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Research Article

Effect of Sprouting on Amino Acids, Protein Solubility and Availability in Green and White Faba Bean (*Vicia faba* L.)

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Abstract: The changes in crude protein, free amino acids, amino acid composition, protein solubility, protein fractionation and protein availability after sprouting of faba bean were investigated. Green and white faba bean were soaked for 20 h followed by germination for 72 h; the results revealed that crude protein and free amino acids in raw faba beans ranged from 8.72 to 10.54% and 0.76 to 1.21 mg/g, respectively. After sprouting, crude protein was decreased and free amino acids were increased. There was an increase in content of valine and phenylalanine amino acids after sprouting. On the other hand, there was a decrease in most of amino acids after sprouting. After sprouting protein solubility was significantly increased. Regarding protein fractions, there was an increase in albumin, globulin, kafirin and glutelin proteins and a decrease in cross linked kafirin and cross linked glutelin after sprouting.

Keywords: Availability, faba bean, solubility, sprouting

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 energy, proteins, carbohydrates, vitamins and minerals. Faba bean components, especially its protein is less digestible for human and mono-gastric animals, because of its anti-nutritional factors such as tannins and phytic acid. Removal of these undesirable components is essential to improve the nutritional quality of faba bean and effectively utilize its potential as human food or animal feed (Concepcion *et al*., 2002).

Interaction between tannins and faba bean proteins reduces both protein and starch digestibility. This is important in both human and animal nutrition. The formation of complexes between faba bean proteins and tannins is thought to render the proteins indigestible as well as inhibit digestive enzymes. Proteins rich in proline bind more faba bean tannins than other proteins (Chung *et al*., 1998).

The low digestibility of faba bean proteins is presumably due to the high protein cross linking. Good quality proteins are those that are readily digestible and contain the essential amino acids in quantities that correspond to human requirements (El-Beltagi, 2011; Zhao *et al*., 2008).

The *in vitro* pepsin digestion assay is mimics the digestive system and are widely used to study the structural changes, digestibility and release of food components under simulated gastrointestinal conditions. The most frequently used biological molecules included in the digestion models were digestive enzymes, bile salts and mucin (Coles *et al*., 2005; Hur *et al*., 2011).

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; Luo *et al*., 2012). As germination proceeds and enzymes trigger elaborate biochemical changes (Zielinski *et al*., 2005). According to Ghavidel and Prakash (2007) 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. Sprouting is widely used in legumes and cereals to increase their palatability and nutritional value, particularly through the breakdown of certain anti-nutrients, such as phytate and protease inhibitors (Steiner *et al*., 2007). Sprouting triggers the enzymatic activity of sprouting grains, leading to the breakdown of proteins, carbohydrates and lipids into

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simpler forms. This processing method activates proteases which are active in degrading proteins, thereby increasing nutrient bioavailability (Elkhalifa and Bernhardt, 2010).

In our previous study, we studied effect of soaking and sprouting on iron and zinc availability in green and white faba bean (Luo and Xie, 2014). But we did not study the effects on protein characteristics in faba bean. So the objective of this study was to enhance faba bean nutritional value via sprouting, identify of faba bean protein characteristics, such as protein digestibility, protein solubility and protein fractionation as well as amino acids contents.

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, pancreatin,-amylase and L-aspartic acid 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.

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 (Luo *et al*., 2012). 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:

Determination of crude protein: Crude protein contents of raw sorghum and treatments were determined according to the methods of AOAC (2000).

Determination of free amino acids: Free amino acids were determined using the method outlined by Rosen (1957). Ninhydrin reagent used for the determination of free amino acids. The free amino acids were calculated as mg/g DW from the standard curve which prepared by using L-aspartic acid as standard.

Determination of amino acids composition: Amino acids composition of samples was determined by using amino acid analyzer (Biochrom 30) according to the method outlined in AOAC (2000). An aliquot sample, were weighed and digested with 25 mL of 6 N HCl at 110°C for 24 h. Then HCl was removed by evaporation; the remaining solid fraction was dissolved with 0.2 N sodium citrate buffer (pH 2.2). One milliliter of the solution was filtered through 0.45 μm Millipore membrane filters. The standard amino acids (consist of 17 amino acids) was treated as the same as of the samples. Amino acids were expressed as g/100 g protein on dry weight basis.

