

EFFECT OF SPROUTING TIME ON PROXIMATE COMPOSITION AND ASCORBIC ACID LEVEL OF MUNG BEAN (*VIGNA RADIATE L.*) AND CHICKPEA (*CICER ARIETINUM L.*) SEEDS

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ABSTRACT

This study was conducted to investigate the effect of different sprouting times on proximate composition, total energy and ascorbic acid content of mung bean (*Vigna radiate L.*) and chickpea (*Cicer arietinum L.*) seeds. Seeds were sprouted for 24, 48, 72, 96 and 120 h under dark at 25 °C ±2. Moisture contents, crude protein, ash and crude fiber contents were significantly increased from 9.75 %, 20.65 %, 2.75 % and 5.65 % to 62.67 %, 26.80 %, 3.67 % and 8.96 % respectively after 120 h of sprouting. While crude fat, carbohydrate and total energy content significantly decreased from 3.79 %, 57.41 % and 346.3 kcal 100 g⁻¹ to 2.97 %, 47.75 % and 325.3 kcal 100 g⁻¹ respectively after 120 h of sprouting. Higher percent improvement over control in moisture, ash and crude protein was recorded in chickpea than mung bean seeds. In contrast more % losses over control in crude fat, carbohydrates and total energy were found in mung bean than chickpea seeds after 120 h sprouting. Ascorbic acid contents in mung bean and chickpea increased significantly from zero to 20.78 mg 100 g⁻¹ and 9.94 mg 100 g⁻¹ respectively after 120 h of sprouting. Improvement in ascorbic acid over control samples was greater in mung bean (3700 %) than chickpea sprouts (994 %). It can be concluded from the study that sprouting for 120 h brought maximum improvement in the nutritional quality of mung bean and chickpea.

Keywords: mung bean, chickpea, sprouting time, proximate composition, ascorbic acid

INTRODUCTION

Legumes are widely grown throughout the world and remained part of the human diet since early ages of Agriculture. It is important and irreplaceable source of dietary proteins in the diets of people living in tropical and subtropical areas (Khatoun and Prakash, 2005). Pulses are important source of vegetable protein in Pakistan. They are cultivated on 5 % of the total cropped area. The total area under major pulse crops in Pakistan is about 1.5 Million ha. Among pulses, chickpea is the major winter grain legume and mung bean is the major summer legume. These two legumes constitute the main component of traditional dishes throughout the country. These pulses are consumed in many ways: seedlings and tender leaves are eaten as salads, fresh and green pods provide green vegetable and dry seeds are cooked in various dishes. They can also be eaten as roasted. The protein content of local cultivar of mung bean and chickpea varied between 19.5-31.3 % (Anwar *et al.* 2007; Shah *et al.* 2011) and 22.9-24.8 % (Iqbal *et al.* 2005) respectively. Hence these two pulses not only add variety to the diet of locals but also serve as a cheap source of protein for a large and poor population of Pakistan. They can be considered as the Poor man's meat in developing countries. However, unfortunately legumes contain greater types of toxic and undesirable constituents than other plant family. These toxic compounds are flavonoid,

tannins, phytates, alkaloids and trypsin inhibitors that limit their utilization as more acceptable source of inexpensive protein. For human consumption legumes are processed in many ways including roasting, cooking, fermentation and fortification. Among processing technologies sprouting is a procedure that has been developed to significantly increase the bioavailability of nutrients to ensure the nutritional security of population for developing countries. Sprouting or germination is a complex metabolic process during which storage proteins, carbohydrates and lipids are broken down to provide energy necessary for the developing plant (Ziegler, 1995). As seeds are soaked, enzyme inhibitors are disabled and the seed explodes to life. Germination unfolds, and enzymes trigger elaborate biochemical changes. Ascorbic acid has many vital biological functions in plants and animals (Ginter, 1989; Gosh *et al.* 1997; Jimenez *et al.* 1997). Humans are unable to synthesize this vitamin and it must be taken from external dietary sources (Nishikimi and Yagi, 1996). Ascorbic acid which is practically absent in dry grain legumes (Xu *et al.* 2005) increased in significant amount after sprouting (Sattar *et al.* 1995; Khattak *et al.* 2007)

The metabolic changes during sprouting affect the bioavailability, palatability and digestibility of essential nutrients. However, the effect of sprouting depends on the types of legume and conditions and duration of sprouting process (Savelkoul *et al.* 1992). The

legume sprouts are popular vegetable used in China and Southeast Asia. However, intake of sprouts in the diet of the local population of Pakistan is not common where an increase potential for its production, consumption and export exists. Legumes belong to those food groups that provide sufficient amount of macro and micro nutrients to human body. Therefore, this research aims to investigate the effect of different sprouting time on nutritional quality of locally available cultivars of mung bean and chickpea seeds and also comparison can be drawn between mung bean and chickpea for improvement in its nutritional quality. Availability of such data is valuable as the effect of sprouting on the proximate composition vary greatly with specie, variety and sprouting conditions such as temperature, humidity, light and time of sprouting (Sattar *et al.* 1989; Bau *et al.* 1997; Kuo *et al.* 2003). As sprouting is very cheap and effective procedure for improvement of nutritional quality of legume seeds, it is hoped that sprouts as new functional food can be a contribution to the nutrition of the local people.

MATERIALS AND METHODS

Sample collection and preparation: Samples of Mung bean (Var. KM-1) and Chickpea (Var. Lawaghar, 2000) were obtained from Ahmad Wala Research Station Karak, Khyber Pakhtunkhwa, Pakistan. Samples were manually cleaned from impurities. Control (un-sprouted) samples were ground and stored in airtight bottles in desiccators for further analysis. The remaining seeds were used for the sprouting experiment.

Soaking and Sprouting: Grains were disinfected by soaking in a solution of 1% sodium hypochlorite for 20 minutes and rinsed twice with distilled water. Sanitized grains were soaked in distilled water (1:3 w/v) for 24 h. After draining water, grains were dried with adsorbent paper. Treatments were applied in triplicate, for the period of 48, 72, 96 and 120 h in darkness, on seeds placed in petri dishes containing moist filter paper for sprouting in a germinator at $25^{\circ}\text{C}\pm 2$. Adequate seed moisture is necessary for sprouting, which was maintained through daily spray of distilled water (Machaiyah *et al.* 1999).

