for incubations (pH 5.0, 55 °C) were fixed after confirmation that they were permitted to be active, given their different pH and temperature optima (pH 5.0 and 50−55 °C for faba bean and pH 5.0 and 55 °C for wheat). Next, faba bean flour was incubated for a short period (1 h) and for a long period (3 h) to obtain samples containing different levels of phytates and, in a further case, to achieve total phytate degradation in the sample incubated for the long period with exogenous phytases. At the end of the incubation period, the mixtures were cooled to 4 °C and flours were separated by centrifugation at 5000*g* for 15 min. The pellets were then freeze-dried and milled with a laboratory pestle and mortar.

**Faba Bean Fractions with Low and High Fiber and Tannin Contents.** Two faba bean fractions were obtained by dehulling hull from whole faba bean, on one hand; dehulled faba bean with low fiber and tannin contents; and, on the other hand, hull with high fiber and tannin contents. Dehulling was done by using a laboratory dehuller (TM 050, Satake, Stockport, U.K.). Flours of fractions were prepared in a hammer-mill type grinder (HY-04B, Beijing Xinhuanya, China) and sieved through a 1 mm screen.

**Dephytinized Faba Bean Fractions.** Thirty grams of each fraction were incubated with the previously described exogenous phytase solution for 4 h at 55 °C under low shaking (60 rpm) in an incubator to obtain total phytate degradation. At the end of the incubation period, the mixtures were cooled to 4 °C and flours were separated by centrifugation at 5000 *g* for 15 min. The pellets were then freeze-dried and milled with a laboratory pestle and mortar.

**Analytical Methods.** *Phytate*. Phytate contents were determined by the method of Haug and Lantzsch (*9*). 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) sulfate−12 $H_2O$ was dissolved in 100 mL of 2 N HCl, and volume was made up to 1000 mL with distilled water). Phytate content in the supernatant was measured as the decrease in absorbance of iron content using 2,2-bipyridine (Dissolve 10 g of 2,2′-bipyridine and 10 mL of thioglycollic acid in distilled water and make up to 1000 mL) at 419 nm.

*Dietary Fiber Determination*. Mes-Tris AOAC method 991.43 was used for DF determination (*10*). Two replicates of each sample were taken to complete the six-sample analysis method. The principle of the method was based on the use of three enzymes (heat-stable α-amylase, protease, and amyloglucosidase) under different incubation conditions in order to remove starch and protein components. Dietary fiber fractions were obtained as indigestible residues after enzymatic digestion of nondietary fiber components; the insoluble residues were isolated by filtration, and soluble fiber was precipitated with ethanol. Dried residues correspond to insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), respectively. Determination of residual ash and proteins (as Kjeldahl N x 5.40) was carried out in the residues for corresponding corrections. Total dietary fiber (TDF) was calculated as the sum of IDF and SDF. Kjeldahl nitrogen and ash contents were assayed according to standard procedures (*10*).

*Determination of Tannin Content*. Total tannin was determined by the method of Anonymous (*11*). 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 analyzed for total phenolics by spectrophotometrical methods using the Folin-Ciocalteu's phenol reagent. Total phenolic compounds were calculated from a prepared standard curve of tannic acid under the same set of conditions. Tannin was complexed with polyvinylpolypyrrolidone (Sigma), and unbound nontannin phenolics were determined as above (*11*). Total tannin was calculated by subtracting nontannin phenolics from total phenolics. The phytate, tannin, insoluble dietary fiber (IDF), and soluble dietary fiber (SDF) contents of faba bean flours and legume fractions are shown in **Table 2**.

