Food Research 3 (5) : 501 - 505 (October 2019)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Potency of mung bean with different soaking times as protein source for breastfeeding women in Indonesia

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Article history:
Received: 28 February 2019
Received in revised form: 6 April 2019
Accepted: 6 April 2019
Available Online: 8 April 2019

Keywords:
Mung beans,
Soaking time,
Protein,
Breastfeeding women in Indonesia

DOI:
https://doi.org/10.26656/fr.2017.3(5).105

Abstract

This research studied the changes of protein and amino acid profile in mung bean during soaking. The whole mung bean seeds were subjected to five different treatments of soaking, without soaking (control), with soaking for 2, 4, 6 and 8 hrs. Crude protein content, soluble protein content and amino acid profile of the mung beans were analyzed. The results showed that crude protein content of mung bean without soaking (26.64% db) was higher than those of soaked mung beans (in a range of 23.52-25.33%). The results indicated that the crude protein contents were significantly different among the different soaking times. The soluble protein contents tend to increase during soaking treatment and be optimal on 6 hrs soaking time. The amino acids profile showed a trend increasing up to 6 hrs soaking time. Based on these results, the suggested soaking time was 6 hrs. Those findings of amino acids profile, crude protein and soluble protein contents revealed that the soaked mung beans were potential as a good and inexpensive source of protein for breastfeeding women, especially in combination with rice which is the staple food of most of the Indonesian people.

1. Introduction

Legume is known as an important protein source in developing countries. Protein content (20-25%) in legume seeds is 2-3 times higher than that in cereals (Deraz and Khalil, 2008). Mung bean (*Vigna radiata*) is one of the most important vegetable protein sources after soybeans and peanuts. Guillon and Champ (2002) reported that legumes give their mutual compatibility to cereals by giving lysine. Rice is a cereal commonly consumed as a staple food by most of the Indonesian people. The combination of rice and mung bean in diet menu may increase the chemical score of protein compare to each commodity. Mung bean is a cheaper protein source than animal-based protein, making it become an affordable source of protein for low-income people, especially vulnerable group to nutrition such as pregnant and breastfeeding women. Human milk production needs high protein supply because protein composed 1.63% (w/v) of human milk (Agostoni *et al.*, 2000a). Protein was also needed to provide mothers health recovery after pregnancy period and babies delivery.

Protein is the second largest compound after carbohydrates in mung bean. Mung bean is also a good source of minerals, vitamins and dietary fiber (Mubarak, 2005). Mung bean (*V. Radiata*) as one of the mung groups (Vigna species) is rich in lysine and the aromatic amino acids and limited in S-containing amino acids (Deraz and Khalil, 2008). Based on the solubility classification, legume storage proteins are primarily globulins (soluble in dilute salt solutions) followed by the albumins (water soluble proteins). Sashikala *et al.* (2015) reported that the green gram cultivars contained high amounts of salt-soluble globulin fractions (79-85%) of the total protein. Mung bean as well other legumes provide an important role in human nutrition, but their role been limited because of several factors including protease inhibitors, anti-nutritive phytic acid, and flatulence-causing oligosaccharides (Hussain and Burhanddin, 2011). Traditionally mung beans need to be soaked first to reduce the content of this anti-nutrition substance (Kaur *et al*., 2015). Soaking was provided to increase the nutritional value of mung beans because it can reduce the amount of phytic acid and flatulence-causing oligosaccharides.

2. Materials and methods

2.1 Materials and chemicals

Mung bean seeds (*Vigna radiata* L) were obtained from a local market in Surabaya, East Java, Indonesia.

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eISSN: 2550-2166 /

All the chemicals were analytical grade purchased from local distributors.

2.2 Experimental design

The experimental design used in this study was a completely randomized design. The factor in this study was the soaking time of whole mung bean seeds with five-factor levels, namely without soaking (control), with soaking for 2, 4, 6 and 8 hrs. The experiment was conducted in triplicate. Analyzed variables were crude protein content, soluble protein content and amino acid profile.