After every 24 h, dishes of germinated seeds were collected, kept in liquid nitrogen for one hour and then dried in ventilated oven at $70^{\circ}\text{C}\pm 2$. Dried seed samples were ground by a stainless steel grinder (JFS-13A, Xian Jiang Equipment Co., Hangzhou, Zhejiang, China) and the resulting flour was stored under refrigeration temperature before further chemical analysis.

Chemical Analysis: The analytical work was carried out in the laboratories of Agricultural Chemistry, The University of Agriculture Peshawar, Pakistan and Crop

Physiology, College of Agriculture, Nanjing Agricultural University P.R. China.

Moisture was determined by drying the control and sprouted samples at $105^{\circ}\text{C}\pm 2$ in an oven until constant weight was achieved. Crude Ash was analyzed by igniting the flour samples in muffle furnace at 550°C for 8 h. Nitrogen was measured by Kjeldhal method and crude protein was calculated as $\text{N} \times 6.25$. Crude Fat and crude fiber were estimated by standard methods (A.O.A.C., 2000). Carbohydrates were calculated by difference i.e., $100 - (\text{moisture} + \text{protein} + \text{ether extract} + \text{ash} + \text{crude fiber}) = \text{NFE}$. The energy value of control and sprouted mung bean and chickpea flour was calculated by Atwater factor method [$(9 \times \text{fat}) + (4 \times \text{carbohydrate}) + (4 \times \text{protein})$] as described by Osborne and Voogt (Osborne and Voogt, 1978). The percent values of protein, fat and carbohydrate in the flour were multiplied by their physiological fuel values of 4, 9 and 4 kcal, respectively and their sum was taken as total energy in the flour sample. Ascorbic acid was determined in the fresh sprouted samples by titrimetric method, which was based on the measurement of the extent to which 2,6-dichlorophenol-indophenol solution was decolorized by ascorbic acid in sample extracts and standard ascorbic acid solutions (A.O.A.C., 2000).

Statistical Analysis: The data were statistically analyzed using MSTAT-C computer package (Gomez and Gomez, 1976) for the analysis of variance with two factors CRD (complete randomized design). The mean and standard deviation (S.D) of the data were calculated through MS Excel 2007. Means were separated by Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Proximate composition

Moisture: Estimation of moisture content is an important part of proximate composition analysis of food samples. Data regarding the moisture content of control and sprouted mung bean and chickpea as a function of sprouting time is depicted in Table 1. Moisture contents of sprouts were significantly ($p < 0.01$) influenced by sprouting time showing almost linear upward trend with the passage of sprouting time. The mean value of moisture content in un-sprouted seeds was 9.75% which increased to 62.67% in sprouted seeds after 120 h sprouting time. The two legumes were significantly ($p < 0.01$) different in moisture at different sprouting intervals with mean values of 51.60 and 45.55% for mung bean and chickpea respectively. Interaction of legume type with sprouting time was also found significant ($p < 0.01$) for moisture content. The mean values for moisture concentration in control samples of mung bean and chickpea were 10.5% and 9.0%, which increased to 68.63% and 56.70% respectively after 120 h sprouting

time. The percent increase over control in moisture value was slightly higher in mung bean (561.49%) than chickpea (530.04%) after 120 h sprouting. Results regarding the effect of sprouting on moisture content were truly in line with Shah *et al.* (2011) and Khalil *et al.* (2007) who found significant increase in moisture level of mung bean cultivars and desi and kabuli chickpea seeds after sprouting for 96 h. The increase in water uptake of a seed with time depends on the number of cells within the seed to be hydrated (Nonogaki *et al.* 2010).

Ash: Results related to the ash contents of control and sprouted mung bean and chickpea samples are presented in Table 2. Statistical analysis of the data revealed that sprouting time had significant ($p < 0.01$) effect on ash content. Although the increase was not regular during different sprouting periods yet it ended in a higher mean value (3.67%) in samples sprouted for 120 h as compared to un-sprouted samples (2.75%). Mung bean and chickpea differed significantly for ash content during sprouting with mean value of 3.67% and 2.59% respectively. Interaction analysis revealed significant ($p < 0.01$) results for ash content. The increasing tendency was not uniform in both legumes. Chickpea showed higher percent increase over control (92.5%) than mung bean (3.9%) after 120 h sprouting time (Figure 2). A significant ($p < 0.05$) decrease in ash content was observed by Sood *et al.* (2002) and Barakoti, (2004) in chickpea and mung bean respectively after germination supported present findings.

Fat: Mean values for crude fat in control and sprouted mung bean and chickpea samples are given in Table 3. Statistical analysis of the data revealed that sprouting treatments had significant effect on the ether extract irrespective of legume type. Sprouting caused reduction in crude fat content. The un-sprouted samples had the highest average value (3.79%) for ether extract which was reduced to 2.97% after 120 h sprouting time (Table 3). Two legumes were significantly ($p < 0.01$) different in crude fat content during entire sprouting process. Mung bean had lower mean value (1.54%) as compared to chickpea (5.26%) averaged across sprouting periods. Interaction effect between sprouting time and legume type was also significant ($p < 0.01$). The fat content in mung bean and chickpea seeds decreased from initial values of 1.79 and 5.80% to 1.32 and 4.62% after 120 h sprouting time. Although mung bean had the lower values (1.79-1.32%) than chickpea (5.80-4.62%) throughout sprouting period but the overall % reduction was higher in mung bean samples (26.31%) than in chickpea (20.24%) after 120 h sprouting time (Figure 3). During sprouting of mung bean (Shah *et al.* 2011) and rapeseed (Dogra *et al.* 2001), a total lipid loss was found. The loss in crude fat could be due to total solid loss (Wang *et al.* 1997) or it had been used in providing

energy for the germination process (El-Adawy, 2002). Similarly significant increase in fat content was reported while germinating canola seeds (Chang and Harrold, 1988). These results are contradictory to the present findings in chickpea and mung bean where significant decrease was noted in fat content after 120 h sprouting.