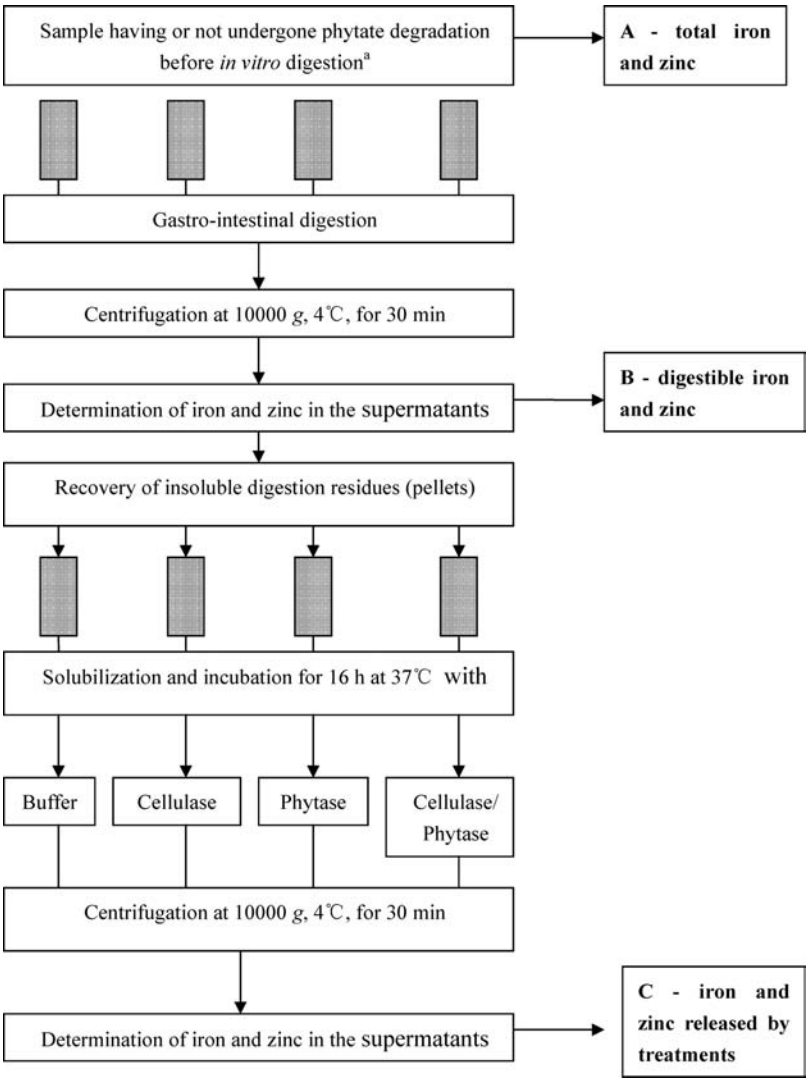


**Figure 1.** Experimental design. For the totally dephytinized samples, only the treatments with buffer and cellulose solution were carried out.

*Total Iron and Zinc*. Iron and zinc contents in materials were analyzed 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.

*In Vitro Soluble Iron and Zinc Contents*. *In vitro* soluble iron and zinc was defined as the relative amount of iron and zinc that becomes soluble after enzymatic treatment. faba bean 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. (*12*) . Mixtures were centrifuged at 5000*g* for 15 min at 4 °C. The resulting supernatant was filtered (0.45 $\mu$m 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 analyzed by atomic absorption spectrophotometry. Each sample was enzymatically extracted in duplicate. *In vitro* soluble iron and zinc contents were determined on three independent digests.

*Treatments of the Insoluble Residues of in Vitro Digestion*. To identify the factors responsible for inhibiting intestinal absorption of iron and zinc, the insoluble *in vitro* digestion residues were incubated with phytase and/or cellulase. A solution of cellulase (Sunson, Beijing China) at 12000 units/L (120 units/g of sample), a solution of phytase from wheat (Sunson, Beijing China) with an activity of 6000 units/L (500 units/g of sample), and a solution of cellulase and phytases (containing the same quantities of enzyme as the preceding enzyme solutions) were prepared in 0.1 M acetate buffer at pH 5.0. The different treatments applied to the insoluble *in vitro* digestion residues of a sample are summarized in **Figure 1**.

A total of 12 independent gastrointestinal digestions were carried out per sample to obtain 12 insoluble residues on which three replications of each of the four different treatments were carried out: incubation in 0.1 M acetate buffer at pH 5.0 without the enzyme, in cellulase solution, in phytase solution, or in cellulase and phytase solution. Each residue, obtained from 2 g of sample, was solubilized in 20 mL of solution. After homogenization, the mixtures were incubated under magnetic stirring (100 rpm) in a shaking water bath at 37 °C for 16 h. Suspensions were then centrifuged at 10000*g* for 30 min at 4 °C. The supernatants were recovered in silica caps to determine the quantities of iron and zinc released by each treatment using atomic absorption spectrophotometry.

