EFFECT OF GERMINATION OF TWO VARIETIES OF MUNG BEAN SEEDS (Vigna radiatus L.) ON THEIR FREE SUGARS, ANTINUTRITIONAL FACTORS AND NUTRITIONAL QUALITY

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ABSTRACT: The effect of germination of two varieties of mung bean seeds on the free sugar content, nutritional quality and antinutritional factors was studied. Verbascose represented the highest ratio in both varieties, followed by sucrose. The germination of mung bean seeds for 72hrs. had a marked effect on the reduction of the galactoside sugars. The most of essential amino acids, except methionine were increased after germination. In general, germination process was found to enhance the nutritional quality of protein in both varieties. The antinutritional factors in mung bean seeds was reduced by germination.

Keywords: Free sugars, germinated mung bean, nutritional quality, antinutritional factors.

INTRODUCTION

The rapid increase of human population especially in the poor countries makes food shortage a major world problem (Frias et al., 1996).

Legumes have been used as human foods for more than 80 centuries and are still one of the most important and inexpensive source of dietary protein where the animal products are scarce (El-Shimi et al., 1984; El-Mahdy et al., 1985 and El-Rify et al., 2000). However, legumes contain large amounts of antinutritional factors (i.e. trypsin inhibitors, alpha-galactosides, inositol phosphates) in the raw seeds that need to be reduced by processing before consumption (Augustin & Klein, 1989).

Germination has been proposed as a means by which the nutritional quality of legume seeds might be improved (Finney, 1983 and El-Shimi, 2000). The sprouting of legume seeds as food for human consumption has been a common practice in many cultures for several centuries, as it is an easy process that can be done at home or at a commercial scale and effective for achieving desirable changes in nutritional quality (Feng, 1997).

Legume sprouts have been used in culinary preparations in salad and to produce flours of high nutritional value. Many legume sprouts are now offered in the markets and in health food shops. These include alfa alfa, lentil, mung bean, soybean, pea, adzuki bean and chickpea (Ghorpade and Kadam, 1989 and Kuo et al., 2004).

In Egypt, mung bean has been introduced recently by the Ministry of Agriculture as a promising legume crops (El-Adawy, 1996).

The objective of this study was to determine the effect of germination on the nutritional value and free sugars, especially, the α -galactose oligosaccharrides of two varieties of mung bean as a new legume crop in Egypt.

MATERIALS AND METHODS

1- Materials:

- a-Two varieties of mung bean seeds (Vigna radiata L.) named G1000 and G2010 were obtained from the National Research Center El-Dokki, Giza, Egypt.
- b-Germinated seeds: The mung bean seeds were firstly soaked for 12hrs. in water at ratio of 1:5 (w:w). the soaked seeds were washed with tap water and then spread on wet cheese cloth in stainless steel baskets and germinated for 72hrs. at 25-28°C in the dark. The seeds were sprayed with 0.02% sodium hypochlorite solution twice a day to inhibit the microbial growth (Domoney et al., 1995). The sprouts were collected and dried at 55°C for ~30hrs.

2-Methods:

Moisture content and crude protein were determined according to A.O.A.C. method .The total soluble sugars were determined using the method of Dubois et al. (1956). Individual free sugars were extracted by 70% ethanol and separated by paper chromatography and identified by comparison with known standards as described by Lineback and Ke (1975). The separated free sugars were eluted with water and quantified as glucose using the method of Dubois et al. (1956).

Amino acids composition of germinated mung bean protein, except tryptophan was determined after acid hydrolysis using HPLC Shimadzu LG-10AD Rf-10 A fluorescence detector according to the method of A.O.A.C. (1995).

Tryptophan was determined colorimetrically according to the method of Sastry and Tummuru (1985).

The chemical score was calculated according to Bhanu et al. (1991) as follow:

= mg of essential amino acid in one g test protein mg of essential amino acid in one g reference protein

Computation of A/E ratio:

The relationship between the content of an individual essential amino acid in the food protein (A) and the total essential amino acids content (E) was calculated according to FAO (1965) as follows:

$$A/E$$
 ratio = $\frac{mg \text{ of the individual essential amino acid}}{g \text{ of total essential amino acid}}$

Computation of protein efficiency ratio (PER):

Protein efficiency ratio was calculated using the equation suggested by Alsmeyer et al. (1974) as follows:

PER= - 0.684 + 0.456 (leucine) - 0.047 (proline) (g/100g protein

Computation of biological value (BV):

Biological value of mung bean protein (BV) was calculated according to the equation of Oser (1959) as follows:

BV = 49.09 + 10.53 (PER).