Protein: Crude protein content was significantly ($p < 0.01$) influenced by sprouting treatments. Results indicated increment in protein concentration with sprouting time from an average value of 20.65% in un-sprouted samples to 26.80% in samples after 120 h sprouting time (Table 4). Sprouted mung bean and chickpea samples had significantly ($p < 0.01$) different values for crude protein. High mean values for protein content was noted in sprouted mung bean (26.82%) as compared to chickpea (20.83%) over different sprouting intervals (Table 4). The interaction effect of sprouting time with legume type was also significant ($p < 0.01$) for protein content. Sharp increase was observed after 24 h sprouting time in both legumes but then it became gradual with further progress in sprouting time. In mung bean the percent increase over control was lower (29.50%) than chickpea (31.27%) after 120 h sprouting time (Figure 4). Improvement in protein content in mung bean and chickpea seeds was recorded with the advancement in germination time (Khalil *et al.* 2007; Shah *et al.* 2011). The increasing trend in protein content with progress in sprouting was almost similar to the results of the current study. Rise in crude protein could be attributed to the synthesis of new proteins (e.g., proteases) by germinating seeds and to the compositional change after degradation of other constituents (Bau *et al.* 1997). Many researchers reported increase in percent protein in germinated grains (Dogra *et al.* 2001; Sood *et al.* 2002; Urbano *et al.* 2005; Khatoon and Prakash, 2006; Ghavidel and Prakash, 2007; Kaushik *et al.* 2010). However, decrease was also observed in crude protein with germination (King and Puwastien, 1987; Torres *et al.* 2007; Veluppillai *et al.* 2009) and had given the reason that the decrease in total protein content is concurrent with increase amino acid content caused by high level of protease activity during germination. The difference in results for protein content of sprouts not only depends on cultivar but also on germination conditions (Dagnia *et al.* 1992; Urbano *et al.* 2005; Torres *et al.* 2007).

Fiber: Results regarding crude fiber content of control and sprouted mung bean and chickpea seeds are presented in Table 5. Data showed highly significant ($p < 0.01$) impact of sprouting time on crude fiber content with mean values of 5.65% and 8.96% in the control and 120 h sprouted samples respectively. The two legume sprouts were also significantly different in crude fiber content throughout sprouting time. Higher mean value (7.41%) for crude fiber was recorded in chickpea samples

than in mung bean (7.20%) over the entire period of sprouting (Table 5). The interaction of sprouting time with legume type was also highly significant ($p < 0.01$). Mung bean showed more linear upward increase in crude fiber concentration than chickpea with progress in sprouting time. The increase over control in crude fiber was more noticeable in mung bean sprouts (109.34%) than chickpea sprouts (20.82%) after 120 h sprouting time (Figure 5). Germination of mung bean and chickpea for 12, 16, 20 hours and 36, 48, 60 hours at room temperature respectively resulted in significant increase in crude fiber content in both legumes (Uppal and Bains, 2011). The increase in fiber was in the range of 13 to 17% and 19 to 26%, in chickpea and mung bean respectively. The difference in the extent of increment in fiber content during sprouting of mung bean and chickpea as compared to present results may be due to the difference in the length of sprouting time and varieties. The results were also in close proximity with the findings of Chavan and Kadam, (1989) where they found that germination improves the crude fiber content in seeds due to the consumption of starch. The crude fiber which is a major component of cell wall increases with the synthesis of structural carbohydrates, such as cellulose and hemicellulose (Peer and Leeson, 1985; Cuddeford, 1989).

Nitrogen Free Extract (NFE): Nitrogen free extract which represents digestible carbohydrates of mung bean and chickpea seeds were significantly affected ($p < 0.01$) by sprouting treatments. The mean value for control samples was 57.41% which was reduced to 47.75% after 120 h sprouting interval (Table 6). The two legumes sprouts also differed significantly in nitrogen free extract with chickpea having higher mean value (54.91%) than mung bean (50.23%) sprouts. Interaction effect between sprouting time and legume type was also highly significant ($p < 0.01$). The reduction trend was similar in mung bean and chickpea up to 96 h sprouting but mung bean exhibited more drastic reduction in NFE contents (21.32%) than chickpea (12.58%) after 120 h of sprouting (Figure 6). Mung bean had higher percent loss over control in NFE content (21.32%) than chickpea (12.58%) after 120 h sprouting time. Significant reduction in carbohydrates was observed during germination (Mubarak, 2005) which corresponded well to the results of the present study. This could be due to enhanced hydrolytic enzyme activities that promoted starch digestibilities. Decrease in carbohydrates during germination made the sprouts popular in developed countries due to low carbohydrates and rich in vitamins (Stephens, 2003).

Total energy: Results regarding the total energy content of mung bean and chickpea at different sprouting intervals and percent reduction over control are given in Table 7 and Figure 7 respectively. Sprouting significantly ($p < 0.01$) decreased the energy values from an initial

mean value of 346.3 kcal 100 g⁻¹ for un-sprouted seeds to 325.3 kcal 100 g⁻¹ after 120 h sprouting time. The two legumes were significantly different for total energy value during different sprouting time, with higher mean value (350.28 kcal 100 g⁻¹) observed in chickpea than in mung bean samples (322.24 kcal 100 g⁻¹). Interaction between sprouting time and legume type was also significant ($p < 0.01$). Linear reduction in energy values was observed in both legumes with the passage of sprouting time. The mean energy value of control chickpea samples (359.7 kcal 100 g⁻¹) was higher than mung bean (333.0 kcal 100 g⁻¹), however percent reduction over control was higher in mung bean (7.21%) than chickpea (5.01%) after 120 h sprouting time (Figure 7). Energy value of 336.65 kcal 100g⁻¹ for raw dehulled mung bean flour had been reported in earlier study (Blessing and Gregory, 2010) and it was observed that decrease in energy value of mung bean sprouted for 72 h was not regular. The low energy value of sprouted legumes was due to low levels of fat and carbohydrates in the sprouted samples (Uppal and Bains, 2011). Profound changes occurred in proximate composition but such changes in nutrient profile were misleading, as they were only related to the alterations in the ratio of nutrients during seed germination. Seed sprouting involves energy use, which is provided by break down of starch to sugars and lipids to free fatty acids resulting in shift in nutrient profile. Any experiment related to nutritional composition after sprouting must deal with such changes in nutrient composition (Peer and Leeson, 1985).