**Statistical Analysis.** The quantities of iron and zinc [total (A), digestible (B), and released by the different treatments (C)] were expressed as percentages of dry matter (**Figure 1**). The mean and standard deviation of means were calculated after three replications of each treatment and calculated from the quantities of released iron or zinc.

The results are presented in two ways: (a) histograms representing iron or zinc solubility of insoluble residues after the different treatments and (b) tables giving iron or zinc solubility after the cumulated effect of *in vitro* gastrointestinal digestion and treatment of the insoluble residues.

The solubility rates shown in the histograms were calculated according to the following equation: solubility H (%) $= \frac{C}{A-B} \times 100$where $A$ is the total quantity of iron (or zinc) in 2 g of sample, $B$ is the quantity of iron (or zinc) solubilized from 2 g of sample during *in vitro* digestion, and $C$ is the quantity of iron (or zinc) released by a treatment after *in vitro* digestion of 2 g of sample.

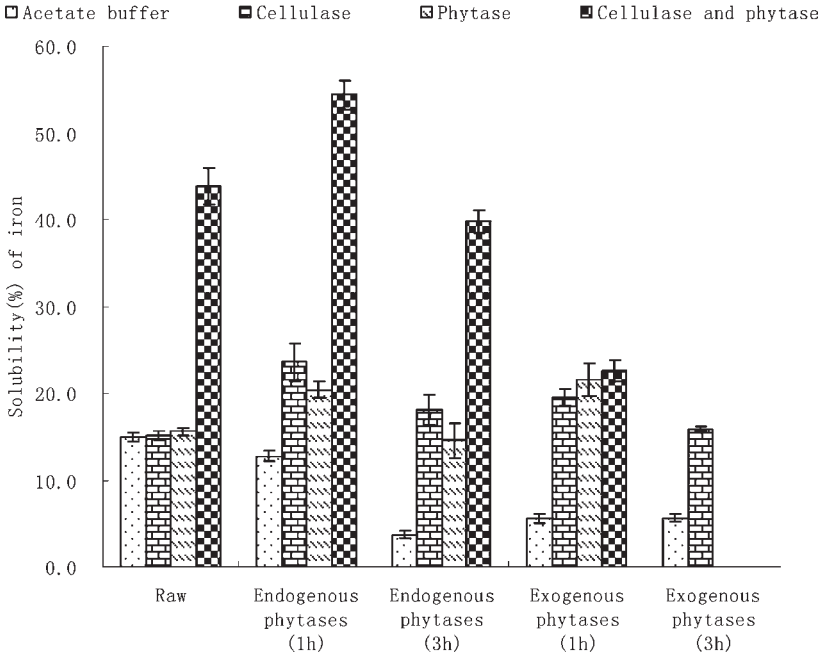


**Figure 2.** Solubility of indigestible iron (%) of faba bean flours after treatment of insoluble digestion residues with acetate buffer, cellulase, phytase, or cellulase in combination with phytase.

**Table 3.** Solubility of Iron (%) after Gastrointestinal Digestion and Treatment of Insoluble Residues of Faba Bean Flours with Decreased Phytate Contents[a]

| faba bean flours | digestion + buffer | digestion + cellulase | digestion + phytase | digestion + cellulase and phytase |
|---|---|---|---|---|
| raw | 32.1 ± 1.3 b | 32.6 ± 1.6 b | 32.4 ± 2.5 b | 49.8 ± 2.5 c |
| endogenous phytases (1 h) | 37.4 ± 1.5 a | 45.2 ± 2.8 a | 41.6 ± 2.4 a | 62.5 ± 3.0 a |
| endogenous phytases (3 h) | 35.6 ± 0.8 a | 44.2 ± 2.6 a | 43.1 ± 1.8 a | 57.6 ± 2.4 b |
| exogenous phytases (1 h) | 31.9 ± 2.4 b | 44.5 ± 3.1 a | 42.8 ± 1.9 a | 45.6 ± 2.7 c |
| exogenous phytases (3 h) | 34.2 ± 2.1 ab | 42.3 ± 1.4 a | | |

[a] Values within a column without common superscripts are significantly different ($P < 0.05$).

The solubility rates shown in the tables were calculated according to the following equation: solubility T (%) $= \frac{B+C}{A} \times 100$where $A$ is the total quantity of iron (or zinc) in 2 g of sample, $B$ is the quantity of iron (or zinc) solubilized from 2 g of sample during *in vitro* digestion, and $C$ is the quantity of iron (or zinc) released by a treatment after *in vitro* digestion of 2 g of sample.