In-Vitro protein digestibility(IVPD):

The in-vitro protein digestibility of mung bean was carried out according to the method of Singh and Jambunathan (1981) using pepsin and pancreatin enzymes.

Antinutritional factors:

Tannins was measured by the method of A.O.A.C. (1975) using Folin-Denis reagent after the samples being extracted by MeOH. Phytic acid was determined by the method of Thompson and Erdman (1982). Trypsin inhibitor activity was determined according to the method of Roy and Roa (1971) and expressed as trypsin inhibited units mg⁻¹ sample (TIU mg⁻¹).

Statistical Analysis:

was carried out according to Senedecor and Cochran(1967), using Duncan's Range Test .

RESULTS AND DISCUSSIONS

Data in Table (1) show the free sugar content of both varieties of mung bean G1000 and G2010 before and after germination. Verbascose represented the highest ratio of free sugar in both varieties 42-75 and 38-99% followed by sucrose, 27.91 and 25.24% in G1000 and G2010 respectively. Both varieties contained almost the same amount of raffinose. Galactose, fructose and glucose were the minor free sugars in raw mung bean seeds. From the results in Table (1), the germination of mung bean for 72hrs led to reduce the verbascose to a minimum level. Both raffinose and stachyose were reduced by about 50% in both varieties. On the other hand,

glucose was highly increased from 0.14g/100g to 1.93g/100g and 0.13g/100g to 1.8g/100g in G1000 and G2010 varieties respectively. Fructose was noticed to increase in both varieties from 0.13q/100q to 1.09q/100q and from 0.23g/100g to 0.7g/100g in G1000 and G2010 respectively. Also, a little increase in sucrose content of germinated mung bean seeds was observed in both varieties. These results indicate that, the germinated mung bean contain a high amount of sucrose. Similar to that occurred in lupin and differ than other legumes as reported by Donangelo et al. (1995). El-Mahdy and El-Sebaiy (1983) reported that a substantial increase in sucrose was found after germination of fenugreek seeds for 96hrs. On the other hand, α-galactosides were present in mung bean at a lower level than that reported for lupin. soybean and black bean (Donangelo et al., 1995 and Trugo et al., 2000). Ei-Mahdy and El-Sebaiy (1983) noticed that raffinose and stachyose content of fenugreek seeds decreased to 53% and 78% of their original values after germination for 96hrs. However, the decrease in a-galactosides may be attributed to the higher activity of a-galactosidase during the germination of mung bean. Chung et al. (2001) reported that five isoforms of galactosidase were isolated from five days germinated mung bean; three of these forms have a molecular mass of 87 Kda and comprising of two non identical subunits.

Table (1): Effect of germination* on the free sugar content of mung bean (on dry weight basis)

Sugar	G1000				G2010				
	Raw		Germinated		Raw		Germinated		
	g/100g	%FS	g/100g	%FS	g/100g	%FS	g/100g	%FS	
Stachyose	0.59	12.21	0.27	4.85	0.71	16.33	0.37	7.31	
Verbascose	2.07	42.75	0.00	0.00	1.68	38.99	0.08	1.54	
Raffinose	0.50	10.39	0.19	3.47	0.42	9.75	0.21	4.24	
Galactose	0.06	1.28	0.23	4.15	0.06	1.44	0.15	3.08	
Sucrose	1.35	27.91	1.73	31.06	1.09	25.24	1.60	31.97	
Glucose	0.14	2.88	1.93	34.66	0.13	2.89	1.80	35.96	
Fructose	0.13	2.58	1.08	19.39	0.23	5.35	0.70	13.98	
unknown	-	-	0.14	2.42	-	-	0.10	1.92	

^{*} Germination for 72 hrs. **% FS=%of free sugar from total soluble sugars

As a result, the carbohydrates available for fermentation in the large intestine in human will decrease. These results were found in agreement with many studies on other legumes, revealed that during germination process several enzyme systems become active and bring about profound changes in

the nutritive value of pulses (Udaysekhara and Belavady, 1978; Mohd et al., 1980, and Ibrahim et al., 2002).

The protein analysis revealed that, although there was an increase in crude protein from 26.18 and 26.33% to29.24 and 31..40 % in G1000 and G2010 variety respectively, true protein was reduced from 21.3and 24.65% to 19.48 and 20.01% in both varieties. The breakdown of protein and synthesis of the amino acids and other nitrogenous compounds may be behind these changes.

