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Nutritional improvement of an Egyptian breed of mung bean by probiotic lactobacilli

Ashraf A. Khalil

Protein Technology, Genetic Engineering and Biotechnology Research Institute, Mubarak City for Scientific Research, Alexandria, Egypt. E-mail: egyprot@yahoo.com. Tel/Fax: +202 222 0262.

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Germination and/or fermentation processes for Egyptian breeds of mung seeds were carried out with three *Lactobacillus* strains namely, *L. reuteri, L. case,* and *L. heleviticus*. Results revealed increase in protein content, nitrogen solubility and *in vitro* digestibility for all treated mung meals. Treated mung proteins contained most of the essential amino acids in concentrations comparable to those recommended by the FAO/WHO, with the exception of the sulphur-containing amino acids. Trypsin inhibitor units (TIU) and phytate content of untreated MB were 6.98 TIU/mg dry sample and 5.61 mg phytate/g dry sample, respectively. Fermentation treatments were effective in decreasing the phytate content as well as reducing trypsin inhibitor activity, while *L. casei* was the most efficient probiotic culture in reducing those antinutritional factors. The combined treatment of germination and lactic fermentation utilizing *Lactobacillus* species is recommended process for enhancing the nutritional quality of legume protein.

Key words: Mung bean, protein extract, *Lactobacillus*, fermentation, germination, nutritive value.

INTRODUCTION

Protein is one of the five basic components in an adequate diet. In developing countries, there is an urgent need of nutritious foods to meet the nutritional requirements of the ever-increasing populations (Prakash and Misra, 1988). Since legumes seeds are important sources of protein, complex carbohydrate and dietary fiber in the diet (Morrow, 1991), there has been a worldwide interest in searching for potential utilization of unconventional legumes (Siddhuraju et al., 1996). The observed increase in the search for alternative or additional food and feed ingredients, especially for the developing countries, is of paramount importance for two reasons: (1) the low production of oil seeds and grains; and (2) the stiff competition between man and the livestock industry for existing food and feed materials (Siddhuraju et al., 2000). Today, the Egyptian diet is supposed to be high in fiber and low in fat and calories.

Legumes are the basic ingredients of such staff-of-life dishes (Amine et al., 1990). As the need for pulses in Egypt is increasing with the increase in population, there is emphasis on introducing new types of legumes like mung bean (*Vigna radiate* L.). Mung beans (MB) are a good source of energy, proteins, vitamins, minerals and dietary fiber. They are relatively inexpensive compared to meat foods and they have high carbohydrate content. MB can be useful as high energy foods for peasants and nomadic farmers. The high lysine content of MB makes them an excellent enhancer of protein quality when combined with cereal grain proteins.

Most developing countries of the world today face the problem of inadequate protein intake by a large section of their population. However, utilization of legume protein is below their potential partly due to the deficiency of some of the essential amino acids in their proteins and also due to the presence of some antinutritional factors associated with their protein (Kavas and Nehir, 1992). Protease inhibitors, lectins, gossypol, phytate and others are among the antinutritional factors associated with the legume meal proteins (Liener and Kakade, 1980).

Food fermentation processes are reliant on both endogenous and microbial enzymatic activities for the

Abbreviations: EAA, Essential amino acids; MB, mung bean and EAA, essential amino acids.

Table 1. Different treatments carried on mung seeds.

Treatment		Bacterial culture	Abbreviation
Germination	Fermentation		
No	No	-	M
Yes	Yes	*L. casei subsp casei* NRRL B-1922	MGc
Yes	Yes	*L. helveticus* NRRL B-4526	MGh
Yes	Yes	*L. reuteri* NRRL B-14171	MGr
No	Yes	*L. casei subsp casei* NRRL B-1922	MUGc
No	Yes	*L. helveticus* NRRL B-4526	MUGh
No	Yes	*L. reuteri* NRRL B-14171	MUGr

degradation of starches, lipids, proteins, antinutritional and toxic factors. Fermented legumes have increasingly gained interest in food applications because of its possible nutritional and health benefits and the potential use as a food flavoring agent. Examples of indigenous food fermentations are mung sauce (shoyu), Japanese miso, Indonesian tempeh, Indonesian tape ke ten, Japanese sake (Steinkraus, 1983), and Egyptian Kishk and Bouza (Morcos et al., 1973). Unlike fermented soy foods, which have been extensively studied, no previous studies on fermented mung foods been carried out. Published work on locally-breeding Egyptian mung has focused on their proximate composition and protein availability and on the effects of modifying basic processing parameters such as soaking, dehulling and heat treatment. Alternative processing methods for Egyptian MB have not been given much attention in the past. Therefore, there is a dearth of information on MB extract and its fermented products. Fermentation is a low-cost and the most economical technique of production and preservation of foods in developing countries.

