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Effect of Soaking and Germination Temperatures on Selected Nutrients and Antinutrients of Mungbean

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ABSTRACT

Effects of different temperatures, during soaking and germination on selected nutrients and antinutrients of mungbean, were studied. Comparison of the coefficients of variability revealed striking differences in their values as a result of soaking and germination. Soaking at 55°C resulted in larger decreases in the phytate and trypsin inhibitor activity than at 27°C whereas protein was little affected. Biosynthesis of protein, ascorbic acid and riboflavin was generally greater, and biodegradation of phytate and trypsin inhibitor higher, during germination at ambient conditions (20–35°C) than at low temperature (20°C) while thiamine and amino acid contents were less affected. Maximum contents of ascorbic acid (47·0 mg/100 g), riboflavin (3·54 μg/g) and thiamine (4·37 μg/g) were detected during germination for 48–72 h irrespective of temperature. A slight increasing trend in the protein content (18·4–23·5%) but decreasing trends in the phytate (216–105 mg/100 g) and trypsin inhibitor (2460–497 TIU/g) values, were observed during germination regardless of the temperatures tested.

INTRODUCTION

Individually, the plant foods are not sufficiently balanced in regard to essential nutrients and invariably contain harmful biochemical factors such as phytate and trypsin inhibitors. In Pakistan legumes, mungbeans are an especially important source of dietary proteins and other nutrients.

However, their role appears to be limited because of several factors, including low protein digestibility and ability of phytate to complex and reduce the bioavailability of certain essential metals (Underwood, 1975). In many parts of the world legumes are often consumed after soaking and germination, during which the nutritional value is increased (Khan & Ghafoor, 1978). It has been reported that certain vitamins (Sattar *et al.*, (1985), protein (Alexander, 1983), amino acids and sugars (Chavan *et al.*, 1981), were increased whereas phytic acid and trypsin inhibitor (Eskin & Wiebe, 1983; Kaur & Bhatia, 1984), decreased during germination of food grains. Most workers have invariably involved ambient temperatures during soaking or germination for improvement of the nutritional quality of legumes. However, patterns of changes in the constituents taking place under different experimental conditions have not been investigated. The objective of this study was to examine the comparative influence of ambient and controlled temperatures during soaking and germination on selected nutrients and harmful biochemical factors of mungbean.

MATERIALS AND METHODS

Mungbeans (*Phaseolus aureus*) of commercial variety (19–19), were obtained from the Mutation Breeding Division of this Institute. The seeds were dried under sunlight to a moisture level of about 10%.

Soaking and germination

For experiments on soaking, the cleaned seeds were soaked in 4–5 volumes of water (22–25°C) for 3–12 h under ambient laboratory conditions as well as at 55°C in an oven. In the experiments on germination, the seeds were initially soaked in 4–5 volumes of water and subsequently sprouted on filter papers in plastic trays (30 × 45 cm) for 24–120 h at ambient (20–35°C) and low temperatures (20°C) under prevailing light and dark conditions during the day and night, respectively.

Sample preparation

Germinated seeds, along with roots and shoots, were taken every 24 h, dried at about 70°C in an air-oven and ground in a Wiley mill to pass through a 40 mesh screen. The samples were stored in plastic bottles in a deep freezer until analysed for different nutrients and antinutrients except ascorbic acid which was determined in the fresh samples.