Ascorbic Acid: Several enzyme systems become active during sprouting that brings about profound changes in the nutritional quality of pulses. Data regarding the ascorbic acid content of control and sprouted mung bean and chickpea seeds is summarized in Table 8 and Figure 8. Statistical analysis of the data revealed significant ($p < 0.01$) effect of sprouting time on ascorbic acid level of mung bean and chickpea seeds. Dry seeds have no ascorbic acid but phenomenal linear increase has been observed in mung bean and chickpea with the progress in sprouting. The value of ascorbic acid increased from 4.83 mg 100 g⁻¹ after 24 h sprouting to 28.50 mg 100 g⁻¹ after 120 h sprouting. The two legumes differed significantly ($p < 0.01$) in ascorbic acid content throughout sprouting periods. Mung bean has the higher value (20.78 mg 100 g⁻¹) of ascorbic acid than chickpea (9.94 mg 100 g⁻¹) after 120 h sprouting time. Interaction effect between sprouting time and legume type was also significant ($p < 0.01$). Increase was higher in mung bean with mean value ranged from 4.67 to 37 mg 100 g⁻¹ than chickpea (5.0 to 9.94 mg 100 g⁻¹) during sprouting for 120 h (Table 8). The percent increase over control in ascorbic content was also higher in mung bean (3700%) than chickpea sprouts (994%) after 120 h sprouting time (Figure 8).

Although ascorbic acid is in abundance in fruits and vegetables (Loewus and Loewus, 1987) yet its availability is limited due to seasonal availability of fruits and vegetables and losses during storage, preparation and cooking methods (Davey *et al.* 2000). Therefore, enhancement of ascorbic acid in vegetables and fruits by different processing techniques including sprouting is beneficial for human health. The ascorbic acid content increased with germination time as obvious from the observations made by Khattak *et al.* (2007) who found linear relationship between germination time and ascorbic acid content for seeds of desi type chickpea cultivar. Ascorbic acid content of chickpea with different sprouting times increased substantially after 24 h sprouting with mean value of 5.7 mg 100 g⁻¹ to 17.5 mg 100 g⁻¹ after 96 h sprouting time (Khalil *et al.* 2007). Present results are well in line with the findings reported for sprouted mung bean cultivars (Shah *et al.* 2011) where rapid increase was found in vitamin C from an average initial value of 112.9 mg 100 g⁻¹ after 24 h sprouting to maximum value of 23.2 mg 100 g⁻¹. Initially vitamin C content was absent in raw pigeon pea but germination for 4 days synthesized 14 mg 100g⁻¹ of it (Torres *et al.* 2007). Vitamin C content of soybean increased by 91.3% after sprouting for 3 days (Ahmad and Pathak, 2000) while 300% increase observed in ascorbic acid content of white beans germinated for 5 days (Sangronis and Machado, 2007). Therefore, sprouted legumes can be considered a rich source of ascorbic acid. The difference in level of biosynthesis of ascorbic acid with sprouting might be attributed to the legume type, maturity, climatic conditions, light conditions, harvesting and storage methods (Davey *et al.* 2000; Macrae *et al.* 1993). The reason for accumulation of ascorbic acid during sprouting in grain legumes was due to reactivation of enzyme (L-Galactono- lactone dehydrogenase) involved in the oxidation of L-galactono-1, 4-lactone to ascorbic acid. The activity of this enzyme increased with germination in soybean seeds with parallel increase in ascorbic acid confirmed its involvement in the biosynthesis of ascorbic acid during germination (Xu *et al.* 2005).

Table 1. Moisture content (%) of control and sprouted mung bean and chickpea seed

Sprouting time (Hours)	Legumes		Mean
	Mung bean	Chickpea	
0 (control)	10.5±0.13 ^h	9.00±0.22 ^h	9.75 ^c
24	55.13±1.14 ^{de}	50.22±0.53 ^g	52.67 ^d
48	56.92±0.74 ^{cd}	51.19±2.22 ^g	54.05 ^{cd}
72	57.85±0.92 ^c	52.17±0.24 ^{fg}	55.01 ^c
96	60.57±2.18 ^b	54.02±0.09 ^{ef}	57.30 ^b
120	68.63±3.15 ^a	56.70±0.19 ^{cd}	62.67 ^a
Mean	51.60 ^a	45.55 ^b	

♦ Values are means of three determination ± Standard deviation.

♦ Mean values followed by different letters in the same column are significantly (p< 0.01) different from each other.

♦ Values in the interaction matrix followed by different letters are significantly (p< 0.01) different from each other.

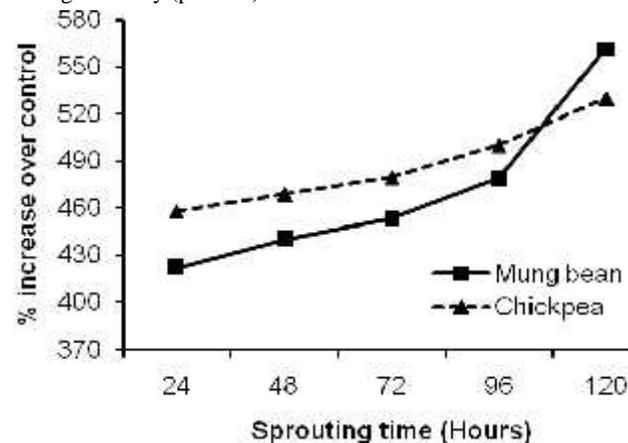


Fig. 1. Increase (%) over control in moisture content of mung bean and chickpea during sprouting

Table 2. Ash content (%) of control and sprouted mung bean and chickpea seed

Sprouting time (Hours)	Legumes		Mean
	Mung bean	Chickpea	
0 (control)	3.67±0.03 ^{bc}	1.83±0.08 ^h	2.75 ^d
24	3.64±0.03 ^{cd}	1.72±0.16 ^h	2.68 ^d
48	3.25±0.10 ^e	2.70±0.15 ^g	2.97 ^c
72	3.81±0.05 ^{ab}	2.95±0.10 ^f	3.38 ^b
96	3.81±0.05 ^b	2.84±0.09 ^f	3.33 ^b
120	3.82±0.03 ^a	3.52±0.04 ^d	3.67 ^a
Mean	3.67 ^a	2.59 ^b	

♦ Values are means of three determination ± Standard deviation.