The objective of this study is to produce nutritive protein isolates based on local variety of MB. The effect of germination and/or fermentation on the protein value, antioxidant activity and antinutritional factors of mung meals was studied. The study is expected to give an insight into the impacts of combined effect of germination and fermentation on the nutrient status and functionality of the seed product.

MATERIALS AND METHODS

Cultures, seeds, and chemicals

Lactobacillus strains in this study were used namely *L. casei* sub sp *casei* NRRL B-1922, *L. helveticus* NRRL B- 4526, and *L. reuteri* NRRL B-14171. The cultures were obtained from ARS Culture Collection, Peoria, IL 61604, USA. Fully grown locally-breeding mung (*Vigna radiate* L.) seeds variety Qawmy 1 were obtained within 4 months of being harvested as a kind gift from Prof N. Ashour at the National Research Center, Egypt. All chemical reagents used in the experimental analysis were analytic grade.

Seeds pretreatment

The seeds were cleaned by rinsing two times with distilled water, sterilized with 1% sodium hypochlorite solution for 10 min, rinsed three times with fresh distilled water, dried in a ventilated oven at 40°C and divided into three sets (Table 1); one was kept as raw sample (untreated), one used for germination treatment (germinated), and the third used for a combined germination and fermentation treatment (germinated-fermented). Mung seeds were crushed to smaller fragments and then milled in a Tecator Cyclotec mill using 1-mm sieve to flour. Break and reduction flours were collected together and blended thoroughly. The blended flours were used throughout this research. MBs were milled to flour directly in a Tecator Cyclotec mill. All flour samples were stored in air-tight plastic containers at -20°C until used.

Germination

Mung seeds were soaked in tap water (1:3, w/v) for 8 h. Germination was carried out by spreading the soaked seeds in wet blotting paper and kept at 25°C for 72 h to germinate with 8 h of daylight per day. The materials were kept wet throughout germination by spraying them with distilled water every 12 h. The unimbibed seeds were discarded and the sprouts were rinsed twice in distilled water and dried in a ventilated chamber at 8°C. The dried sprouts were crushed then milled as indicated above.

Preparation and inoculation of blends

To maintain culture throughout the study, *Lactobacillus* strains were subcultured onto MRS broth twice for 24 h at 37°C, and stored into the same broth at 4°C. Inoculum was prepared by sub culturing twice under same conditions, then the cells were resuspended in sterile distilled water to obtain an approximate cell concentration of 1-5 x 10^8 cfu/ml. Flours of MB (either germinated or ungerminated) were sterilized by autoclaving for 20 min at 121°C, and then mixed for 2 min with sterile water (40°C) in a blender to obtain slurries with dry legume content of 50%. Slurries were individually inoculated with 5% of active culture and incubated at 37°C for 48 h. The fermented slurries were freeze-dried and subjected for protein extraction.

Legume seed protein extracts

The powders of untreated (raw) and treated (germinated and/or hydrolyzed) samples were subjected to defatting in a Soxhlet apparatus using n-hexane (1:3 w/v) at a temperature of about 40°C until the fat content was reduced to about 5%. Defatted samples

Table 2. Protein and *in vitro* digestibility of ungerminated and germinated mung fermented with *Lactobacillus* strains[a].

Sample[b]	Total protein (%)	Difference (%)	Digestibility (%)	Difference (%)
M	24.86	–	67.12	–
MUGc	27.49	10.6	68.23	1.7
MUGh	26.09	4.9	67.95	1.2
MUGr	26.35	6.0	68.81	2.5
MGc	25.96	4.4	69.37	3.4
MGh	25.17	1.2	69.78	3.9
MGr	25.81	3.8	70.46	5.0

[a]Values are means of three replicates.
[b]M, Mung; MUGc, ungerminated MB fermented with *L. casei*; MUGh, ungerminated MB fermented with *L. helveticus*; MUGr, ungerminated MB fermented with *L. reuteri*; MGc, germinated MB fermented with *L. casei*; MGh, germinated MB fermented with *L. helveticus*; MGr, germinated MB fermented with *L. reuteri*.

were spread to dry at room temperature until all traces of solvent were removed, and saved for further analysis.