2.3 Soaking procedure

Mung bean seeds were sorted to get round, dry, non-moldy and intact seeds. After sorting, the whole mung bean seeds were rinsed under running tap water and then soaked with the ratio of mung bean seeds: distilled water of 1: 5 (w/v) at 30°C. After various soaking times, the mung bean seeds were drained, and then freeze-dried to 2-3% of moisture content. The dried mung bean seeds were ground, wrapped in an airtight plastic container and aluminum foil bag as secondary packaging, and then stored in a refrigerator. Powdered mung bean samples were then subjected to the crude protein content, soluble protein content and amino acid profile analysis.

2.4 Chemical analysis

Crude protein content analysis of treated mung bean seeds was conducted according to the standard method of the Association of Official Analytical Chemists i.e. AOAC 923.03 (AOAC, 2000). The samples were subjected to destruction and distillation in a Kjeldahl apparatus and titration. The analysis was conducted in triplicate. Crude protein content was calculated with a conversion factor of 6.25.

The analysis of soluble protein content was conducted according to Bradford method (Kruger, 2002). The method is based on the ability of proteins to bind Coomassie brilliant blue dyes. The blue anion portion of the dye will be bound to arginyl and lysyl residues of the protein. The absorbance of the solution was measured at 595 nm. The results obtained were plotted into the regression equation of the BSA G250 standard to obtain the soluble protein content of samples.

Amino acid composition analysis was done by LC-MS/MS equipped with The Waters Xevo TQD (Tandem Quadruple Detector) and C18 column according to Chandrachood *et al*. (2011). Powdered mung bean samples were prepared by hydrolyzing the protein with 6N HCl in an autoclave (110°C, 12 hrs), neutralized with 6N NaOH, and filtered with 0.22 µm of membrane filter. The filtrates were used in LC-MS/MS injection for amino acid detection. The mobile phase consisted of eluent A and B with a flow rate of 0.6 mL/min. Eluent A was 0.1% penta deca fluoro octanoic acid (PDFOA) 99.5%: 0.5% water/CH_3CN with 0.1% formic acid. Eluent B was 0.1% PDFOA 10%:90% water/CH_3CN with 0.1% formic acid. Samples carried through the column with injected volume 2 µL. Samples that have gone through the chromatography column, move towards mass spectrometry. Specification of mass analyzer was two high-resolution, high-stability quadruple analyzers (MS1/MS2), plus pre-filters to maximize resolution and transmission while preventing contamination of the main analyzer. Before samples were injected, standard amino acids were injected for LC-MS/MS to obtain the spectra with varying retention times of each amino acid. Then, the obtained spectra of each amino acid from samples compared with the standard amino acid spectra, and the quantity was calculated and stated in mg amino acid/g protein. Analysis of amino acids was done in duplicate.

2.5 *Statistical analysis*

The analysis of data was performed by using software SPSS (version 19). One-way analysis of variance (ANOVA) at $p<0.05$ was used for the analytical variation. Least significant difference (LSD) test as multiple comparison methods was used to determine differences between means of the sample with a level of significance of 0.05.

3. Results and discussion

Soaking is the preliminary step in almost of legumes processing, which may influence metabolism in the seed. The metabolic activity of resting seeds increased as soon as they are hydrated during soaking (Dipnaik and Bathere, 2017). Penetration of water into the seeds could affect biochemical reactions in the seeds since the hydrolyzing enzyme, such as protease and transaminase were activated. As a result, changes protein of mung bean seeds during soaking occurred. Crude protein and soluble protein contents of the treated mung bean seeds are presented in Figures 1 and 2, respectively. There were significant differences ($p<0.05$) in each variable among those treatments.

