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Impact of sprouting pretreatment on phytic acid and polyphenol level of faba bean (*Vicia faba* L.) flour

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Abstract

The seeds of two cultivars of faba bean (*Vicia faba* L.), namely Qidou 2 and Big Qinpi, were germinated for 6 days to obtain 2-, 4- and 6-day-old sprouts. Faba bean sprout (5% and 10%) was added to faba bean flour. The mixtures were incubated at 30°C with shaking for 30, 60, 90 and 120 min. Phytic acid and polyphenol contents were assayed for all treatments. The results revealed that phytic acid and polyphenol contents were significantly ($P < 0.05$) reduced. When a mixture containing 10%, 4-day-old malt and faba bean flour was incubated for 120 min, it significantly ($P < 0.05$) reduced phytic and polyphenol contents by 92% and 98%, respectively, for Qidou 2 cultivar, while for Big Qinpi they were reduced by 93% and 96%, respectively. The rate of reduction of phytic acid and polyphenol content increased with incubation time and sprout age and concentration.

Introduction

The faba bean is one of the oldest crops that ranks sixth in production among the different legumes grown in the world (Concepcion *et al.*, 1998). And the bean is also a staple legume food in many Asian countries and it contains a reasonable amount of dietary fibers (10-12%) and carbohydrate (50-65%) with a dry matter ranging from 85.6% to 92.5%. Faba bean nutritional quality is dictated mainly by its chemical composition and one of the constraints on the utilisation of faba bean as food is the occurrence of phytic acid and polyphenol. 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 including faba bean. Phytic acid binds minerals that are necessary as cofactors, thus interfering with several essential metabolic processes, especially the utilisation of protein (Harland and Morris, 1995) and also minerals that are not cofactors, e.g. calcium and iron. Polyphenol or their oxidized products can form complexes with essential amino acids, enzymes and other proteins, thus lowering their nutritional value and protein digestibility. These anti-nutrients can be fully or partially removed by processing. Sprouting of cereals and legumes is a processing procedure traditionally used in many Asian countries for improving the nutritional quality of cereals and grain legumes. Sprouting is a process involving germination and drying of legume seeds, the prime objective being to promote the development of hydrolytic enzymes that are not active in raw seeds (Dewar *et al*., 1997). Attempts to reduce the phytic acid content have been tried by different means including milling (Mahgoub and Elhag, 1998) and soaking of sorghum grains (Elmaki *et al.*, 1999), malting of oats (Larsson and Sandberg, 1995) and pea (Beal and Mehta, 1985), fermentation of sorghum, maize, soybean, faba bean, cowpea and yam (Marfo *et al*., 1990) and activation of the indigenous enzyme phytase and/or addition of microbial phytase (Barrier *et al*., 1996). Extractable polyphenol content was markedly reduced when faba beans were soaked in water and germinated for different periods of time. In feeding trials with rats (Yasaman *et al*., 1990) and chicks (Teeter *et al*., 1986), polyphenols reduced weight gain and feed conversion depending on the type of animal and feeding system. Elimination or inactivation of such antinutritional compounds is absolutely necessary to improve the nutritional quality of faba bean. Therefore, in this study we would like to evaluate the

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efficiency of sprouting pretreatment on phytic acid and polyphenol level of two faba bean cultivars.

Materials and Methods

Sample preparation

The seeds of two cultivars of faba beans, locally known as Qidou 2 and Big Qinpi, were obtained from Gangzicun Company, Nanjing, Jiangsu Province. The seeds of Qidou 2 cultivars are small in size, oval in shape and slightly brown in colour, while those of Big Qinpi are slightly big in size, oval in shape and white in colour. About 5 kg of each cultivar was cleaned from damaged seeds and foreign objects, then subjected separately to the following treatments:

(1)Three kilograms of whole seed of each cultivar was milled to whole beans flour using a laboratory mill to pass a 0.4- mm sieve to obtain 94% flour yield and kept at 4°C before use.

(2) One kilogram of whole seed of each cultivar was immersed in water overnight and then the beans were spread on trays lined with cloth. It was kept wet by frequent spraying of water. After 2, 4 or 6 days, the germinated beans were removed from the trays, sundried, milled and termed as 2-, 4- and 6 -day-old-sprout.

Addition and incubation of sprout to faba bean flour

2-, 4- or 6-day-old sprout was added to faba bean flour at different concentrations (5% or 10%) in triplicate and mixed well. Water was added to the mixture in the ratio of 1:2 (w/v). Then the mixtures were shaken for 30 min and incubated at 30°C in a shaker for 30, 60, 90 and 120 min, then the mixture was dried at 75°C and ground to pass a 0.4-mm sieve and kept at 4°C before use.