Data were analyzed with SPSS 13.0 for windows. The data were analyzed 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

**Effects of the Antinutritional Factors on Iron Solubility.** *Effects of Phytates on Iron Solubility*. In a digestible residue of raw faba bean flour, degradation of fibers or phytates did not release more iron than the treatment with buffer (**Figure 2**). On the other hand, simultaneous action of cellulase and phytases made it possible to release about 28% units more iron than that released with the treatment without enzymes. Thus, only the simultaneous degradation of fibers and phytates allowed the solubilization of some iron, which indicates that part of the indigestible iron is linked to fiber−phytate complexes.

In samples with decreased phytate contents after the action of endogenous phytases, degradation of fibers or phytates allowed solubilization of about 10% units more iron than that solubilized by treatment with buffer, while simultaneous action of the two enzymes led to the release of 42% units more iron. The degradation of phytates before *in vitro* digestion consequently facilitated iron solubilization by phytases and/or cellulase.

With regard to samples with reduced phytate contents after the action of endogenous and exogenous phytases, degradation of fibers or phytates allowed solubilization of a larger quantity of iron than treatment with buffer, but simultaneous action of the two enzymes did not allow the release of additional iron. Thus, it is possible that exogenous phytases are involved in the formation of complexes with iron, which would then not be released by action of either cellulase or phytases.

Results of the total solubility of iron after the cumulated effect of *in vitro* digestion (B) and treatment of insoluble residues (C) also showed inhibition of iron solubilization in samples treated with exogenous phytase before *in vitro* digestion (**Table 3**).

In short, about 49.8% of iron in raw faba bean flour was solubilized after *in vitro* digestion and simultaneous action of cellulase and phytase, against about 32% after action of only one of the two enzymes. Thus, a high proportion of iron in faba bean flour seems to be linked to fiber−phytate complexes. Partial phytate degradation by action of endogenous phytases before *in vitro* digestion increased the proportion of iron that can be solubilized. On the other hand, treatment of faba bean flour by addition of exogenous phytase before *in vitro* digestion decreased the proportion of iron that can be solubilized, probably because of interactions between added proteins and other compounds of the matrix likely to chelate iron.

*Effects of Fibers and Tannins on Iron Solubility*. Action of cellulase or phytases on insoluble digestion residues of the dehulled faba bean (**Figure 3**) caused more iron to be released than treatment without enzymes (approximately 20% units of

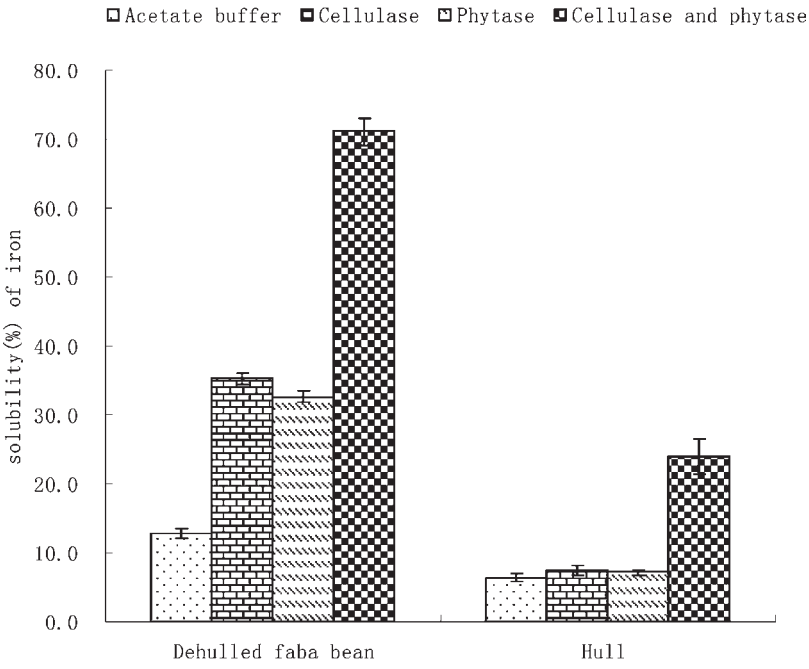


**Figure 3.** Solubility of indigestible iron (%) of faba bean fractions with low (dehulled faba bean) and high (hull) fiber and tannin contents after treatment of insoluble digestion residues.