Crude protein content of mung bean without soaking (control) in this study was 26.64% dry weight, similar to that reported by other researchers i.e. Mubarak (2005) and Wongsiri *et al*. (2015) found protein contents of 27-30% and 25.58%, respectively. Those differences might be due to the mung bean plant growing conditions and soil types. The crude protein contents of soaked mung bean were in a range of 23.52 and 25.33% dry weight

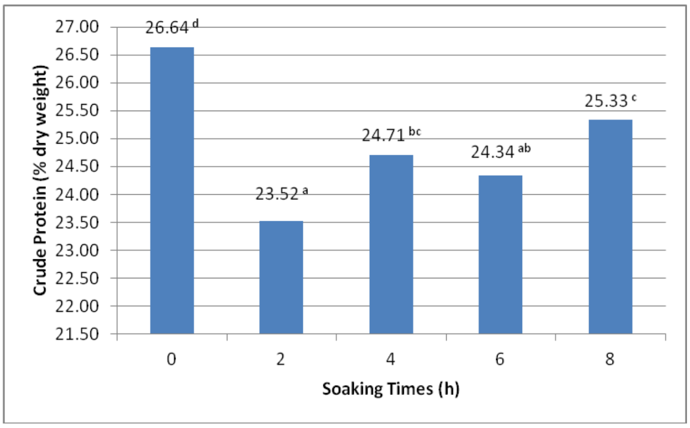

Figure 1. Effect of soaking time on the crude protein content of mung bean

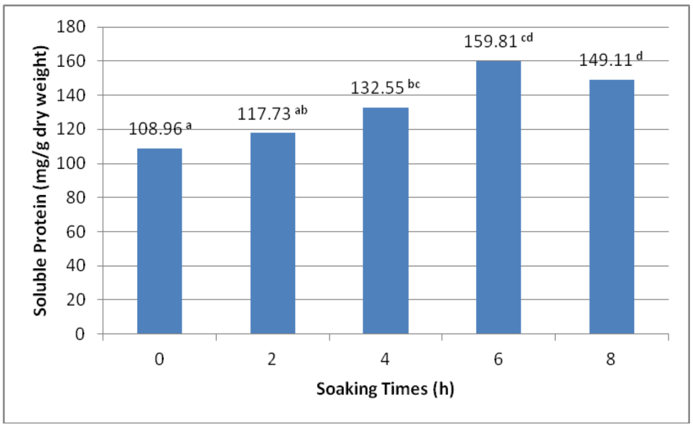

Figure 2. Effect of soaking time on the soluble protein content of mung bean

(Figure 1), lower than that of the control. This might be due to leaching of soluble protein into soaking water.

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There was an increase of crude protein content during soaking for 4-8 hrs compared to 2 hrs soaking time. This phenomenon showed that protein synthesis occurred after 2 hrs soaking times. Meanwhile, there was leaching of water-soluble protein in a significant amount during the 2 hrs soaking. Nonogaki *et al.* (2010) also stated that protein synthesis occurred during inhibition.

The measurement of soluble protein in mung bean seeds aims to determine the change of polypeptides into simpler peptides that are more soluble so that they could be an indicator of readiness of proteins to be digested. According to the data in Figure 2, the soluble protein was significantly different among all the treatments and it showed a trend of soluble protein increasing except on 8 hours soaking treatment.

The composition of amino acid in food ingredients could determine the protein quality. The mung beans protein is rich in some essential amino acids, including aromatic amino acids, leucine, isoleucine and valine, as well as glutamic acid (Tang *et al*., 2009). The results showed that amino acid composition changed during soaking as shown in Figures 3 and 4. In this study, all essential amino acids were measured except tryptophan. Tryptophan in raw mung beans was 6.4 mg/g protein (Yi -Shen *et al*., 2018). The non-essential amino acids tend to increase more during soaking than the total essential amino acids (without tryptophan) because the utilization of non-essential amino acids occurred in a smaller amount by seeds tissue.