Determination of soaking and sprouting losses

Soaking and sprouting losses were indicated by the ratio of the weight difference before and after soaking of the seeds or addition of sprout to that of the original flour weight (on dry weight basis).

Determination of phytic acid and phosphorus

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 H_2O 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.

The colorimetric method AOAC 995.11(Horwitz, 2000) was used to determine phosphorus levels. 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).

Determination of polyphenol

Quantitative estimation of polyphenols was carried out using the modified vanillin-HCl method (Price *et al*., 1978). A 200 mg sample was extracted using 10 mL 1% (v/v) concentrated HCl in methanol for 20 min in capped rotating test tubes. Vanillin reagent (0.5%, 5 mL) was added to the extract (1 mL) and the absorbance of the colour developed after 20 min at 30°C was measured at 500 nm.

Statistical analysis

Data were analysed with SPSS 13.0 for windows XP. 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

For both cultivars (Qidou 2 and Big Qinpi), the loss in dry matter occurred when the whole seeds were soaked prior to germination because germination itself had no effect on dry matter of the sample. As the period of germination increased, the percentage of loss in dry matter was minor and constant, therefore, the loss was found to be due to soaking prior to germination. Soaking of the cultivars seeds overnight caused a slight reduction in the dry matter and it was found to be of no significant effect. Moreover, in this study although sprouting had no effect on dry matter, we apply only 5% and 10% of the sprout produced and in the meantime reduced the incubation period to minimize the loss in dry matter. Results obtained for dry matter loss are similar to those reported by Duhan *et al*. (2002) on pigeon seeds. Table 1 shows phytic acid, polyphenol, phosphate contents and phytic acid/P percentage of untreated faba bean cultivars. Results showed that phytic acid, polyphenol and phosphate contents of Qidou 2 cultivar were greater than those of Big Qinpi. The percentages of phytic acid/total P for Qidou 2 and Big Qinpi cultivars were 87% and 82%, respectively. The percentage of phytic acid/ P obtained in this study agree with Chauhan

Table 1. Antinutritional factors and phosphorus content of raw seeds of two faba bean cultivars

Parameter	Cultivars	
	Qidou 2	Big Qinpi
Dry matter (%)	88.60 ± 0.2b	91.25 ± 0.3a
Phytic acid (mg 100 g^{-1})	825 ± 12.1a	779 ± 7.9b
Phosphorus (mg 100 g^{-1})	1006 ± 5.3a	895 ± 5.8b
Polyphenol (%)	0.85 ± 0.05a	0.46 ± 0.02b

Values are means ±SD . Means not sharing a common superscript letter in a row are significantly different at ($P < 0.05$) as assessed by Duncan's multiple range test.

and Mahjan's (1988) finding who stated that phytic acid represents more than 70% of total P in cereals and those of Raboy *et al*. (1991) who concluded that, in various seeds, phytic acid positively correlates with total P, correlation coefficients being greater than 0.90. Variation in phytic acid/ P percentage between the two cultivars is likely due to variation in soil as well as fertilizers applied which affect total P content and accordingly influences phytic acid concentration (Miller *et al.*, 1980). Sprout was found to contain traces of phytic acid and polyphenol and low level of phytic acid in the sprout is likely due to the action of the enzyme phytase on phytic acid while that of polyphenol is due to leaching during soaking and germination of the seeds as indicated by the significant browning of supporting filter paper during germination. Figure 1 shows the effect of sprouting pretreatment (5%) followed by incubation for different periods of time on phytic acid level of Qidou 2 and Big Qinpi cultivars. Addition of 5% sprouts of different age to Qidou 2 cultivar flour with incubation for different periods of time (30, 60, 90 or 120 min) were found to decrease the phytic acid content with time (Figure 1a). A maximum reduction level (from 265 to 66 mg 100 g^{-1}) was obtained when the 4-day-old sprout was added to faba bean flour and incubated for 120 min. The reduction of phytic acid content is more likely due to incubation time rather than addition of sprout. Phytic acid content of Big Qinpi cultivar (Figure 1b) was also significantly ($P < 0.05$) affected by the addition of sprout but the rate of reduction was slightly low compared with Qidou 2 cultivar. Phytic acid level of Big Qinpi cultivar was decreased from 233 to 77 mg 100 g^{-1} during incubation of the flour with sprout (4 days old) for 120 min (Figure 1b). Increment of malt concentration to 10%, significantly ($P < 0.05$) reduced phytic acid content with increasing time of incubation (Figure 2). Incubation of Qidou 2 flour withsprout (10%) for 120 min reduced phytic acid content from 265 to 21 mg 100 g^{-1}, i.e. about 92% was lost (Figure 2a), while that of Big Qinpi reduced from 233 to 16 mg 100 g^{-1}, i.e. about 93% was lost (Figure 2b). The results indicated that phytic acid reduction was significantly affected