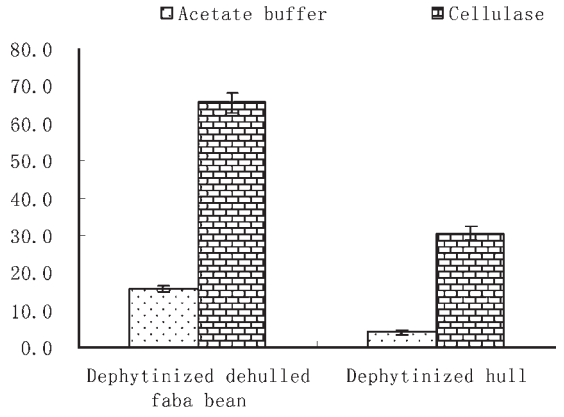


**Figure 4.** Solubility of indigestible iron (%) of dephytinized faba bean fractions after treatment of insoluble digestion residues.

additional soluble iron). Concerning residues of the hull fraction, there was no significant increase in iron solubility ($P > 0.05$) after action of cellulase or phytases. Simultaneous action of the two enzymes led to the release of more than 60 and 18% units of additional iron for residues of dehulled faba bean and hull fractions, respectively.

These results are comparable with those obtained on residues of dephytinized fractions (**Figure 4**), because, in both fractions, the quantities of iron released by treatment with buffer were very low, whereas the action of cellulase led to the release of more than 50 and 25% units of iron for the dehulled faba bean and hull fractions, respectively.

Thus, it appears that there is no great difference in the nature of the iron-containing complexes present in dehulled faba bean and hull fractions, because, in both fractions, iron mainly appears to be rendered insoluble by complexes in which both phytates and fibers play a role.

When looking at the sum of digestible iron (B) and iron released from insoluble residues (C), simultaneous action of cellulase and phytases allowed solubilization of 72 and 41% of iron for dehulled faba bean and hull fractions, respectively (**Table 4**). These results are similar to those obtained after treatment of dephytinized dehulled faba bean and hull fractions with cellulase (79 and 39%, respectively). The marked difference in iron solubility between the dehulled faba bean and hull fractions after these treatments is probably due to the fact that the iron−tannins compound content is much greater in the hull fraction than in the dehulled faba bean fraction (1.28 versus 0.26 g/100 g of DM, **Table 1**). Indeed, some studies have already shown that some phenolic compounds were involved in the reduction of iron solubility (*13*−*16*) and that their inhibiting effect was dose-dependent (*17*, *18*).

Enzymatic degradation of phytates and fibers enabled solubilization of around 72 and 41% of iron contained in dehulled faba bean and hull fractions, respectively, against around 50 and 31% for dehulled faba bean and hull fractions, respectively, after action of one of the two enzymes. The results of the study of these fractions tend to confirm the presence of fiber−phytate−iron complexes in faba bean flours and also indicate the presence of tannin−iron complexes that render the majority of iron contained in the hull of faba bean.

**Effects of the Antinutritional Factors on Zinc Solubility.** *Effects of Phytates on Zinc Solubility*. In insoluble residues of raw faba bean (**Figure 5**), degradation of fibers did not allow the release of more zinc than the treatment with buffer. On the other hand, phytate degradation allowed the release of about 11% units more zinc than that released by the treatment with buffer, while simultaneous action of cellulase and phytases did not lead to an additional release of zinc compared to the action of the single phytases. It thus appears that phytates are responsible for the inhibition of zinc solubilization.

In samples whose phytate contents were reduced by action of endogenous phytases, the greater the degree of phytate degradation, the greater the quantities of zinc released by the different treatments. This can be attributed to the stronger hydrolysis of phytate molecules that decreased from six or five phosphates to less than five phosphates (IP4, IP3, IP2, and IP1), which, according to Lönnerdal et al. (*19*), does not appear to have any negative effect on zinc bioavailability.