Biochemical analysis

Moisture was determined by drying at 105°C. Crude protein (%N × 5·7), was determined by the micro Kjeldahl method (AOAC, 1984). Extraction, precipitation and analysis of phytate phosphorus was carried out according to the method of Wheeler & Ferrel (1971) assuming an iron–phosphorus ratio of 4:6 in the ferric phytate. For the estimation of trypsin inhibitor activity (TIA) the method of Kakade *et al.* (1974) was used. Vitamin assays were performed according to the methods of the Association of Vitamin Chemists (1985). Ascorbic acid was determined by titration with 2,6-dichlorophenolindophenol and riboflavin and thiamine by fluorimetry using a Turner model 111 fluorimeter with 2A and 47B as primary filters and 2A12 as a secondary filter for riboflavin and 7–60 as a primary and 47B and 2A secondary filters for thiamine. For amino acid assays, dried samples were de-fatted by extraction with petroleum ether (b.p. 40–60°C) for 3 h. The residue was air-dried in an oven at 60°C for 4 h, ground in a stainless steel Wiley mill to pass through a 60 mesh sieve and then placed in a vacuum oven maintained at 65°C for 4 h. A procedure described by Blackburn (1968), was used for amino acid assay. The dried material, 40–45 mg in each case, was weighed into a glass tube and 10 ml of 6N HCl was used to hydrolyse the sample for 24 h after sealing the tube under vacuum in an oven at 105°C. The resultant amino acids were resolved on a single column (30 × 0·6 cm) of resin using an amino acid analyser (model LKB 4101) fitted with an automatic loader.

Statistical analysis

An estimate of relative variation in nutrients and antinutrients in relation to soaking and germination was made by determining the coefficient of variation (CV), which is a ratio in percentage of standard deviation to the mean (Little & Hills, 1972).

RESULTS AND DISCUSSION

The untreated mungbean seeds contained protein 18·4%, riboflavin 1·94 μg/g, thiamine 3·66 μg/g, phytate 216 mg/100 g and trypsin inhibitor (TI) 2460 TIU/g. Since the antinutrients and protein content were considered to be of interest during the soaking process, results concerning the influence of varying soaking time and temperatures on protein, phytate and trypsin inhibitor activity of mungbean are presented in Table 1. These treatments resulted in slight increases in the protein values. However, a

TABLE 1
Effect of Soaking on Protein, Phytate and Trypsin Inhibitor of Mungbean

Soaking time (h)	*Protein (%)*		*Phytate (mg/100 g)*		*Trypsin inhibitor (TIU/g)*	
	22–25°C	*55°C*	*22–25°C*	*55°C*	*22–25°C*	*55°C*
Unsoaked control	18·4	18·4	216	216	2460	2460
3	19·2	20·3	213	192	2000	1760
6	19·4	20·3	194	180	1960	1120
9	19·6	20·7	193	174	1080	892
12	20·0	20·3	180	168	908	553
Mean	19·3	20·1	199	186	1682	1357
CV	3·04	4·81	7·59	9·13	39·3	55·9

profound decreasing trend in the phytate and TI activity occurred with increasing time and temperature of soaking. The protein content increased from 18·4 to 19·3% and 18·4 to 20·1% after 12 h of soaking at 22–25°C and 55°C, respectively. The phytate values decreased from 216 to 199 and 186·0 mg/100 g while TI decreased 2460 to 1682 and 1357 TIU/g through soaking for 3–12 h at 22–25°C and 55°C, respectively.

The marginal increases in the total protein are not in fact real ones but merely the result of dissolution of starch content into the soaking medium. A decrease in the starch content (from 40 to 32%) during soaking of Fenugreek seeds was observed by El-Shimi *et al.* (1984). Similarly a decreasing pattern of starch has been reported during soaking/germination of corn at ambient conditions (Sattar *et al.*, 1985). An increase in the non-protein nitrogen as a result of soaking has also been shown (El-Shimi *et al.*, 1984; Youssef *et al.*, 1987). Although no change in the electrophoretic pattern was detected, soaking beyond 12 h led to the disappearance of one of the bands (Youseff *et al.*, 1987). Partial destruction of trypsin inhibitor and decomposition of phytate in relation to processing has been invariably observed in several legumes including mungbean (Haider & Chughtai, 1981; Gad *et al.*, 1982). Faster degradation of phytate during soaking at 55°C than 22–25°C in these studies is attributed to the combined effects of phytase and diffusion. El-Mahdy & El-Sebaiy (1982) indeed reported greater activity of phytase and phosphatase at 52°C than 32°C throughout soaking of Fenugreek seeds. An increase in the phytase activity with decrease in phytate, as a result of soaking and germination of Fababean, has also been observed (Eskin & Wiebe, 1983). In addition to the enzymatic hydrolysis of phytate, Chang *et al.* (1977) proposed diffusion to be an important process for the removal of phytate in dry beans. Extraction patterns of phytin-P in green gram revealed