Results in Table (2) show the amino acid composition of raw and germinated mung bean seeds protein. It is noticed from the tabulated data that mung bean protein like other legumes is deficient in sulfur containing amino acids after germination for 72hrs. Germination process was led to slight increase in some amino acids, while decreased some others like methionine. The changes in amino acids content of germinated mung bean were found to be dependent on the variety. Glutamic acid was the predominate amino acid followed by leucine, phenylalanine and lysine in germinated mung bean seed proteins of both varieties. These changes may attributed to difference in breakdown and synthesis of proteins during germination process. The results obtained herein were in accordance with Buimindik et al. (1978), El-Rify et al. (2000) and Kuo et.al. (2004).

Table (2): Effect of germination on the amino acid pattern of mung bean seeds (g AA/100g protein).

G1000 G2010 FAO Amino acid Raw Germinated Raw Germinated Pattern I. leucine 4.95 5.00 5.46 5.51 4.57 Leucine 8.95 8.51 8.78 9.28 5.23 Lysine 7.14 7.35 7.18 9.04 8.21 Methionine 0.78 0.44 1.05 0.58 1.12 Phenylalanine 7.21 8.00 6.24 7.84 3.63 Threonine 4.08 4.86 4.03 4.74 3.68 Tryptophan 0.78 0.48 0.82 0.91 0.80 Valine 6.48 6.95 6.95 7.09 6.19 Alanine 4.68 4.82 4.20 5.09 Arginine 6.96 8.34 7.88 8.43 6.52 Aspartic 15.09 14.47 11.44 10.97 Cystine 0.74 0.50 0.90 0.78 1.09 Glutamic 18.53 15.87 17.64 16.81 Glycine 4.96 4.82 4.31 4.59 Histidine 4.21 4.31 3.99 4.00 3.90 Proline 4.56 4.60 3.96 4.92 Serine 5.43 5.77 5.03 5.41 Tyrosine 3.00 3.36 2.94 3.08 2.89

Abdel-Hamid (1980) found that germination of chickpea for 72hrs increased most of the essential amino acids content of the protein, especially methionine, phenylalanine, threonine, leucine and valine. In general, the germination process was found to enhance the nutritional quality of mung bean seeds proteins of both varieties.

The results in Table (3) reveal that, the A/E ratio of the germinated mung bean seeds did not improved for most essential amino acids. The comparison of the A/E ratio of the individual essential amino acids of the germinated mung bean with FAO requirement revealed that, the germinated mung bean seeds considered a rich source of the essential amino acids except methionine and tryptophan.

Table (3): A/E ratio of raw and germinated mung bean seeds.

Essential amino acid	G1000		G2010		FAO/WHO/UNU (1985)	
(EAA)	Raw	Germinated	Raw	Germinated	School child 10-12Yr	Adult
I. Leucine	121.69ª	118.04 ^b	135.69°	123.85 ^d	126.00	117.00
Leucine	221.70°	219.07 ^b	211.48°	197.35 ^d	198.00	171.00
Lysine	176.86ª	173.51 ^b	178.43ª	203.19 ^d	198.00	144.00
Methionine	19.32 ^a	10.38 ^b	26.09°	13.03 ^d	99.00	153.00
Ph-alanine	178.59ª	188.85 ^b	155.07°	176.22 ^d	99.00	171.00
Threonine	99.28°	114.73 ^b	100.15°	106.54 ^d	126.00	81.00
tryptophan	19.32ª	11.33 ^b	20.33ªc	20.45ac ^d	40.00	45.00
Valine	160.51ª	164.07 ^b	172.71°	159.36 ^{ad}	112.00	117.00
Computed PER	3.18	3.33	3.01	3.09		
Biological value (BV)	82.58ª	84.15 ^{ab}	80.79 ^{ac}	81.63 ^{abcd}		

In general, the obtained results are in agreement with those obtained by El-Rify et al. (2000), in spit of these values were slightly lower.

The results of calculated protein efficiency ratio (PER) and biological value (BV) of raw and germinated mung bean seeds of both varieties indicate that germination process for 72hrs improved the calculated PER and the BV of the protein by 10, 9.6% and 4.1 and 3.98% in G1000 and G2010 respectively. These results were in accordance with that of Chandrasekhar et al. (1981), Prabhaval (1988), El-Rify et al. (2000) and Mubarak (2005) who found that PER of mung bean was improved by germination.