Determination of protein solubility: Protein solubility was determined by the method of Sathe and Salunkhe (1981). One gram of samples was dispersed in 25 mL of 1 M NaOH. The obtained suspensions were mixed and stirred in an orbital Shaker at 150 rpm for 12 h at room temperature and then centrifuged at 3000 g for 20 min. Soluble proteins in supernatants were determined by Lowry *et al*. (1951). Bovine Serum Albumin was used as standard protein. Soluble protein was expressed as g/100 g DW sample.

Determination of faba bean protein fractions: Protein fractionation was performed according to the method modified from Landry and Moureaux (1970). Two grams of faba bean were sequentially extracted with the six solvents listed below (20 mL at 25°C and centrifuged at 18,900 g for 10 min at 4°C after each step). Initially, sorghum was extracted with deionized water for 20 min (albumins, fraction 1) and the pellet sequentially extracted with the following solutions: 0.5 M NaCl for 60 min (globulins, fraction 2); 60% 2-propanol (v/v) for 4 h (kafirins, fraction 3); 0.1 M borate buffer, pH 10.8, for 4 h (glutelin, fraction 4); 60% 2-propanol with 1% Dithiothreitol (DTT) for 4 h (cross-linked kafirins, fraction 5); and 0.1 M borate buffer, pH 10.8, containing 1% DTT and 1% Sodium Dodecyl Sulfate (SDS) for 18 h at 4°C (cross-linked glutelins, fraction 6). Soluble protein in supernatants was determined by Lowry *et al*. (1951) for fractions 1 to 4 and by de Wreede and Stegemann (Sangronis and Machado, 2007) from fractions 5 and 6. Protein fractions were expressed as g/100 g protein on dry weight basis.

Determination of *in vitro* protein availability: *In vitro* protein availability was determined according to the method of Akeson and Stahmanna (1964). One gram samples added to HCl (15 mL, 0.1 M), containing 1.5 mg pepsin then the incubated at 37 uC for 3 h. The obtained suspension was neutralized with NaOH (7.5 mL, 0.2 M), then treated with 4 mg of pancreatin in 7.5 mL 0.2 M phosphate buffer (pH 8.0). One milliliter of toluene was added to prevent microbial growth and the mixture was gently shaken and incubated for additional 24 h at 37°C. After incubation, the sample was treated

with 10 mL of 10% TCA to remove undigested protein and larger peptides and centrifuged at 50000 g for 20 min at room temperature. Protein in the supernatant was estimated using the Kjeldahl method (AOAC, 2000). The percentage of protein availability was calculated by the ratio of protein in supernatant to protein in sample as equation:

Protein availability % = Nitrogene (in supernatant) - Nitrogene (in blank) /Nitrogene (in sample) ×100

Statistic analysis: The experiments were conducted in triplicates. Data were analyzed with SPSS (Statistical Package for the Social Sciences) 13.0 for windows. The mean and standard deviation of means were calculated. 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 a probability $p<0.05$.

RESULTS AND DISCUSSION

Effect of sprouting of faba bean on crude protein and free amino acids: Table 1 presents crude protein and free amino acids content in faba bean before and after sprouting. Protein content were 8.72, 10.54% in raw green and white faba bean, respectively. These results are in agreement with Johnson *et al*. (2010) who found that crude protein content in whole faba bean grain is ranged from 7 to 15% or 10.30 to 14.90%.

The crude protein was significantly decreased after germination compared with raw Faba bean. These results are agreed with Shaker *et al*. (1995) who reported that nutrients loss might be attributed to the leaching of soluble nitrogen, mineral and other nutrients into desired solution.

Free amino acids content were 0.76 and 1.21 mg/g in raw green and white faba bean, respectively. After sprouting free amino acids content were increased to 8.57 and 9.68 mg/g, respectively. And this may be due to the activity of proteolytic enzymes. Chavan *et al*. (1981) also found that free amino acids content decreased to 1.20 mg/g in germinated sorghum after germination (72 h).