higher extraction in 5N HCl than water (Kumar *et al.*, 1978). However, in the case of beans, all the phytin-P was found to be water-soluble (Lolas & Markakis, 1977). This indicated that the removal of phytate during soaking depends upon the nature of phytin, which may be in the form of K, Ca or Mg salts. Higher destruction of TI at 55°C than 22–25°C on soaking during these studies is supported by earlier observation where higher temperatures eliminated the TI more than relatively low temperature treatments (Ologhobo & Fertuga, 1984).

Haider & Chughtai (1981) observed 28·5% destruction of TI on soaking of green gram while the dry and moist heat treatments reduced it by 43·6 and 59·6%, respectively. Although some fractions of trypsin inhibitors have been reported to be resistant to dry heat (Tan & Wong, 1982), larger decreases at higher temperature may have involved heat-labile inhibitors. In order to make an estimate of dispersion of the amount of individual constituent in relation to time of soaking, the coefficient of variation (CV) was measured. This revealed slight changes in the protein values and generally larger differences in the contents of antinutrients as a result of soaking at varying temperatures.

As germination/sprouting of food grains and tubers is generally affected by environmental temperature, it was considered worthwhile to study the effect of different temperatures on certain nutrients and antinutrients during germination of mungbean. Since ascorbic acid is important in fresh food materials, germinating mungbeans were analysed for this vitamin while, for the determination of other constituents, the air-dried material was employed. The data regarding the influence of germination temperatures on selected nutrients and antinutrients are shown in Table 2. Ascorbic acid was not detectable in the seeds and this vitamin increased markedly during germination and the maximum values of 39·9 and 47·0 mg/100 g were observed after 48 h of germination at ambient temperatures (20–35°C) and 20°C, respectively. The biosynthesis of riboflavin and thiamine was faster at ambient than low temperature and their maximum values of 3·54 and 4·37 μg/g were observed during 48 or 72 h of germination against the initial values of 1·97 and 3·65 μg/g respectively. Synthesis of riboflavin was found to be greater than thiamine during germination. Similarly, protein content increased during germination, reaching a maximum of 23·5 and 22·8% at ambient and low temperatures, respectively, against an initial value of 18·4%.

The amino acid profiles of different unsprouted and sprouted samples are given in Table 3. Comparison among the samples showed differences in most of the amino acids including lysine, phenylalanine and isoleucine, which increased during germination. The influence of germination temperature was variable, resulting in increases in certain amino acids and decreases in

TABLE 2
Effect of Germination on Selected Nutrients and Antinutrients of Mungbean

Germination time (h)	*Protein (%)*		*Ascorbic acid (mg/100 g)*		*Riboflavin (μg/g)*		*Thiamine (μg/g)*		*Phytate (mg/g)*		*Trypsin inhibitor (TIU/g)*	
	Ambient 20–35°C	*20°C*	*Ambient 20–35°C*	*20°C*	*Ambient 20–35°C*	*20°C*	*Ambient 20–35°C*	*20°C*	*Ambient 20–35°C*	*20°C*	*Ambient 20–35°C*	*20°C*
Ungerminated control	18·4	18·4	—	—	1·97	1·97	3·65	3·65	216	216	2460	2460
24	21·0	20·3	16·0	14·8	2·50	1·97	3·73	3·73	194	213	1986	1989
48	21·4	21·0	32·9	47·0	3·54	2·17	4·14	4·13	174	210	1230	1420
72	21·5	21·4	29·1	25·1	3·22	2·80	4·37	4·06	165	193	987	1012
96	22·6	22·1	26·4	21·3	2·80	1·05	4·37	3·94	144	165	709	709
120	23·5	22·8	26·4	16·2	1·54	0·98	3·94	3·44	105	147	497	500
Mean	21·4	20·8	21·8	20·7	2·59	1·83	4·03	3·83	166	191	1311	1348
CV	8·07	7·27	55·3	77·3	29·1	38·1	3·73	6·90	23·3	15·0	58·2	56·4