♦ Mean values followed by different letters in the same column are significantly (p< 0.01) different from each other.

♦ Values in the interaction matrix followed by different letters are significantly (p< 0.01) different from each other.

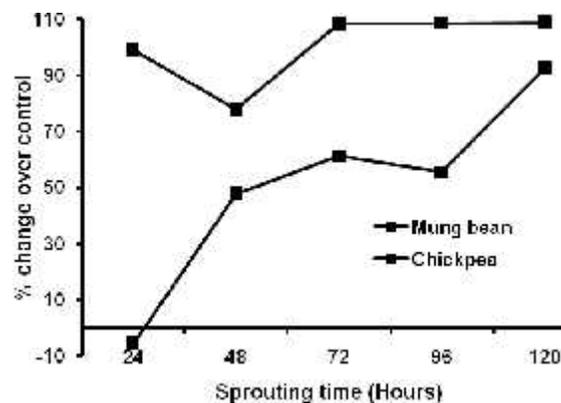


Fig. 2. Change (%) over control in ash content of mung bean and chickpea during sprouting

Table 3. Crude fat (%) of control and sprouted mung bean and chickpea seed

Sprouting time (h)	Legumes		Mean
	Mung bean	Chickpea	
0 (control)	1.79±0.02 ^g	5.80±0.01 ^a	3.79 ^a
24	1.62±0.03 ^h	5.73±0.03 ^b	3.67 ^b
48	1.56±0.03 ⁱ	5.37±0.03 ^c	3.46 ^c
72	1.51±0.01 ^j	5.09±0.01 ^d	3.30 ^d
96	1.43±0.03 ^k	4.93±0.03 ^e	3.18 ^e
120	1.32±0.03 ^l	4.62±0.04 ^f	2.97 ^f
Mean	1.54 ^a	5.26 ^b	

♦ Values are means of three determination ± Standard deviation.
 ♦ Mean values followed by different letters in the same column are significantly (p< 0.01) different from each other.
 ♦ Values in the interaction matrix followed by different letters are significantly (p< 0.01) different from each other.

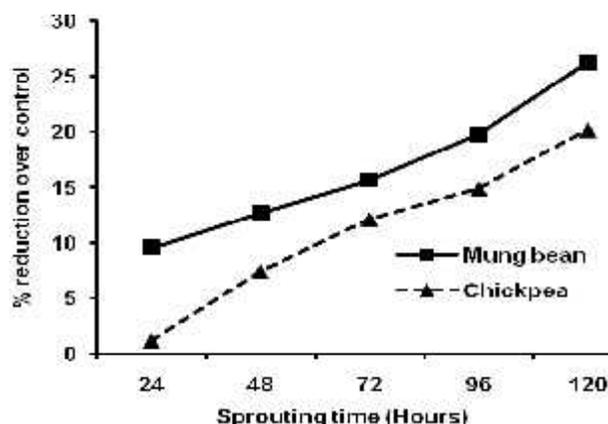


Fig. 3. Reduction (%) over control in crude fat content of mung bean and chickpea during sprouting

Table 4. Crude Protein content (%) of control and sprouted mung bean and chickpea seed

Sprouting time (Hours)	Legumes		Mean
	Mung bean	Chickpea	
0 (control)	23.5±0.26 ^f	17.80±0.20 ^k	20.65 ^f
24	25.53±0.15 ^e	18.43±0.15 ^j	21.98 ^e
48	26.77±0.15 ^d	21.03±0.12 ⁱ	23.90 ^d
72	27.17±0.15 ^c	21.6±0.20 ^h	24.38 ^c
96	27.80±0.20 ^b	22.77±0.15 ^g	25.28 ^b
120	30.43±0.15 ^a	23.37±0.06 ^f	26.80 ^a
Mean	26.82 ^a	20.83 ^b	

♦ Values are means of three determination ± Standard deviation.
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 ♦ Values in the interaction matrix followed by different letters are significantly (p< 0.01) different from each other.

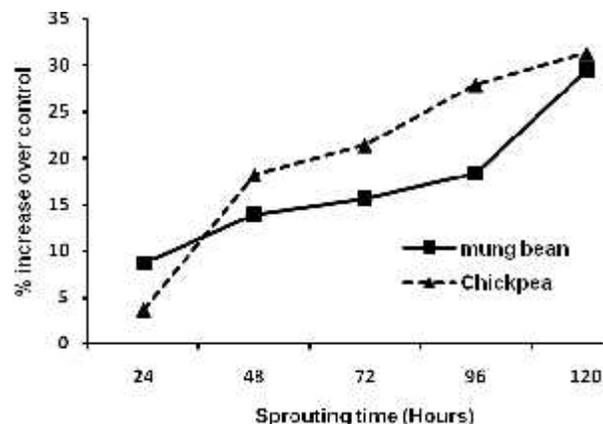


Fig. 4. Increase (%) over control in crude protein content of mung bean and chickpea during sprouting

Table 5. Crude Fiber (%) of control and sprouted mung bean and chickpea seed

Sprouting time (Hours)	Legumes		Mean
	Mung bean	Chickpea	
0 (control)	4.82±0.07 ^h	6.48±0.03 ^f	5.65 ^f
24	5.31±0.04 ^g	7.15±0.05 ^e	6.23 ^e
48	6.60±0.10 ^f	7.51±0.08 ^d	7.06 ^d
72	7.77±0.09 ^c	7.67±0.13 ^{cd}	7.72 ^c
96	8.58±0.20 ^b	7.79±0.16 ^c	8.18 ^b
120	10.09±0.28 ^a	7.83±0.15 ^c	8.96 ^a
Mean	7.20 ^a	7.41 ^b	

♦ Values are means of three determination ± Standard deviation.
 ♦ Mean values followed by different letters in the same column are significantly (p< 0.01) different from each other.
 ♦ Values in the interaction matrix followed by different letters are significantly (p< 0.01) different from each other.