Analysis of legume extracts

Nitrogen content was determined by the microkjeldahl technique according to AOCS (1998). The conventional nitrogen-to-protein conversion factor of 6.25 was used for estimating the protein content. Nitrogen solubility at various pH values was determined by the following procedure according to Lyman et al. (1953). *In vitro* digestibility was calculated from the regression equation of Hsu et al. (1977), $Y = 210.464 – 18.103\ X$, where Y = protein digestibility (%) and X = pH of protein suspension after 10 min digestion.

Amino acids contents were determined as described by Moore et al. (1958). The analysis was performed at Central Service Unit, National Research Centre, Egypt using LC3000 amino-acid analyzer (Eppendorf-Biotronik, Germany). Cysteine and methionine were determined as cysteic acid and methionine sulfone, respectively, after oxidation with performic acid (Schram et al., 1954). An amino acid standard containing cys was treated parallel with the samples and used to quantify the cys content. The amino acid content of the reference protein was taken from FAO/WHO (1991). The essential amino acid (EAA) score was also calculated.

Trypsin inhibitor activity was determined in quadruplicate according to Hammerstrand et al. (1981) with benzoyel-D, L-arginine *p*-nitroanilide. Phytate content as described by method of Wheeler and Ferrell (1971) and as modified by Alonso (1995). Results are expressed as percentage phytic acid by using standard phytic acid.

RESULTS AND DISCUSSION

The new trend in food processing promotes the use of biotechnological methods over chemical methods, as well as additives from natural sources rather than synthetic ones. From this point of view, this study aimed to investigate the application of germination and fermentation as means to improve the nutritional value as well as enhance the antioxidant activity of locally-breeding legume namely, mung. Up to the best of our knowledge, *Lactobacillus* strains used were not reported before for this particular purpose especially with MB.

Protein content, nitrogen solubility and *in vitro* digestibility

Fermentation of germinated or ungerminated MB with the three *Lactobacillus* strains resulted in an increase of protein content and *in vitro* protein digestibility (Table 2). Protein content increased in all treated MB samples. The highest (%) protein increase was obtained in ungerminated MB samples fermented with *L. casei* (MUGc) while the lowest was observed for germinated samples treated with *L. helveticus*. Germination increased protein content of cotton seed, mung and sunflower, due to the utilization of carbohydrates as an energy source (Liener and Kakade, 1980). Evidence of protein synthesis during germination was presented earlier by Kylen and Mccready (1975). They found the protein content of all sprouts was higher than that of the ungerminated seeds. Highest improvements in *in vitro* protein digestibility were observed in germinated and fermented samples regardless the bacterial strain used. *In vitro* digestibility is a useful method for protein quality evaluation (Akeson and Stahmann 1964). Generally, fermentation of MB with *L. reuteri* followed by *L. helveticus* gave the best improvements in protein characteristics. Figures 1 and 2 show the nitrogen solubilities for untreated and treated MB samples. For all samples, lowest and highest solubilities were recorded at pH 4 and 9, respectively. The present study shows increased solubility both above and below pH 4, which is the isoelectric point. This behavior is similar to many other seed flours reported by Akobundu et al. (1982). It was observed that alkaline pH supports the greater extraction of soluble protein (Ma and Harwalkar, 1984),