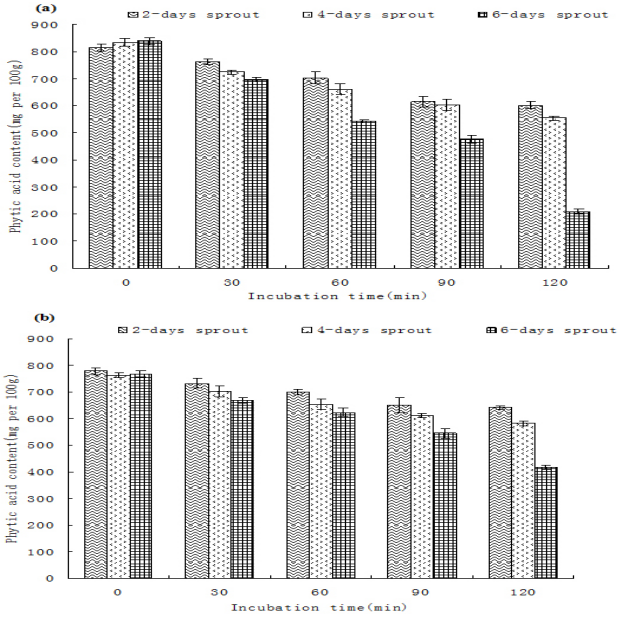

Figure 1. Effect of incubation of faba bean flour with 5% sprout on phytic acid content (mg per 100 g) of (a) Qidou 2 and (b) Big Qinpi cultivars

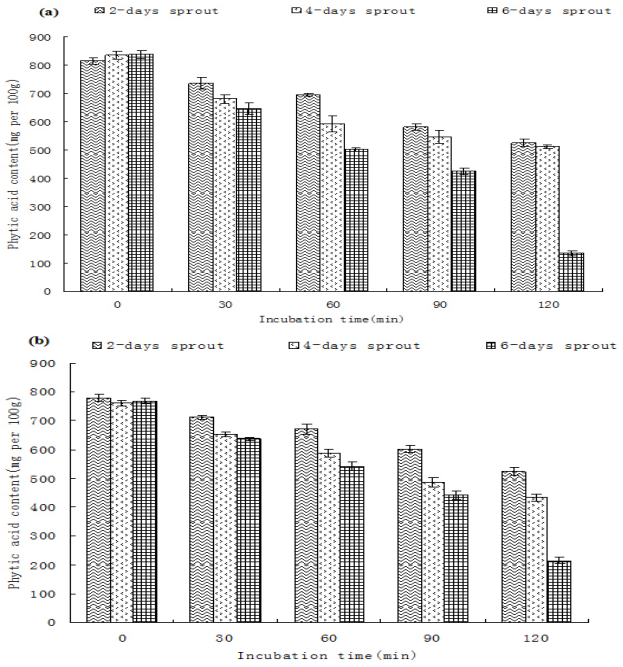

Figure 2. Effect of incubation of faba bean flour with 10% sprout on phytic acid content (mg 100 $^{-1}$) of (a) Qidou 2 and (b) Big Qinpi cultivars

by the addition of sprout because the germination stage had a substantial effect on the reduction in phytic acid content as a result of the action of endogenous phytases obtained during germination that degrade the phytic acid into inorganic phosphorus and inositol and its intermediate forms. The rate of reduction depends upon the age as well as the amount of malt (Elkhalil *et al*., 2001). Valverde *et al.* (1994) reported

that germination of lentils greatly reduced phytic acid content compared with soaking or cooking. Similar results were also reported when sorghum flour was treated with malt and incubated for different time intervals (Elkhalil *et al*., 2001). Figure 3 shows the effect of sprouting pretreatment on polyphenol level during incubation of faba bean flour with 5% malt (1, 2 or 4 days old) for different periods of time (30, 60, 90 or 120 min). Qidou 2 cultivar, which can be classified as a high-polyphenol cultivar as indicated by the seed coat colour, showed a progressive reduction in polyphenol content with incubation time (Figure 3a). Polyphenol level of the cultivar reduced from 0.96% to 0.13%, when 5% malt was added to the flour and incubated for 120 min. Although Big Qinpi cultivar was low in polyphenol content but the level of reduction in polyphenol was similar to that of Qidou 2 cultivar (Figure 3b).