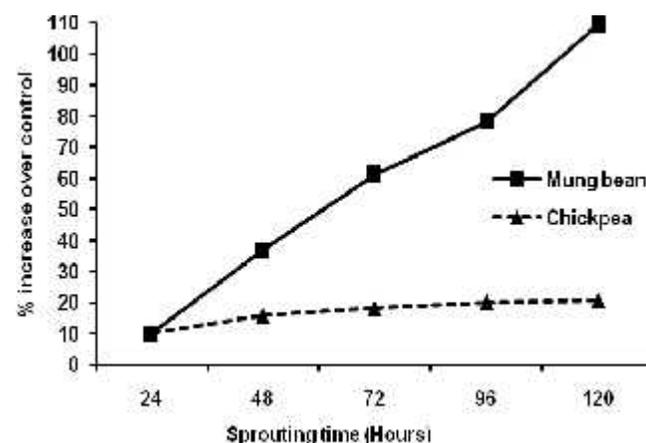


Fig. 5. Increase (%) over control in crude fiber content of mung bean and chickpea during sprouting

Table 6. NFE content (%) of control and sprouted mung bean and chickpea seed

Sprouting time (Hours)	Legumes		Mean
	Mung bean	Chickpea	
0 (control)	55.72±0.28 ^c	59.09±0.03 ^a	57.41 ^a
24	53.40±0.17 ^e	57.96±0.16 ^b	55.68 ^b
48	51.32±0.22 ^g	54.39±0.03 ^d	52.85 ^c
72	49.25±0.30 ^h	53.69±0.31 ^e	51.47 ^d
96	47.87±0.11 ⁱ	52.67±0.14 ^f	50.27 ^e
120	43.84±0.41 ^j	51.66±0.12 ^g	47.75 ^f
Mean	50.23 ^a	54.91 ^b	

♦ Values are means of three determination ± Standard deviation.
 ♦ Mean values followed by different letters in the same column are significantly (p< 0.01) different from each other.
 ♦ Values in the interaction matrix followed by different letters are significantly (p< 0.01) different from each other.

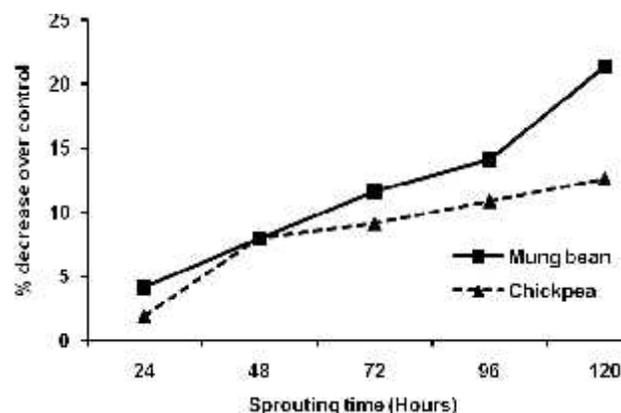


Fig. 6. Reduction (%) over control in NFE of mung bean and chickpea during sprouting

Table 7. Total energy content (kcal 100 g⁻¹) of control and sprouted mung bean and chickpea seed

Sprouting time (Hours)	Legumes		Mean
	Mung bean	Chickpea	
0 (control)	333.0±0.34 ^f	359.7±0.16 ^a	346.3 ^a
24	330.3±0.42 ^g	357.1±0.23 ^b	343.7 ^b
48	326.4±0.13 ^h	350.0±0.75 ^c	338.2 ^c
72	319.2±0.75 ⁱ	347.0±0.45 ^d	333.1 ^d
96	315.6±0.70 ^j	346.1±0.34 ^d	330.9 ^e
120	309.0±1.18 ^k	341.7±0.65 ^e	325.3 ^f
Mean	322.24 ^a	350.28 ^b	

♦ Values are means of three determination ± Standard deviation.
 ♦ Mean values followed by different letters in the same column are significantly (p< 0.01) different from each other.
 ♦ Values in the interaction matrix followed by different letters are significantly (p< 0.01) different from each other.

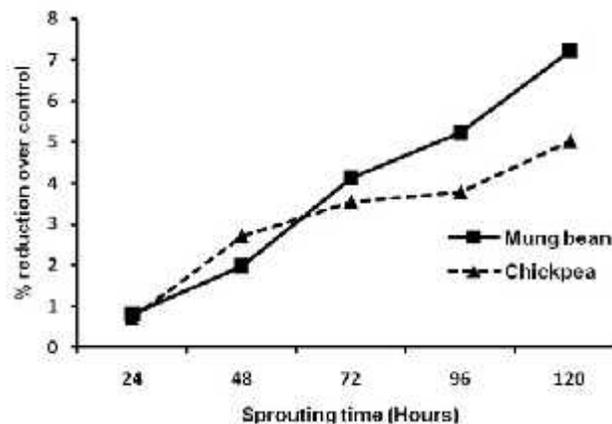


Fig. 7. Reduction (%) over control in total energy content of mung bean and chickpea during sprouting

Table 8. Ascorbic acid content (mg 100g⁻¹) of control and sprouted mung bean and chickpea seed

Sprouting time (Hours)	Legumes		Mean
	Mung bean	Chickpea	
0 (control)	0.0 ^f	0.0 ^f	0.0 ^f
24	4.67±1.1 ^e	5.0±0.1 ^e	4.83 ^e
48	18.17±1.3 ^c	7.17±1.0 ^{de}	12.67 ^d
72	31.0±3.0 ^b	10.33±1.6 ^d	20.67 ^c
96	33.83±4.9 ^{ab}	17.17±3.3 ^c	25.50 ^b
120	37.0±1.5 ^a	20.0±0.5 ^c	28.50 ^a
Mean	20.78 ^a	9.94 ^b	

♦ Values are means of three determination ± Standard deviation.
 ♦ Mean values followed by different letters in the same column are significantly (p< 0.01) different from each other.
 ♦ Values in the interaction matrix followed by different letters are significantly (p< 0.01) different from each other.