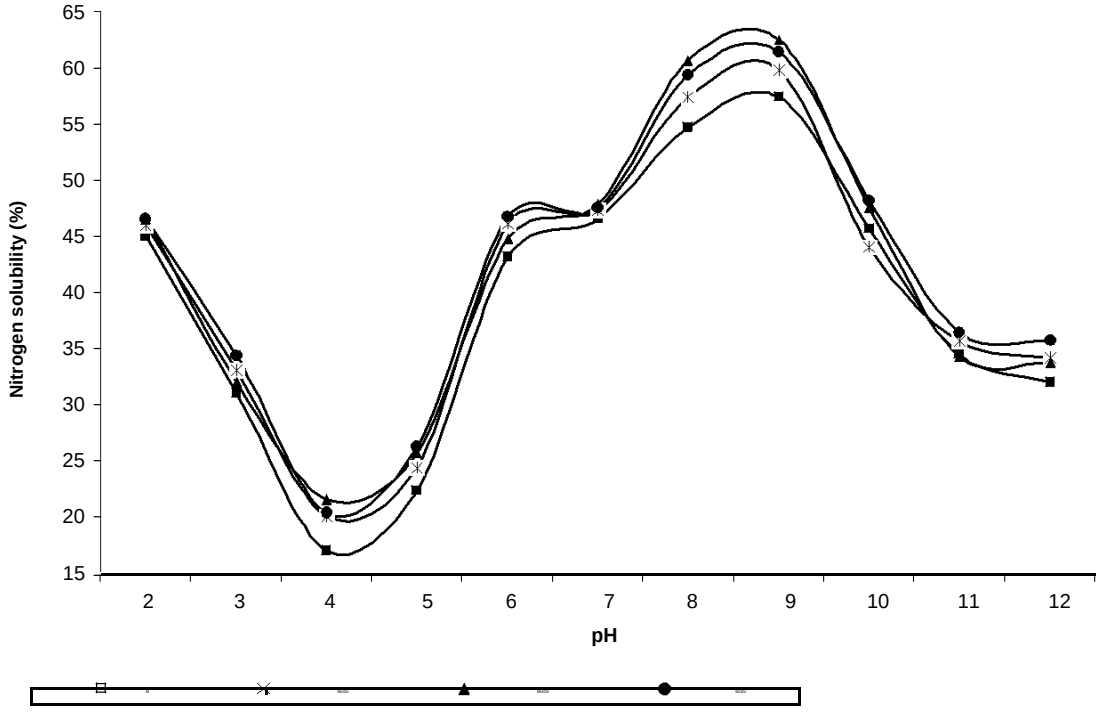

Figure 1. Nitrogen solubilities of ungerminated mung bean samples fermented with *Lactobacillus* species. MUGc, ungerminated MB fermented with *L. casei*; MUGh, ungerminated MB fermented with *L. helveticus* ; MUGr, ungerminated MB fermented with *L. reuteri*; MGc, germinated MB fermented with *L. casei*; MGh, germinated MB fermented with *L. helveticus*; MGr, germinated MB fermented with *L. reuteri.*