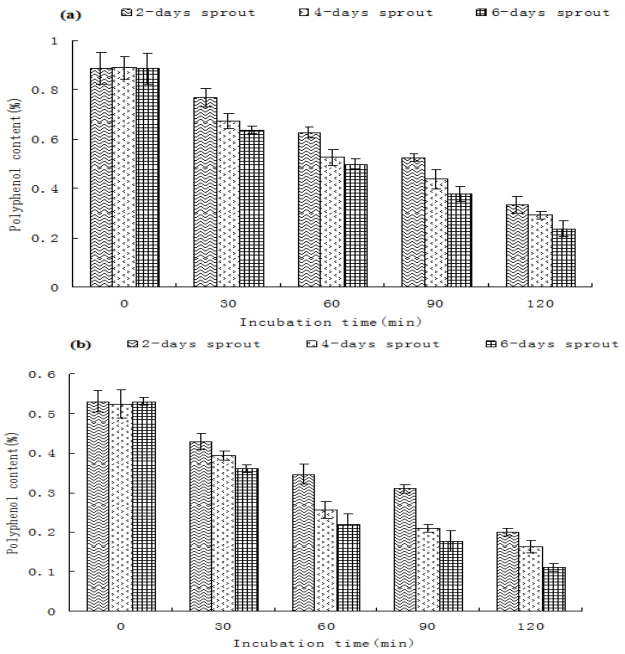

Figure 3. Effect of incubation of faba bean flour with 5% sprout on polyphenol content (%) of (a) Qidou 2 and (b) Big Qinpi cultivars

Polyphenol level of Big Qinpi cultivar reduced from 0.35% to 0.03%, indicating that most of polyphenol of the cultivar was soluble and about 90% of it leached out. Increment of sprout concentration significantly ($P < 0.05$) reduced polyphenol content with increasing time of incubation (Figure 4). Incubation of Qidou 2 flour with sprout (10%) for 120 min reduced polyphenol content from 0.96% to 0.02%, i.e. 98% of polyphenol was leached out (Figure 4a), while that of Big Qinpi reduced from 0.35% to 0.01%, i.e. 96% was leached out (Figure 4b). Results indicated that sprouting of faba bean flour was very efficient in reducing polyphenol content. It has been reported that soaking of seeds of sorghum grains for 10 h, followed by germination significantly reduced the polyphenol content and the rate of reduction depends on germination time. When the grains were germinated for 24 h, about 74% of the total polyphenol was leached out from the grains and the loss significantly increased up to 98% after 72 h germination (Elmaki *et al*., 1999) because polyphenols are located in the seed coat (Jambunathan and Mertz, 1973). The loss of polyphenol during sprouting can be attributed to solubilisation by enzymes because the prime objective of sprouting is to promote the development of hydrolytic enzymes that are not active in raw seeds (Dewar *et al.*, 1997).

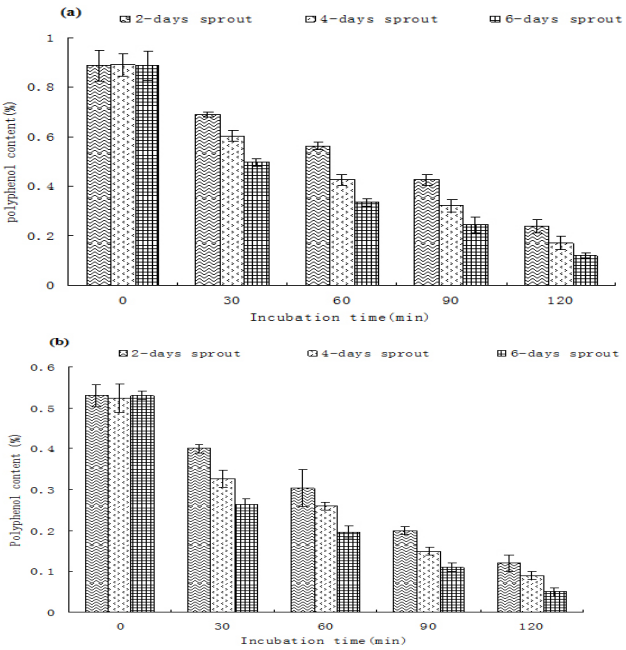

Figure 4. Effect of incubation of faba bean flour with 10% sprout on polyphenol content (%) of (a) Qidou 2 and (b) Big Qinpi cultivars

Conclusion

Utilisation of faba bean sprout to lower phytic acid and polyphenol contents is a promising and simple method. The rate of reduction of phytic acid and polyphenol depends on the sprout age and concentration as well as the incubation period. Short incubation period was useful to avoid the loss of dry matter.

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