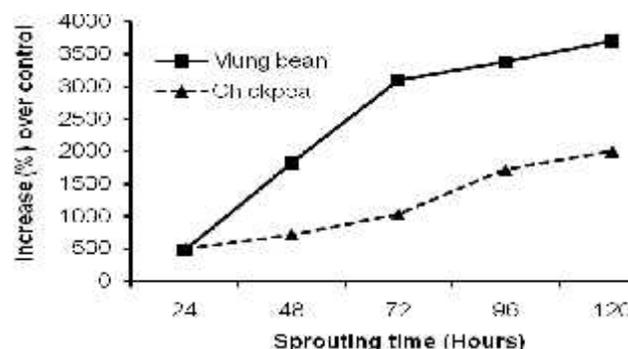


Fig. 8. Increase (%) over control in ascorbic acid content of mung bean and chickpea seeds during sprouting

Conclusions and recommendations: It was concluded from the study that sprouting for 120 h brought maximum

improvement in the nutritional quality of mung bean and chickpea. These biochemical changes were more pronounced in mung bean than chickpea seeds. Therefore mung bean is more appropriate to be used as sprouts. While 48 h sprouting brought maximum improvement in nutritional quality of mung bean and chickpea with minimum loss of dry matter, which can be used as seeds in making different dishes.

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REFERENCES

- A.O.A.C. (2000). Official Methods of Analysis of the Association of Official Analytical Chemists International 17th Ed. Published by the Association of Official Analytical Chemists International, Suite 400 2200 Wilson Boulevard, Arlington, Virginia, 22201-3301.
- Ahmad, S. and D. Pathak (2000). Nutritional changes in soybean during germination. *J Food Sci. Technol.* 37(6): 665-666.
- Anwar, F., S. Latif, R. Przybylski, B. Sultana and M. Ashraf (2007). Chemical composition and antioxidant activity of seeds of different cultivars of mung bean. *J. Food Sci.* 72: 503-510.
- Barakoti, L. (2004). M.Sc. Thesis, Development of recipes to enhance the bioavailability of iron from mung bean (*Vigna radiata*). Punjab Agricultural University, Ludhiana, India
- Bau, H. M., C. Villanme, J. P. Nicolos and L. Mejean (1997). Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soy bean (*Glycine max*) seeds. *J. Sci. Food Agri.* 73: 1-9.
- Blessing, I. A. and I. O. Gregory (2010). Effect of Processing on the Proximate Composition of the Dehulled and Undehulled Mung bean [*Vigna radiata* L.] Wilczek] Flours. *Pak. J. Nutr.* 9 (10): 1006-1016.
- Chang, K.C. and R.L. Harrold (1988). Changes in selected biochemical components, *in vitro* protein digestibility and amino acids in two bean cultivars during germination. *J. Food Sci.* 53: 783-804.
- Chavan, J. and S.S. Kadam (1989). Nutritional importance of cereals by sprouting. *Critical Rev Food Sci. Nutr.* 28, 349-400.
- Cuddeford, D. (1989). Hydroponic grass. In practice, 11, 211-214.
- Dagnia, S.G., D.S. Petterson, R.R. Bell and F.V. Flanagan (1992). Germination alters the nutritional value of lupin seed. *J. Sci. Food Agri.* 60: 419-423.
- Davey, M. W., M. U. Montagu, D. Inze, M. Sanmartin, A. Kanellis and N. Smirnoff (2000). Plant L-ascorbic acid: chemistry, function, metabolism, Bioavailability and effects of processing. *J Sci. Food Agri.* 80: 825-850.
- Dogra, J., Y.S. Dhaliwal and M. Kalia (2001). Effect of soaking, germination, heating and roasting on the chemical composition and nutritional quality of Soybean and Its Utilization in Various Indian leavened products. *J. Food Sci. Technol.* 38 (5): 453-456.
- El-Adawy, T.A. (2002). Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. *Plant Foods Hum. Nutr.* 57:83-97.
- Ghavidel, R.A. and J. Prakash (2007). The impact of germination and dehulling on nutrients, antinutrients, *in vitro* iron and calcium bioavailability and *in vitro* starch and protein digestibility of some legume seeds. *LWT - Food Sci Technol.* 40: 1292-1299.
- Ghosh, M. K., M. Mukhopadhyay and J. B. Chatterjee (1997). NADPH-initiated cytochrome P450-dependent free ion-independent microsomal lipid peroxidation: specific prevention by ascorbic acid. *Molecular Cell Biochem.* 166: 457-462.
- Ginter, E. (1989). Ascorbic acid in cholesterol metabolism and in detoxification of xenobiotic substances: problem of optimum vitamin C intake. *Nutrition.* 5: 369-374.
- Gomez, K.A. and A.A. Gomez (1976). Statistical procedures for agricultural research with emphasis on rice. Los Banos, Philippines Int'l Rice Res. Institute.
- Iqbal A., I.A. Khalil, N. Ateeq and M.S. Khan (2005). Nutritional quality of important food legumes. *Food Chem.* 97: 331-335.
- Jimenez, A., J.A. Hernandez, L.M. R. B. Am sandalio, L.A. Del Rio and F. Sevilla (1997). Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. *Physiol. Plant.* 104: 689-692.
- Kaushik, G., S. Satya and S.N. Naik (2010). Effect of domestic processing techniques on the nutritional quality of Soybean. *Mediterr. J. Nutr. Metab.* 3(1):39-46.
- Khalil, A. W., A. Zeb, F. Mahmood, S. Tariq, A. B. Khattak and H. Shah (2007). Impact of