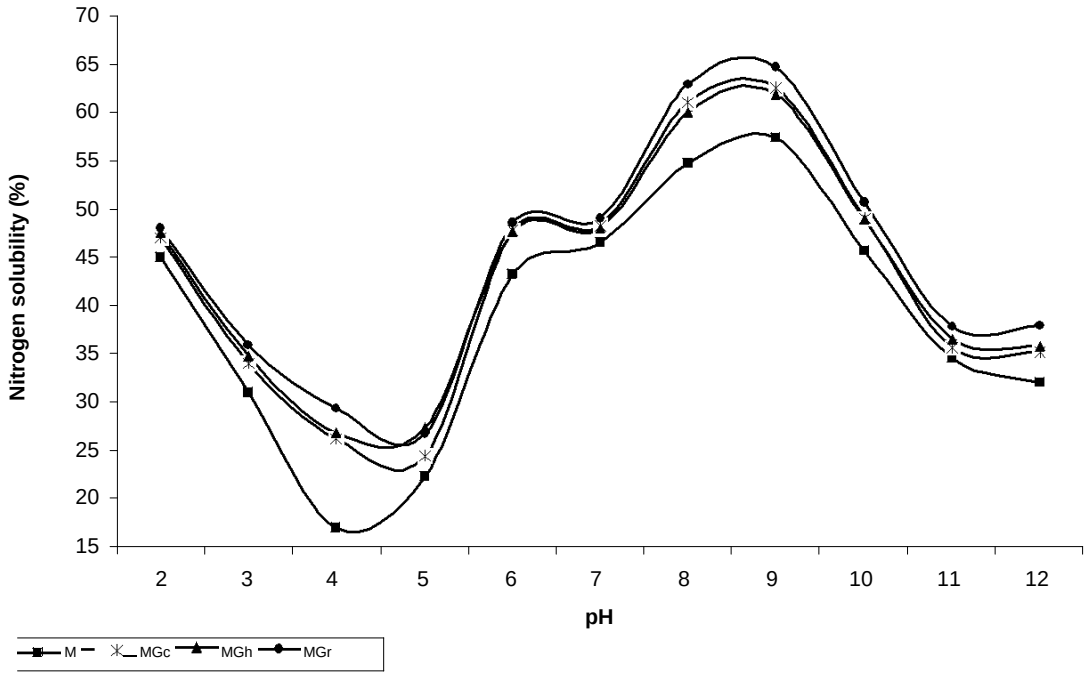

Figure 2. Nitrogen solubilities of germinated mung bean samples fermented with *Lactobacillus* species. MUGc, ungerminated MB fermented with *L. casei*; MUGh, ungerminated MB fermented with *L. helveticus*; MUGr, ungerminated MB fermented with *L. reuteri*; MGc, germinated MB fermented with *L. casei*; MGh, germinated MB fermented with *L. helveticus*; MGr, germinated MB fermented with *L. reuteri.*

Table 3. Trypsin inhibitor units (TIU) and phytate contents of ungerminated and germinated mung fermented with *Lactobacillus* strains[a]

Sample[b]	Trypsin inhibitor units	Difference (%)	Phytate contents	Difference (%)
M	6.98	–	5.61	–
MUGc	5.62	19.45	3.48	37.85
MUGh	5.81	16.7	4.01	28.47
MUGr	2.57	63.2	3.94	29.63
MGLc	1.17	83.26	1.84	67.24
MGLh	2.39	65.72	3.64	35.11
MGLr	1.47	78.91	2.85	49.18

[a]Values are means of three replicates.
[b]M, Mung; MUGc, ungerminated MB fermented with *L. casei*; MUGh, ungerminated MB fermented with *L. helveticus*; MUGr, ungerminated MB fermented with *L. reuteri*; MGc, germinated MB fermented with *L. casei*; MGh, germinated MB fermented with *L. helveticus*; MGr, germinated MB fermented with *L. reuteri*.

which is in agreement with the results shown in Figures 1 and 2. The combined treatment of germination and fermentation brought an increase in nitrogen solubility both at acid and alkaline pH.

The increase in solubility and digestibility in fermented seeds could be attributed to the proteolytic activity of *Lactobacillus* (Fadda et al., 1999). It was postulated that the increase of protein susceptibility to proteolytic enzymes, due to partial protein denaturation and pH during fermentation, leading to increased solubility and digestibility (Czarnecki et al., 1993). However, Lactic acid fermentation did not improve protein digestibility of pea seeds, while fermentation and/or extrusion of bean seeds significantly increased protein digestibility (Czarnecka et al., 1998). Kiers and co-workers (2000) reported a tremendous increase in solubility and *in vitro* digestibility in fermented mung, cowpea and maize with *Rhizopus* spp.

Antinutritional factors

Table 3 shows the reduction (%) in TIA and phytates as a result of germination and/or fermentation of MB seeds. Trypsin inhibitor units (TIU) and phytate content of untreated MB were 6.98 TIU/mg dry sample and 5.61 mg phytate/g dry sample, respectively. The combined treatment of germination and fermentation reduced the antinutritional factors compared to the fermentation alone. The highest inactivation of trypsin inhibitor was obtained in germinated MBs fermented with *L. casei* (MGc) followed by *L. reuteri* (MGr). Germination is involved in the breakdown of seed reserves, owing to the expected increase in the enzymes activity (Bagley et al., 1963). Activation of inherent enzymes during the process of fermentation results in degradation of antinutrients (Fretzdorff and Brummer, 1992). Rattansi and Dikshit (1997) observed a decrease in protease inhibitors during fermentation with a linear relationship between the reduction in protease inhibitor activity and the improvement in digestibility. In the present study, fermentation reduced phytates content for all treated samples. Phytaes content was reduced during germination of different legume seeds as a result of phytase activity (Gibson and Ullah, 1988).

The treatments were effective in decreasing the phytate content for MB samples. Though, the combined germination and fermentation treatment reduced the phytate contents higher than those ungerminated only. The results are in agreement with previous studies when phytic acid content was halved during mung fermentation (Van Der Reit et al., 1987). However, Azeke et al. (2005) found that antinutrients, except phytic acid, were significantly reduced in African yam bean lactic acid fermentation with *L. plantarum.* Thus, from the results of the present study, it could be inferred that probiotic fermentation increased nutrient availability of the Egyptian MB.