**Table 4.** Solubility of Iron (%) after Gastrointestinal Digestion and Treatment of Insoluble Residues of Nondephytinized and Dephytinized Faba Bean Fractions[a]

| faba bean fractions | digestion + buffer | digestion + cellulase | digestion + phytase | digestion + cellulase and phytase |
|---|---|---|---|---|
| dehulled faba bean | 36.5 ± 2.6 b | 52.4 ± 2.9 b | 48.5 ± 2.8 a | 72.1 ± 4.5 a |
| dephytinized dehulled faba bean | 42.8 ± 2.1 a | 78.6 ± 3.2 a | | |
| hull | 28.5 ± 3.0 c | 31.5 ± 1.6 d | 32.4 ± 3.6 b | 40.7 ± 2.6 b |
| dephytinized hull | 27.6 ± 1.6 c | 38.8 ± 4.1 c | | |

[a] Values within a column without common superscripts are significantly different ($P < 0.05$).

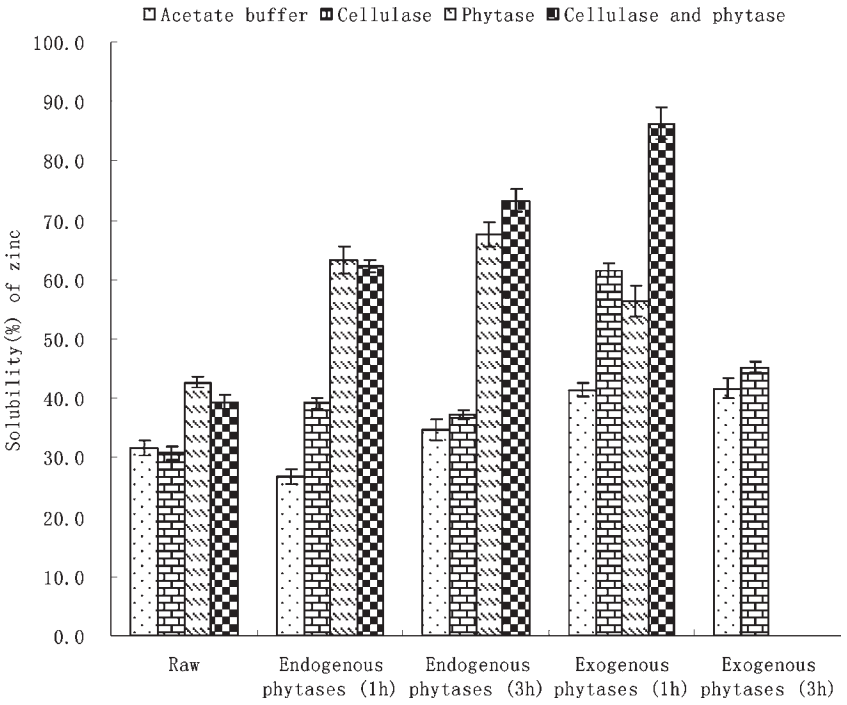


**Figure 5.** Solubility of indigestible zinc (%) of faba bean flours after treatment of insoluble digestion residues with acetate buffer, cellulase, phytase, or cellulase in combination with phytase.

**Table 5.** Solubility of Zinc (%) after Gastrointestinal Digestion and Treatment of Insoluble Residues of Faba Bean Flours with Decreased Phytate Contents[a]

| faba bean flours | digestion + buffer | digestion + cellulase | digestion + phytase | digestion + cellulase and phytase |
|---|---|---|---|---|
| raw | 35.6 ± 1.5 d | 36.4 ± 2.0 c | 48.9 ± 2.2 c | 48.2 ± 2.6 c |
| endogenous phytases (1 h) | 40.1 ± 1.9 c | 52.2 ± 3.2 b | 65.6 ± 2.3 b | 68.2 ± 2.9 b |
| endogenous phytases (3 h) | 48.2 ± 2.1 b | 52.6 ± 2.1 b | 66.2 ± 3.4 ab | 72.1 ± 3.5 b |
| exogenous phytases (1 h) | 45.6 ± 2.4 b | 72.5 ± 3.8 a | 71.6 ± 3.7 a | 86.4 ± 3.7 a |
| exogenous phytases (3 h) | 68.9 ± 3.6 a | 70.6 ± 2.7 a | | |

[a] Values within a column without common superscripts are significantly different ($P < 0.05$).

Furthermore, in samples with decreased phytate contents (endogenous and exogenous phytases), treatment with cellulase allowed slightly more zinc to be solubilized than the treatment with buffer. It thus seems that fibers play a role in the chelation of zinc in faba bean flour.