TABLE 3
Effect of Germination on Amino Acid Composition of Mungbean (g/16 g N)

Amino acids	*Germination*			*CV*
	Ungerminated control	*Ambient 20–35°C*	*20°C*	
Alanine	4·3	4·2	4·3	1·35
Arginine	6·8	6·5	6·5	2·26
Aspartic acid	11·3	11·2	11·3	0·51
Cystine	0·8	0·8	0·8	0·00
Glutamic acid	18·5	17·5	18·1	2·79
Glycine	3·6	3·3	3·3	5·09
Histidine	2·6	2·5	2·3	6·19
Isoleucine	4·5	4·8	4·8	3·68
Leucine	7·8	7·7	7·8	0·74
Lysine	6·3	7·0	6·7	5·27
Methionine	1·5	1·5	1·5	0·00
Phenylalanine	5·7	6·2	5·9	4·24
Proline	4·3	4·3	4·3	0·00
Serine	4·4	4·2	4·2	2·70
Threonine	3·0	3·2	3·2	3·70

others. This was due, primarily, to the increase in protein content. Higher levels of some amino acids in these studies were found not significant when data were expressed on total protein basis. Some workers (Alexander, 1983; Sattar *et al.*, 1985), reported slight to considerable increases in the total protein content during the course of soaking and subsequent sprouting of plant seeds; however, these trends may not be due to increases in the true protein content but the result of elevated values of non-protein nitrogen which indeed has been observed in certain studies (El-Shimi *et al.*, 1984). Recently, Youssef *et al.* (1987), detected the disappearance of some protein bands and appearance of new ones during germination of Faba Beans. Increases in total amino acids (13·6–19·7%), depending upon the germination period of soybeans, when compared with their controls, have also been reported (Mostafa *et al.*, 1987).

The ungerminated mungbeans contained phytate, 216 mg/100 g and TI, 2460 TIU/g. The process of germination profoundly decreased these biochemical factors and the decreases were greater during germination at ambient than low temperature. The phytate content decreased to levels of 104 and 147 mg/100 g and TI 497 and 500 TIU/g at ambient and low temperature conditions, respectively, on 120 h of germination. The measurement of CV indicated striking differences in the content of these

constituents. Determination of CV is especially appropriate under conditions when there are extreme values or when it is desired to express variation as a percentage of the average around which the deviations are taken.

Although the influence of varying germination temperatures on biochemical factors has not been widely studied, a significant destruction of TI and decomposition of phytate was observed in mungbean and other legumes during germination under ambient conditions (Tabekhia & Luh, 1980; Kamalakannan *et al.*, 1981; Chitra & Sadasivam, 1986).

Obviously carbohydrates are mobilized in the formation of non-nitrogenous nutrients. An increase in total protein content was interesting. The nitrogenous compounds in the water used for soaking and germination could be the source of nitrogen build up in germinated seeds. Although the influence of varying germination temperatures on the biosynthesis/biodegradation of seed constituents has not been investigated, accumulation of protein, lipids, fibre and free amino acids during germination at ambient temperatures has already been shown (Tsai *et al.*, 1975; Chavan *et al.*, 1981; Sattar *et al.*, 1985; Mostafa *et al.*, 1987). Increases in the content of ascorbic acid, riboflavin and some other vitamins and the decreases in phytate and TI in relation to germination at the prevailing temperatures have been observed in food grains (Alexander, 1983; Sathe *et al.*, 1983; Ologhobo & Fetuga, 1984; Rahman, 1984). These and previous findings suggest that germinated food grains contain significantly larger amounts of important nutrients and lower values of harmful and antinutrients than their ungerminated originals and hence they can be used to make acceptable food and feed products. Further studies are required to test this process on semi-commercial scales.

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