The increasing level of total aromatic amino acid was the highest among other essential amino acids. Phenylalanine, one of aromatic amino acids group, tend to increase in soaking periods (Figure 3). This seems would be continued when the mung bean seeds germinated as reported by Wongsiri *et al.* (2015). The aromatic amino acids, a major group of the total antioxidative amino acids, increased during the germination (Wongsiri *et al.,* 2015). Randhir *et al.* (2004) explained that one of the substrates for the synthesis of phenolic compound via the phenylpropanoid pathway during germination was phenylalanine.

Lysine and leucine levels in observed mung beans were relatively high, and vice versa a small amount of methionine and cysteine, sulfur-containing amino acids. Mung beans with a high content of lysine combined with cereal grain proteins make them an excellent enhancer of protein quality (Deraz and Khalil, 2008). The content of sulfur amino acids (SAA) which were relatively low in legumes has been reported may provide an advantage of calcium retention because SAA metabolism may cause demineralization of calcium of bone and be excreted through urine (Messina, 1999). Calcium retention gives benefit to both mothers and babies because calcium was needed especially for maintaining bone mass density for mothers and the teeth and skeletal growth for babies and children.

Figure 4 also shows that glutamic acid was the dominant amino acid, followed by aspartic acid. Both amino acids tend to increase during 6 h of soaking. This phenomenon is a benefit for mung beans as a protein source for breastfeeding women because these amino acids were needed for newborn babies. Agostoni *et al.* (2000b) reported that glutamine and glutamic acid increased in human milk through a three-month lactation period. Both of those free amino acids also reported