The values for total zinc solubility after the combined action of *in vitro* digestion and treatment of insoluble residues (**Table 5**) allow the effects of phytates and fibers to be put into consideration. Thus, when phytates were partially degraded by endogenous phytases before *in vitro* digestion, the percentages of soluble zinc after digestion and incubation with buffer increased from 36 to 40 and 48% for samples containing 0.84, 0.43, and 0.27 g of IP6/100 g of DM, respectively (**Table 1**). Furthermore, after the action of cellulase, the solubility of zinc in samples with decreased phytate contents because of the action of endogenous phytases before *in vitro* digestion (endogenous phytases) increased from 40 to 52% for flour incubated for 1 h and from 48 to 53% for flour incubated for 3 h, revealing the significant negative effect of fibers on zinc solubility ($P < 0.05$).

*Effects of Fibers and Tannins*. Separate action of cellulase and phytases on insoluble digestion residues of dehulled faba bean with low fiber and tannin contents led to a greater release of zinc (**Figure 6**) than treatment without enzymes (+5 and +33% units, respectively). The simultaneous action of the two enzymes did not allow more zinc to be released than the action of a single one. Concerning the hull fraction with high fiber and tannin contents, the action of cellulase did not lead to additional zinc solubilization compared to the treatment without enzymes. On the other hand, the action of phytases and the simultaneous action of the two enzymes allowed about 7 and 35% units of additional zinc to be solubilized, respectively, in comparison with treatment with buffer. These results indicate that the nature of the complexes between zinc and antinutritional factors is probably not the same in dehulled faba bean and hull fractions. Zinc from

dehulled faba bean is probably mainly chelated by phytates and only slightly by fibers, whereas that of hull is probably mainly linked to fiber−phytate complexes.

These conclusions on the nature of the complexes were also obtained in the dephytinized fraction (**Figure 7**), with a majority of phytate−zinc complexes and a low proportion of fiber−zinc complexes, although the quantities of solubilized zinc were different. Indeed, in the dephytinized faba bean fraction, all of the zinc was released by the action of phytases followed by that of cellulase, whereas about 27% of the zinc remained insoluble in the case of simultaneous action of the two enzymes on the dephytinized fraction. This result is similar to the results previously obtained on faba bean flours (**Figure 6**), showing that the greater the degree of phytate degradation before *in vitro* digestion, the greater the quantity of zinc released by the different treatments. This could be explained by the major role of IP5 and possibly of IP4 in zinc complexation by phytates (*20*). Conversely, in the hull fraction, the proportion of soluble zinc after the action of cellulase on residues of the dephytinized fraction was lower than that with the simultaneous action by cellulase and phytases on those of the nondephytinized fraction (about 52 versus 73%). This indicates that, like iron, zinc in the hull of faba bean is probably linked to fiber−phytate complexes whose hydrolysis necessitates combined action of cellulase and phytases.

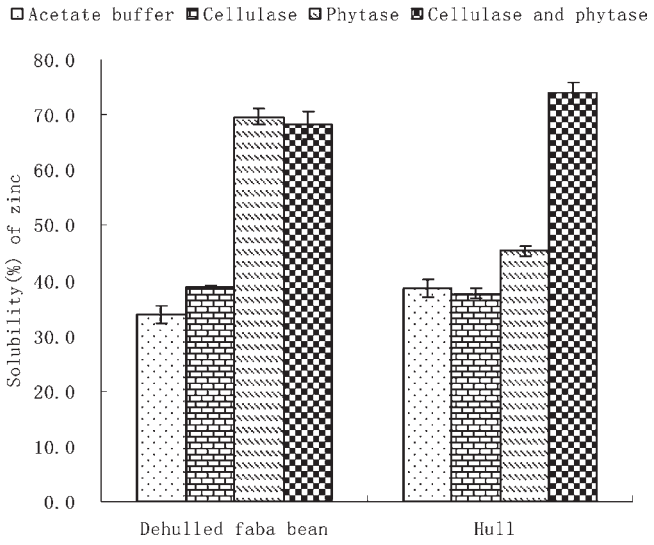


**Figure 6.** Solubility of indigestible zinc (%) of faba bean fractions with low (dehulled faba bean) and high (hull) fiber and tannin contents after treatment of insoluble digestion residues.