Table 1: Crude protein and free amino acids content of faba bean after sprouting[1]

Treatments	Crude protein (%)	Free amino acids (mg/g DW)[2]
Raw		
Green	8.72±0.13^{c}	0.76±0.02^{d}
White	10.54±0.17^{a}	1.21±0.04^{c}
Sprouting		
Green	8.12±0.11^{d}	8.57±0.04^{b}
White	10.04±0.13^{b}	9.68±0.05^{a}

[1]: Values are mean of three replicates ±S.D.; Number in the same column followed by the same letter are not significantly different at $p<0.05$; [2]: mg/g DW = mg per gram dry weight

The nutritive value of food, especially protein mostly would depend not only on its amino acid profile in general but also on the quantities of the essential amino acids content in particular. The nutritive value of dietary protein is determined by the pattern and quantity of essential amino acids present.

Table 2 shows essential amino acids content in faba bean before and after sprouting. Essential amino acids in raw faba bean ranged from 3.29 to 3.86, 11.54 to 13.51, 2.65 to 3.26, 4.53 to 5.12, 4.80 to 5.12, 2.17 to 2.21, 2.83 to 3.04 and 4.26 to 4.36 g/100 g protein for isoleucine, leucine, threonine, valine, phenylalanin, lysine, methionine and tyrosine, respectively.

From the data in Table 2, it could be noticed that after treatments essential amino acids content were changed. There was an increase in content of valine and phenylalanine after sprouting. On the other hand, there was a decrease in most of amino acids after sprouting. These findings are in agreement with Elemo *et al*. (2011) who found that germination significantly increased the essential amino acids except for histidine and sulphur amino acids. Germination of cereals and legumes has been shown to be generally advantageous as it also improves the nutritional qualities of cereals

Table 2: Essential amino acids content of faba bean after sprouting (g/100 g protein)[1]

Treatments	Isoleucine	Leucine	Threonine	Valine	Phenylalanine	Lysine	Methionine	Tyrosine
Raw								
Green	3.29±0.01^{b}	11.54±0.02^{b}	2.65±0.01^{c}	4.53±0.02^{c}	4.80±0.02^{c}	2.17±0.01^{b}	3.04±0.01^{a}	4.26±0.02^{a}
White	3.86±0.01^{a}	13.51±0.01^{a}	3.26±0.01^{a}	5.12±0.01^{b}	5.12±0.02^{b}	2.21±0.01^{ab}	2.83±0.01^{b}	4.36±0.02^{a}
Sprouting								
Green	3.21±0.01^{b}	11.48±0.02^{b}	2.41±0.01^{d}	5.62±0.01^{a}	4.36±0.01^{d}	2.36±0.01^{a}	2.51±0.01^{c}	4.31±0.02^{a}
White	3.81±0.01^{a}	11.29±0.01^{b}	2.83±0.01^{b}	5.31±0.01^{b}	5.51±0.01^{a}	1.98±0.01^{c}	2.42±0.01^{c}	4.17±0.01^{b}

[1]: Values are mean of three replicates ±S.D.; Number in the same column followed by the same letter are not significantly different at $p<0.05$

Table 3: Non essential amino acids content of faba bean after sprouting (g/100 g protein)[1]

Treatments	Arginine	Aspartic	Alanine	Proline	Glutamic	Glycine	Serine	Histidine	Cysteine
Raw									
Green	3.68±0.02^{b}	6.34±0.03^{c}	7.31±0.03^{c}	9.13±0.01^{a}	18.68±0.05^{c}	2.48±0.02^{c}	3.69±0.03^{c}	2.13±0.01^{b}	2.26±0.02^{a}
White	4.12±0.02^{a}	7.26±0.03^{a}	8.93±0.03^{a}	7.16±0.02^{b}	21.63±0.06^{a}	3.15±0.02^{a}	4.27±0.02^{a}	2.47±0.02^{a}	1.76±0.01^{b}
Sprouting									
Green	3.32±0.01^{bc}	6.54±0.02^{c}	8.12±0.03^{b}	9.04±0.02^{a}	20.04±0.05^{b}	2.31±0.01^{c}	3.36±0.02^{d}	2.21±0.02^{b}	2.18±0.01^{a}
White	3.56±0.02^{b}	6.81±0.02^{b}	7.14±0.03^{c}	7.12±0.03^{b}	20.17±0.04^{b}	2.82±0.02^{b}	3.92±0.02^{b}	2.31±0.01^{ab}	1.72±0.01^{b}