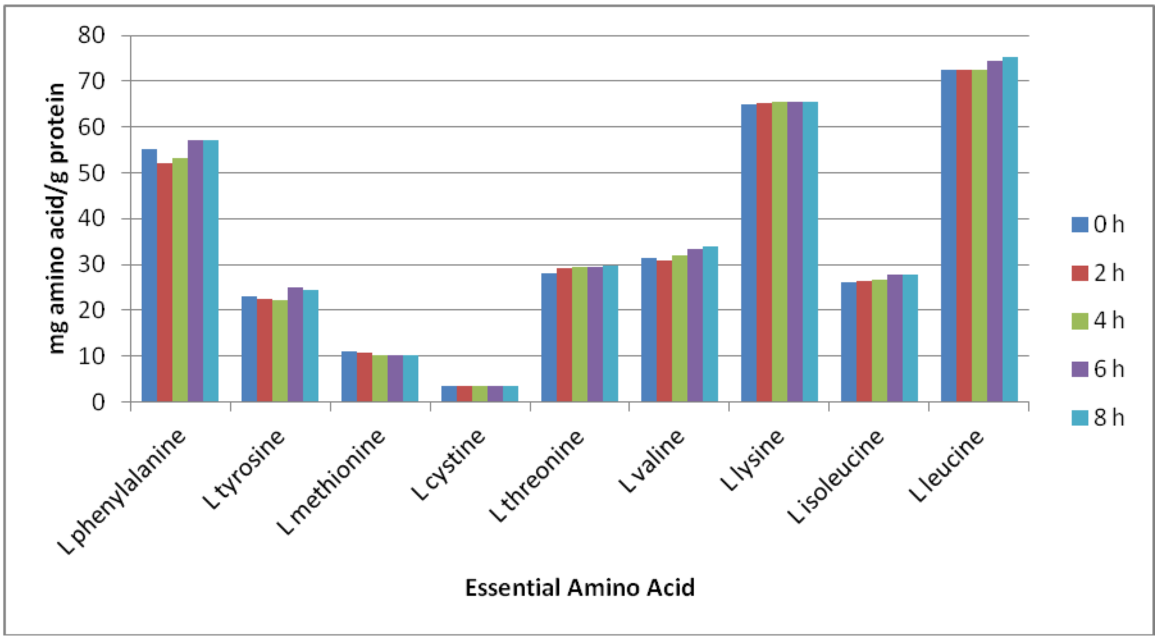

Figure 3. The essential amino acid profile during soaking

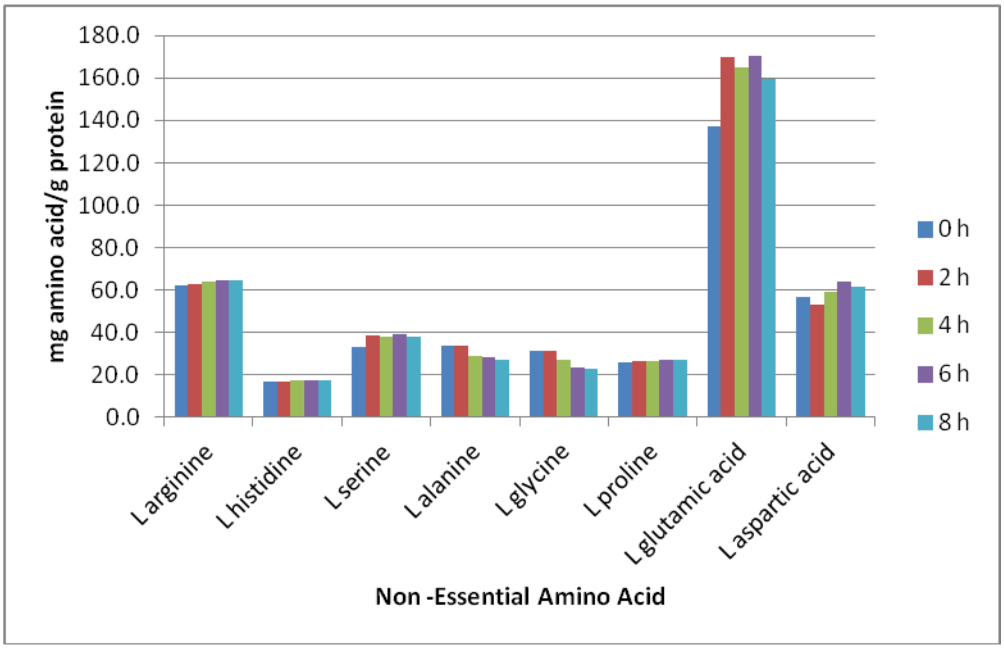

Figure 4. The non-essential amino acid profile during soaking

might have a double role for infants, give protection to intestinal mucosa and act as neurotransmitters. Mucosal intestinal cells preferentially transaminate glutamic acid to yield alanine entering the gluconeogenic pathway, and both glutamic acid and glutamine from the lumen contributed major energy substrate for the intestinal cells. However, the relation of the high content of glutamic acid in mung bean and human milk of breastfeeding women who consumed the mung bean still unknown.

4. Conclusion

The soaking treatment improved the protein quality. In this study, soaking of whole mung bean seeds for 6 hrs was recommended. Those findings of amino acids profile, crude protein and soluble protein contents revealed that the soaked mung beans were potentially good and an inexpensive source of protein for breastfeeding women, especially in combination with rice which is a staple food for most of the Indonesian people.

Acknowledgment

Authors would thank the Directorate of Research and Community Service, Directorate General of Research and Development Strengthening, Ministry of Research; Technology and Higher Education, Republic of Indonesia, for research fund through the Decentralization Research Program of 2018 (Penelitian Dasar Unggulan Perguruan Tinggi with contract number of 115O/ WM01.5/N/2018).

References

Agostoni, C., Carratù, B., Boniglia, C., Lammardo, A.M., Riva, E. and Sanzini, E. (2000a). Free amino acid content in standard infant formulas: comparison with human milk. *Journal of the American College of Nutrition*, 19(4), 434-438. https://doi.org/10.1080/07315724.2000.10718943

Agostoni, C., Carratù, B., Boniglia, C., Lammardo, A.M., Riva, E. and Sanzini, E. (2000b). Free glutamine and glutamic acid increase in human milk through a three-month lactation period. *Journal of Pediatric Gastroenterology and Nutrition,* 31(5), 508-512. https://doi.org/10.1097/00005176-200011000-00011

AOAC. (2000). Official Methods of Analysis of the Association of Official Analytical Chemists. 17th ed. Maryland, USA: AOAC

Chandrachood, P.S., Gadkari, T.V., Bhave, A.A., Torane, R.C., Deore, S., Deshpande, N.R. and Kashakar, R.V. (2011). Detection of amino acids by LC-Mass spectroscopy from the leaves of *Tabernaemontana divaricata*. *Der Pharma Chemica*, 3(4), 449-453.