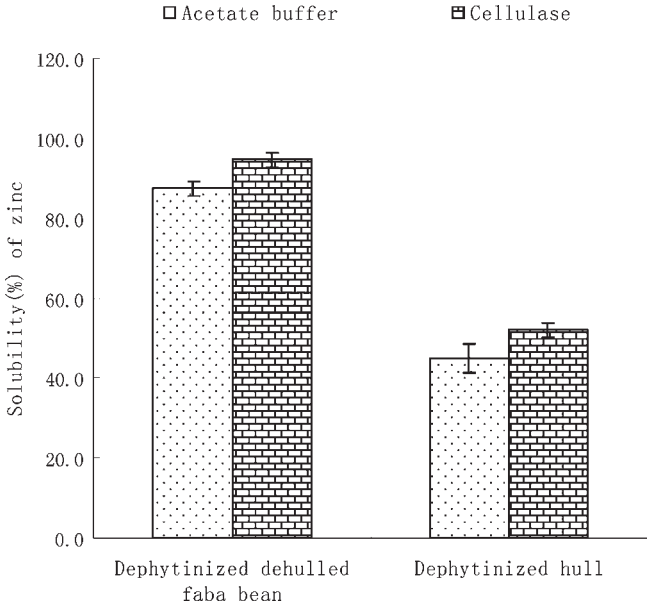


**Figure 7.** Solubility of indigestible zinc (%) of dephytinized faba bean fractions after treatment of insoluble digestion residues.

If we consider the data in **Table 6**, which show total solubility after *in vitro* digestion and treatment of insoluble residues, we can also assume the presence of other complexes in the hull of faba bean. Indeed, a maximum of only 75% of zinc from hull was released by the different treatments, against 100% from the dephytinized dehulled faba bean. This could be due to tannin−zinc or protein−zinc complexes in the hull of faba bean. To our knowledge, these kinds of complexes have little information in the literature, but our results tend to prove their existence. The lack of information on these possible complexes can be explained by the fact that the strong phytate contribution to low zinc bioavailability probably masks these interactions between zinc and other chelator compounds in food matrixes.

Thus, single enzymatic degradation of phytates from dehulled faba bean allowed solubilization from 65 to 93% of zinc, depending upon the treatment, which confirms the major contribution of phytates to low zinc bioavailability in dehulled faba bean. However, the study of the hull revealed that single phytate degradation did not enable an increase in zinc solubility, which indicates the presence of other complexes. Zinc in faba bean hulls is thus probably partly linked to fiber−phytate complexes, and it is also possible that zinc is partially chelated by some tannins or proteins of the matrix, which requires further study.

The iron in faba bean flour was mainly chelated by complexes involving both fibers and phytates, so that this iron was solubilized by simultaneous action of cellulase and phytases. However, in the hull of faba beans that contain high fiber and tannin contents, the majority of iron appeared to be chelated by tannins (iron−tannins). Thus, phytates, fibers, and tannins alike decrease the bioavailability of iron in faba bean flour.

Concerning the zinc of faba bean flour, it was mainly linked to phytates, and the greater the degree of phytate degradation before *in vitro* digestion, the greater the quantity of zinc released. Furthermore, fibers and probably also tannins or some proteins could be involved in the formation of complexes with zinc in the hull of faba bean. Some lower inositol phosphate resulting from phytate hydrolysis (IP5 and IP4) appears to contribute to a considerable extent to low zinc bioavailability in faba bean flour. It would be useful to verify this hypothesis by determining the residual contents in these degradation compounds.

**Table 6.** Solubility of Zinc (%) after Gastrointestinal Digestion and Treatment of Insoluble Residues of Nondephytinized and Dephytinized Faba Bean Fractions[a]

| faba bean fractions | digestion + buffer | digestion + cellulase | digestion + phytase | digestion + cellulase and phytase |
|---|---|---|---|---|
| dehulled faba bean | 32.5 ± 2.3 c | 43.6 ± 3.5 b | 65.2 ± 2.6 a | 62.3 ± 1.8 b |
| dephytinized dehulled faba bean | 92.5 ± 2.6 a | 98.6 ± 3.1 a | | |
| hull | 44.6 ± 1.2 b | 45.8 ± 2.1 b | 63.2 ± 2.2 a | 75.5 ± 1.4 a |
| dephytinized hull | 46.5 ± 1.6 b | 47.2 ± 1.9 b | | |

[a] Values within a column without common superscripts are significantly different ($P < 0.05$).

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