As shown in Table (4) methionine was the first limiting amino acid followed by tryptophan in raw and germinated seeds of both varieties. The chemical score was 24.38 and 43.33, 13.75 and 26.67, 32.81 and 45.56, 18.13 and 50.56 for raw and germinated G1000 and G2010 respectively. The

obtained results confirmed that of Jamalian and Bellet (1968), Khan et al. (1979) and El-Rify et al. (2000) who reported that sulphur containing amino acids were the most limiting amino acids in legumes. Mubarak (2005) found that lysine and sulphur containing amino acids were the first and second limiting amino acid respectively in raw, dehulled and germinated mung bean seeds.

Table (4): Effect of germination on chemical score and limiting amino acid in mung bean seeds.

Essential amino acid	G	1000	G	2010	Whole egg mg EAA/g protein	
(EAA)	Raw	Germinated	Raw	Germinated		
	88.39ª	89.29°	97.50 ^b	98.39 ^b	56	
Leucine	107.23a	111.80 ^b	102.53°	105.78 ^d	83	
Lysine	113.33ª	116.67 ^b	113.97 ^{ac}	143.49 ^d	63	
Methionine	24.38ª	13.75 ^b	32.81°	18.13 ^d	32	
Ph-alanine	141.37*	156.86 ^b	122.35°	153.73b ^d	51	
Threonine	80.00	95.29 ^b	79.02 ^{ac}	92.94 ^{bd}	51	
tryptophan	43.33°	26.67 ^b	45.56 ^{ac}	50.56 ^d	18	
Valine	85.26ª	91.45 ^b	91.45 ^{bcd}	93.29 ^{bcd}	76	
First limiting AA	Met.	Met.	Met.	Met.		
Second limiting AA.	Trp.	Trp.	Trp.	Trp.		

The data in Table (5) show the effect of germination on the antinutritional substances in mung bean seeds. Although both varieties found to contain nearly the same level of tannins, the reduction caused by germination was different and affected by the variety. The reduction in tannins was 18.5 and 5.2% in G1000 and G2010respectively. The trypsin inhibitor activity was reduced by 39.7 and 43% in both G1000 and G2010 respectively. Mubarak (2005) reported that germination of mung seeds for three days reduced their content of tannins and trypsin inhibitor by 66 and 22% respectively.

Phytic acid was greatly reduced by 43.03 and 55.2% in G1000 and G2010 respectively. A similar result was reported for cowpea by Ibrahim *et al.* (2002).

Table (5): Effect of germination* on the antinutritional factors and protein digestibility of mung bean seeds.

digestibility of mun	g bean ac			
Constituent	G	1000	G2010	
	Raw	Germinated	Raw	Germinated
Tannins (mg tannic acid/100g)	16.40ª	13.35 ^b	16.40 ^{ac}	15.54 ^d
Trypsin inhibitor activity (TIU/g)	16427ª	9906 ^b	12551°	7154 ^d
Phytic acid (mg/100g)	674ª	334 ^b	518°	292 ^d
Protein digestibility %	77.35°	88.64 ^b	80.76°	92.58 ^d
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^{*}for 72 hrs.

Parabhavathi and Narasinga Rao (1979) reported that phytate constitute over 60% of the total phosphorus in raw bengal gram was reduced by 12% after two days of germination. This was contributed to the improving of nutrient bioavailability. The iron availability was found to increase eight to twelve folds in some grains (Sankara and Deosthale, 1980).

The enzymatic digestibility of sprouted mung bean seeds was markedly improved by germination for 72hrs compared to that of raw seeds of both varieties Table (5). El-Beltagy (1996) and Mubarak (2005) found that germination improved the in-vitro protein digestibility of mung bean seeds and they attributed this effect to the reduction of trypsin inhibitor and phytic acid. Trugo et al. (2000) found that lupine had a higher tryptic digestibility than soybean and black bean because of its lower trypsin inhibitor activity.

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تأثير الانبات على السكريات الحرة والعوامل المضادة للتغذية والجودة التغذوية في صنفين من بذور المانج

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الملخص العربي

تم دراسة تأثير الاتبات لصنفين من بذور الماتج على محتواهما من السكريات الحرة والجودة التغنوية والعوامل المضادة للتغنية. وقد اوضحت الدراسة ان سكر verbascose يمثل النسبة الاعلى في كلا الصنفين يليه السكروز, كما كان لعملية انبات البذور لمدة 72 ساعة تأثير ملحوظ على خفض السكريات الجالاكتوسيدية وبصفة عامة أدت عملية الاتبات الى زيادة معظم الاحماض الامينية الضرورية عدا الميثيونين كما تحسنت الجودة التغنوية وانخفضت مستويات العوامل المضادة للتغنية.