Deraz, S.F. and Khalil, A.A. (2008). Strategies to improve protein quality and reduce antinutritional factors in mung bean. *Food,* 2(1), 25-38.

Dipnaik, K. and Bathere, D. (2017). Effect of soaking and sprouting on protein content and transaminase activity in pulses. *International Journal of Research in Medical Sciences,* 5(10), 4271-4276. http://dx.doi.org/10.18203/2320-6012.ijrms20174158

Guillon, F. and Champ, M.J. (2002). Carbohydrates fractions of legumes: uses in human nutrition and potential for health. *British Journal of Nutrition*, 88 (S3), 293-306. http://doi.org/10.1079/BJN2002720

Hussain, I. and Burhanddin, M. (2011). Optimization of germination conditions for germinated mung bean flour by response surface methodology. *African Journal of Food Science and Technology*, 2(10), 232-239.

Kaur, M., Sandhu, K.S., Ahlawat, R.P. and Sharma, S. (2015). In vitro starch digestibility, pasting and textural properties of mung bean: effect of different processing methods. *Journal of Food Science and Technology*, 52(3), 1642-1648. http://doi.org/10.1007/s13197-013-1136-2

Kruger, N.J. (2002). The Bradford Method for Protein Quantitation. In Walker, J.M. (Ed.). The Protein Protocols Handbook. 2nd ed. Totowa, New Jersey, USA: Humana Press Inc.

Messina, M.J. (1999). Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition*, 70(3), 439S-450S. http://doi.org/10.1093/ajcn/70.3.439s.

Mubarak, A.E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional process. *Food Chemistry*, 89(1), 489-495. http://doi.org10.1016/j.foodchem.2004.01.007

Nonogaki, H., Bassel, G.W. and Bewley, J.W. (2010). Germination-still a mystery. *Plant Science*, 179(6), 574-581. https://doi.org/10.1016/j.plantsci.2010.02.010

Randhir, R., Lin, Y.T. and Shetty, K. (2004). Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and phytochemical elicitors. *Process Biochemistry*, 39(5), 637-646. https://doi.org/10.1016/S0032-9592(03)00197-3

Sashikala, V.B., Screerama, Y.N., Pratape, V.M. and Narasimha, H.V. (2015). Effect of thermal processing on protein solubility of green gram (*Phaseolus aureus*) legume cultivars. *Journal of Food Science and Technology*, 52(3), 1552-1560. Doi: 10.1007/s13197-013-1149-x.

Tang, C.H., Chen, L. and Ma, C.Y. (2009). Thermal aggregation, amino acid composition and in vitro digestibility of vicilin-rich protein isolates from three Phaseolus legumes: A comparison study. *Food Chemistry*, 113(4), 957-963. https://doi.org/10.1016/j.foodchem.2008.08.038.

Wongsiri, S., Ohshima, T. and Duangmal, K. (2015). Chemical composition, amino acid profile and antioxidant activities of germinated mung beans (*Vigna radiata*). *Journal of Food Processing and Preservation*, 39(6), 1956-1964. https://doi.org/10.1111/jfpp.12434

Yi-Shen, Z., Shuai, S. and FitzGerald, R. (2018). Mung bean proteins and peptides: nutritional, functional and bioactive properties. *Food and Nutrition Research*, 62, 1290. http://dx.doi.org/10.29219/fncv623. 1290.