Amino acid composition

The essential amino acid composition of ungerminated and germinated mung fermented with the three *Lactobacillus* strains; together with the recommended FAO requirements for human adults are presented in Table 4. The contents of essential amino acids (EAA) such as lysine, valine, isoleucine and aromatic acids in treated MB samples were found to be higher than those of the FAO/WHO (1991) recommended pattern. The other EAA, threonine, and leucine, were at levels comparable to the reference pattern. Generally, germination-fermentation process resulted in increased yield of the most amino acids compared to non germinated samples. The consumption of mung proteins can fulfill the essential amino acids requirements with the

Table 4. Amino acid composition and essential amino acid score of ungerminated and germinated mung samples fermented with *Lactobacillus* strains.

Amino Acids[a]	FAO/WHO reference value[b]	Amino acid content (g amino acid/16 g nitrogen)													
		Raw		Ungerminated fermented mung[d]						Germinated fermented mung					
		M	EAA Score[c]	MUGc	EAA Score	MUGh	EAA Score	MUGr	EAA Score	MGc	EAA Score	MGh	EAA Score	MGr	EAA Score
Lys	5.8	6.48	112	6.82	118	6.59	114	6.75	116	6.39	110	6.75	116	6.83	118
Val	3.5	5.28	151	5.24	150	5.48	157	5.67	162	5.96	170	5.30	15	5.57	159
Thr	3.4	4.79	141	4.80	141	4.98	146	4.47	131	4.45	131	4.24	125	4.36	128
Ile	2.8	4.33	155	4.87	174	4.71	168	4.60	164	4.82	172	4.41	158	4.69	168
Leu	6.6	7.96	121	7.97	121	8.29	126	8.38	127	8.03	122	7.82	118	7.95	120
His	1.9	2.68	141	2.88	152	2.35	124	2.91	153	2.46	129	2.69	142	2.95	155
Cys	2.5	0.24	46	0.12	32	0.14	37	0.18	22	0.25	22	0.13	26	0.28	45
Met		0.91		0.68		0.79		0.37		0.31		0.52		0.84	
Phe	6.3	6.19	120	6.54	130	6.12	117	6.46	128	6.61	128	6.73	136	6.93	130
Tyr		1.35		1.59		1.28		1.62		1.47		1.86		1.29	
Trp		ND		ND		ND		ND		ND		ND		ND	
Total EAA	32.8	40.21	123	41.51	127	40.73	124	41.37	126	40.75	124	40.45	123	41.69	127

[a] Analysis performed in duplicates.

[b] Amino acid requirements patterns as suggested by FAO/WHO. Patterns obtained by dividing amino acid requirement (mg/kg per day) by safe level of protein intake (g/kg per day). Safe level of high-quality protein intake is 1.10 for pre-school children, 0.99 for school children and 0.75 for adults

[c] Essential amino acids (EAA) score. Total of aromatic amino acids (Tyr, Phe) as well as sulfur amino acids (Cys, Met) were calculated.

[d] M, Mung; MUGc, ungerminated MB fermented with *L. casei*; MUGh, ungerminated MB fermented with *L. helveticus*; MUGr, ungerminated MB fermented with *L. reuteri*; MGc, germinated MB fermented with *L. casei*; MGh, germinated MB fermented with *L. helveticus*; MGr, germinated MB fermented with *L. reuteri*.

exception of the sulphur-containing amino acids. Available lysine of treated legumes was improved. That increase may be due to the germination process or the stimulation of protease activity in legumes.

Kavas and Nehir (1992) found an increase in the essential amino acids as well as cystine and methionine in both germinated lentils and MBs. It is well accepted that the nutritional value of proteins may differ substantially depending on their (essential) amino acid composition and digestibility (Badau et al., 2005). With respect to the total EAA in the FAO/WHO requirements, all treated MBs seemed to be able to contribute adequately.

CONCLUSION

From the results of the present study, it could be inferred that probiotic fermentation increased nutrient availability of the Egyptian MB. These treatments not only increase protein content, improve digestibility and solubility of the protein, but also reduce the antinutritional factors. Fermented mung “gruel” would be used for preparation of different desirable and nutritive dishes for school children diets in rural areas. Further investigations on the physiological functions and nutritional value of mung seeds using animal feeding experiments are under way.

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