[1]: Values are mean of three replicates ±S.D.; Number in the same column followed by the same letter are not significantly different at $p<0.05$

Table 4: Protein solubility of faba bean after sprouting (g/100 g DW)[1, 2]

Treatments	Protein solubility
Raw	
Green	3.62±0.14[c]
White	3.87±0.17[c]
Sprouting	
Green	4.36±0.21[b]
White	5.17±0.24[a]

[1]: Values are mean of three replicates ±S.D.; Number in the same column followed by the same letter are not significantly different at $p<0.05$; [2]: g/100 g DW = g per 100 g dry weight

and legumes (Elkhalifa and Bernhardt, 2010; Luo *et al*., 2009a, 2012; Sangronis and Machado, 2007).

Table 3 shows non essential amino acids content in faba bean before and after sprouting. Non essential amino acids content in raw faba bean ranged from 3.68 to 4.12, 6.34 to 7.26, 7.31 to 8.93, 7.16 to 9.13, 18.68 to 21.63, 2.48 to 3.15, 3.69 to 4.27, 2.13 to 2.47 and 1.76 to 2.26 g/100 g protein for arginine, aspartic acid, alanine, prolin, glutamic acid, glycine, serine, histidine and cysteine, respectively. Glutamic acid was found to be the major non-essential amino acids in the tested samples, while cystine was the lowest one.

The results are in the same trend with other study, which cited that non essential amino acids in raw faba bean ranged from 5.61 to 6.13, 7.75 to 9.39, 7.60 to 8.32, 16.44 to 16.89, 1.92 to 2.95, 3.12 to 4.04 and 1.90 to 2.24 g/100 g protein for aspartic acid, alanine, prolin, glutamic acid, glycine, serine and histidine, respectively (Traore *et al*., 2004). Essential amino acids content in raw faba bean ranged from 7.21 to 7.58, 7.29 to 9.19, 20.03 to 23.16, 2.58 to 2.92, 1.73 to 2.60, 4.44 to 4.73 and 1.89 to 2.96 g/100 g protein for aspartic acid, proline, glutamic acid, glycine, cysteine, serine and histidine, respectively (Ebadi *et al*., 2005).

After sprouting, most of non essential amino acids content was decreased. But the proline content in green and white faba bean did not change. The breakdown of protease resistant prolamins and the increase of essential amino acids upon germination have been reported (Traore *et al*., 2004).

Effect of sprouting of faba bean on protein solubility and protein fractions: Among the functional properties of proteins, solubility is probably the most critical function. Protein solubility characteristics are influenced by factors such as origin, processing conditions, pH, ionic strength and the presence of other ingredients (Elkhalifa and Bernhardt, 2010; Vinay and Sindhu Kanya, 2008).

Table 4 exhibits the effect of sprouting of faba bean on protein solubilized under alkaline conditions extracted with NaOH as described in materials and methods. Data showed that solubility of raw faba bean protein ranged from 3.62 to 3.87 g/100 g.

There was a significant increase in protein solubility after sprouting. These findings are in agreement with Elkhalifa and Bernhardt (2010), who found that germinated sorghum had a higher protein solubility compared with the un-geminated one. The protein of the germinated sorghum was more soluble than the un-geminated faba bean. This might be due to the high proteolytic activity during germination, which will lead to an increase in the protein solubility resulting from hydrolysis of the storage proteins.

The proteins of the faba bean grain are classically divided, based on solubility in different solvents: water-soluble (albumins), salt-soluble (globulins), aqueous alcohol-soluble (kafirins), acid-soluble (glutelin), aqueous alcohol reducing agent-soluble (cross-linked kafirins) and detergent reducing agent alkaline pH-soluble (cross-linked glutelins).