- germination time on comparative sprout quality characteristics of desi and Kabuli type chickpea cultivars (*Cicer arietinum* L.). *LWT-Food Sci. Technol.* 40(6): 937-945.
- Khatoon, N. and J. Prakash (2006). Nutrient retention in microwave cooked germinated legumes. *Food Chem.* 97: 115-121.
- Khatoon, N. and J. Prakash (2005). Cooking quality and sensory profile of microwave and pressure cooked legumes. *Indian J. Nutr. Diet.* 42, 13-219.
- Khattak A.B., A. Zeb, M. Khan, N. Bibi, I. Ihsanullah and M.S. Khattak (2007). Influence of germination techniques on sprout yield, biosynthesis of ascorbic acid and cooking ability, in chickpea (*Cicer arietinum* L.). *Food Chem.* 103, 115-120.
- King, R.D. and P. Puwastien (1987). Effects of germination on the proximate composition and nutritional quality of Winged bean (*Psophocarpus tetragonolobus*) seeds. *J. Food Sci.* 45(1): 106-108.
- Kuo, Y., P. Rozan, F. Lambein, J. Frias and C. Vidal-Valverde (2003). Effects of different germination conditions on the contents of free protein and non-protein amino acids of commercial legumes. *Food Chem.* 86 (4): 537-545.
- Loewus, F.A. and M.W. Loewus (1987). Biosynthesis and metabolism of ascorbic acid in plants. *CRC Crit. Rev. Plant Sci.* 5:101-119.
- Machaiah, J., M. Pednekar and P. Thomas (1999). Reduction in flatulence factors in mung bean (*Vigna radiata*) using low-dose gamma irradiations. *J. Sci. Food Agric.* 79: 648-652.
- Macrae, R., R. Robinson and M. Sadler (1993). *Encyclopedia of science, food technology and nutrition* (1st Ed). San Diego, CA: Academic Press. 2718-2730.
- Mubarak, A.E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chem.* 89: 489-495.
- Nishikimi, M. and K. Yagi (1996). Biochemistry and molecular biology of ascorbic acid biosynthesis, in *Ascorbic acid: Biochemistry and Biomedical cell Biology*, ed by Harris R.J. Plenum, New York. 17-38.
- Nonogaki, H., G.W. Bassel and J.W. Bewley (2010). Germination-still a mystery. *Plant Science*, 2010, plant sciences, plant-sci. 179, ISSN 574-581. doi:10.1016/j.plantsci.2010.02.010.
- Osborne, D.R. and P. Voogt (1978). *The Analysis of Nutrients in Foods*. Academic press, London, pp:128.
- Peer, D.J. and S. Leeson (1985). Nutrient content of hydroponically sprouted barley. *Anim. Feed. Sci. Technol.* 13: 191-202.
- Sangronis, E. and C.J. Machado (2007). Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. *Food Chem.* 56, 112-120.
- Sattar, A., S.K. Durrani, F. Mahmood, A. Ahmad and I. Khan (1989). Effect of soaking and germination temperatures on selected nutrients and antinutrients of mung bean. *Food Chem.* 34, 111-120.
- Sattar, A., A. Badshah and Z. Aurang (1995). Biosynthesis of ascorbic acid in germinated rapeseed cultivars. *Plant Foods Hum. Nutr.* 47, 63-70.
- Savelkoul, F.H., A.F. Vander Poel and S. Tamminga (1992). The presence and inactivation of trypsin inhibitors, tannins, lectins and amylase inhibitors in legume seeds during germination A review. *Plant Foods Hum. Nutr.* 42:71-85.
- Shah, S.A., A. Zeb, T. Masood, N. Noreen, S. J. Abbas, M. Samiullah, M.A. Alim and A. Muhammad (2011). Effect of sprouting time on biochemical and nutritional qualities of mung bean varieties. *Afr. J. Agril. Res.* 6(22): 5091-5098.
- Sood, M., S.R. Malhotra and B.C. Sood (2002). Effect of processing and cooking on proximate composition of chickpea varieties. *J. Food Sci. Technol.* 39: 69-71.
- Stephens, J. M. (2003). *Bean sprouts- Phaseolus aureus R. and Glycine max.* Series no. HS557, Horticulture Sciences Department, Florida Cooperative Extension Service, Institute of Food Agricultural Sciences, University of Florida, USA. Pp 1-2.
- Torres, A., J. Frias, M. Granito and C. Vidal-Valverde (2007). Germinated *Cajanus cajan* seeds as ingredients in pasta products: Chemical, biological and sensory evaluation. *Food Chem.* 101: 202-211.
- Uppal, V. and K. Bains (2011). Effect of germination periods and hydrothermal treatments on in vitro protein and starch digestibility of germinated legumes. *J. Food Sci. Technol.* 49(2): 184-191.
- Urbano, G., M. L. Jurado, S. Frejnagel, E.G. Villalva, J.M. Porres, J. Frias, C. V. Valverde and P. Aranda (2005). Nutritional assessment of raw and germinated pea (*Pisum sativum* L.) protein and carbohydrate by *in vitro* and *in vivo* techniques. *Nutrition.* 21: 230-239.
- Veluppillai, S., K. Nithyanantharajah, S. Vasantharuba, S. Balakumar and V. arasaratnam (2009). Biochemical changes associated with germinating rice grains and germination improvement. *Rice Sci.* 16(3): 240-242.

- Wang, N., M.J. Lewis, J.G. Brennan and A. Westby (1997). Effect of processing methods on nutrients and antinutritional factors in cowpea. *Food Chem.* 58: 59-68.
- Xu, M. J., J. D. Dong and M. Y. Zhu (2005). Effects of germination conditions on ascorbic acid level and yield of soybean sprouts. *J. Sci. Food Agric.* 85(6): 943-947.
- Ziegler, P. (1995). Carbohydrate degradation during germination. In: Kigel J, Galili G (eds). *Seed development and germination.* pp: 447– 474. Marcel Dekker Inc, New York.