Table 5 presents the effect of sprouting treatment on protein fractions based on solubility for each fraction, into albumins, globulins, kafirins, glutelins, cross linked kafirin and cross linked glutelins. From results, it could be noticed that raw faba bean contain 12.15 to 13.85%, 11.72 to 12.62%, 16.71 to 18.22%, 8.35 to 8.88, 26.16 to 27.82% and 22.14 to 23.55% for albumins, globulins, prolamins, glutline, cross linked kafirins and cross linked glutline, respectively. Distribution of protein in fractions extracted with the different solvents suggested that, the two faba bean varieties different in amount of total extractable protein and this is may be due to the differences in total protein. Cross linked kafirins, represented a considerably greater fraction in faba bean varieties. Results are close to Ejeta *et al*. (1987), who found that fractionated protein in raw faba bean ranged from 10.00 to 24.00%, 6 to 16% and 11.00 to 31.00% for albumins plus globulins, prolamins and cross linked kafirins, respectively. Raw corn contain 19.50 to 26.20%, 20.90 to 35.30% and 15.20 to 23.80% for albumins plus globulins, cross linked kafirins and cross linked

Table 5: Protein fractions (%) of faba bean after sprouting[1]

Treatments	Albumin	Globulin	Kafirin	Glutelin	Cross linked kafirins	Cross linked glutelins
Raw						
Green	12.15±0.25[d]	12.62±0.24[a]	16.71±0.34[b]	8.35±0.24[b]	27.82±0.45[a]	23.55±0.65[a]
White	13.82±0.31[c]	11.72±0.31[b]	18.22±0.37[a]	8.88±0.31[b]	26.16±0.21[a]	22.14±0.54[a]
Sprouting						
Green	16.43±0.24[b]	12.45±0.27[a]	18.63±0.42[a]	9.76±0.27[a]	22.44±0.24[b]	21.76±0.34[a]
White	18.84±0.41[a]	12.86±0.32[a]	18.75±0.28[a]	10.24±0.32[a]	21.87±0.34[b]	17.64±0.27[b]

[1]: Values are mean of three replicates ±S.D.; Number in the same column followed by the same letter are not significantly different at $p<0.05$

Table 6: *In vitro* protein availability (%) of faba bean after sprouting[1]

Treatments	*In vitro* protein availability
Raw	
Green	40.15±0.54[c]
White	41.24±0.64[c]
Sprouting	
Green	64.83±0.68[b]
White	69.58±0.62[a]

[1]: Values are mean of three replicates ±S.D.; Number in the same column followed by the same letter are not significantly different at $p<0.05$

glutline, respectively (Abdel Moueium *et al*., 1996). Since a large percentage of faba bean kafirin storage proteins exist in polymeric forms linked by disulfide bonds in their native state, differences in content of fractions rich in insoluble disulfide proteins, i.e., cross linked kafirin and cross linked glutelin could contribute to protein digestibility differences.

The results revealed an increase in albumin, globulin, kafirin and glutelin fractions and a decrease in cross linked kafirin and cross linked glutelin after germination. These results are close to previous studies, which found that albumin, globulin and glutelin were increased and cross linked kafirin and cross linked glutelin fraction were decreased after germination (Abu Baker *et al*., 2010; Fageer *et al*., 2004).

Effect of sprouting of faba bean on *in vitro* protein availability: The factors that may affect faba bean protein availability, divided in two categories: exogenous factors (i.e., interactions of proteins with non-protein components such as polyphenols, starch, non-starch polysaccharides, phytates and lipids) and endogenous factors (factors arising from the faba bean proteins themselves), concluding that the poor digestibility of faba bean proteins appear to be multi-factorial. The main proteins in sorghum were kafirins. These proteins are known to be peptidase resistant because of their S-S bonds. Digestibility may be used as an indicator of protein availability.

Table 6 presents the *in vitro* protein availability in faba bean before and after sprouting. Data showed that *in vitro* protein availability in raw faba bean ranged from 40.15 to 41.24%. The low digestibility of faba bean proteins is presumably due to the high protein cross-linking.

In addition, *in vitro* protein availability was significantly increased after germination treatments. Germinated white faba bean was higher in protein availability than green faba bean. These results are similar to Correia *et al*. (2010), who found that germination causes activation of intrinsic amylases, proteases, phytases and fiber-degrading enzymes, thereby increasing nutrient availability in sorghum. The activity of intrinsic proteases in germinated grains leads to an increase in *in vitro* protein availability. Germination is effective in increasing protein availability and improving sensory properties Also, processing of faba bean (boiling, germination, fermentation and cooking) greatly improved its nutritive value (Elkhalifa and Bernhardt, 2010; Khalil *et al*., 2007; Luo *et al*., 2009a, b).

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