

*Full Length Research Paper*

## Effects of sprouting time on biochemical and nutritional qualities of Mungbean varieties

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**Effect of sprouting time (0, 24, 48, 72 and 96 h) on the biochemical and nutritional qualities of two mungbean varieties (Ramzan and NM-98) was investigated. Seeds of mungbean varieties were sprouted in laboratory under room conditions (25°C). Starting from the second day of germination, and every day, dishes of germinating seeds were removed, oven-dried, weighed and milled for proximate and chemical analysis. Vitamin C was determined in the fresh samples without drying. Results revealed that in proximate composition, sprouting resulted in increase of most of the parameters except fat and NFE, which were significantly reduced. Sprouting also resulted in almost linear increase in the concentration of vitamin C and protein contents. Phytic acid decreased from an initial average value of 1.88 to 0.33% with 96 h sprouting. It can be concluded that sprouting improved the nutritional worth of the mungbean in terms of higher concentration of nutrients, reduced phytic acid, improved protein content and ascorbic acid.**

**Key words:** Mungbean, germination, proximate composition, phytic acid, ascorbic acid.

### INTRODUCTION

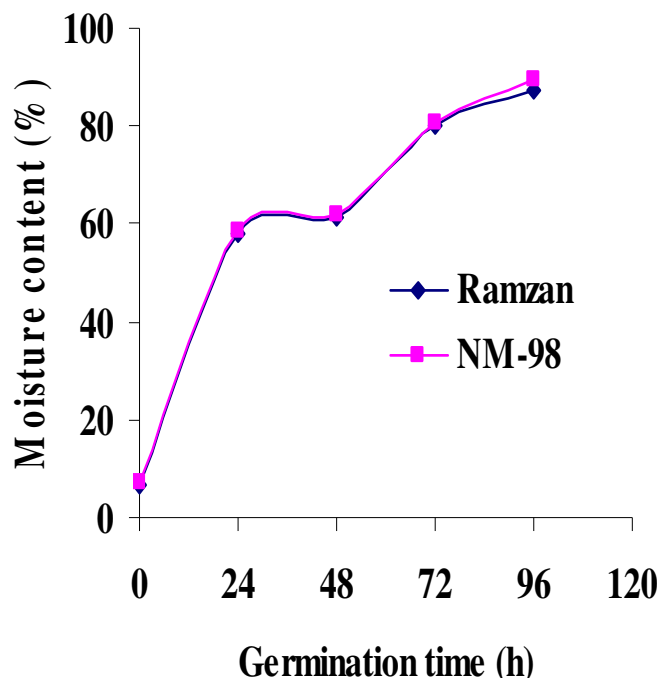
Mungbeans (*Vigna radiate* L) Wilczek is one of the important pulse crops grown and consumed in Pakistan. It is a native of India-Burma and is cultivated extensively in Asia. Its short duration, low water requirement, wide adaptability to fit in different crop rotations and varying cropping patterns can contribute to sustainability in increasing the farm productivity per unit area. It is widely grown in Southeast Asia, Africa, South America and Australia. It is also referred to as green gram, golden gram and chop suey bean. Mungbeans are grown widely for use as a human food (as dry beans or fresh sprouts), but can be used as a green manure crop and as forage for livestock.

Mungbean serves as vital source of vegetable protein (19.1 to 28.3%), minerals (0.18 to 0.21%), and vitamins

particularly in developing countries. However, there are certain problems in proper and economical utilization of mungbeans as staple food. They contain anti-nutritional factors (like flatulence causing oligosaccharides, phytates and polyphenols) which can cause gastro-intestinal discomforts and also result in non-availability of certain nutrients in biological systems (Liener, 1979). Another important problem with legumes is that they need very long time for cooking. Besides requiring more energy, excessive cooking of legumes results in protein losses and in lower availability of lysine (Almas and Bender, 1980)

Mungbean seeds are sprouted for fresh use or canned for shipment to restaurants. Sprouts are high in protein (21 to 28%), calcium, phosphorus and certain vitamins. Sprouts have been reported to have a greater nutritional value than seeds. Germination is an inexpensive and simple method of improving nutritive value and several studies have reported higher levels of nutrients and lower

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**Figure 1.** Effects of sprouting time on moisture content of seed of t Mungbean varieties.

values of anti-nutrients in germinated food grains compared to the un-germinated originals. While searching for new sources of functional foods, special attention has been paid to sprouts from the legumes seeds which are more and more often used in human diets through out the world in general and in the sub-continent in particular. Moreover, the inclusion of sprouted seed in food can further modify its taste and texture. Therefore, the sprouts may thus become a potential source of nutritious food or food ingredients. Recent studies showed that the *cruciferous* sprouts were ready to eat after four days of germination and then they contained an appropriate amount of bioactive compounds such as L-ascorbic acid, reduced glutathione, inositol phosphates, total phenolic compounds (Zielinski et al., 2002., Zielinski et al., 2003., Zielinski et al., 2005). Moreover, radish sprouts were reported to have the highest hydroxyl radical scavenging potency among the 11 kinds of commonly available vegetables (Takaya et al., 2003). It has been recently reported that *cruciferae* sprouts contained higher amounts of Ca, Mg, Cu and Zn compared to the seeds (Zielinski et al., 2005). Khatkhat et al. (2007 a, b) reported a significant increase in ascorbic acid, protein, ash and fat contents and decrease in phytic acid, polyphenols, fiber and nitrogen free extract content in chickpea compared to the original seed. They also observed significant differences in these nutrients and anti-nutrients when the seed was germinated under different type of illuminations. With this perspective in mind, this study was undertaken to investigate and

compare the impact of germination on nutritional quality of two mungbean varieties.

## MATERIALS AND METHODS

The study was carried out in the Nutrition Section of the Food Science Division (FSD) at Nuclear Institute for Food & Agriculture (NIFA) Tarnab, Peshawar. Samples of mungbean varieties Ramzan and NM-98 were obtained from Crop Breeding Division (CBD) of Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar. The samples were cleaned from impurities. The unsoaked samples were ground in stainless steel grinder to pass through a standard 40 mesh screen. The ground samples were kept in glass bottles, placed in desiccators and stored at 4°C. From these bottles, required quantities were taken for chemical determinations.

### Sprouting procedure

The seeds were soaked by submerging in distilled water in glass containers for 24 h at room temperature. After soaking the seeds were taken out from the glass container and the adherent moisture removed by gently rolling them on thick absorbent cloth. A germination experiment was undertaken using 100 g of seeds in Petri dishes lined with filter paper. Sprouting was carried out in triplicate for each treatment (0, 24, 48, 72 and 96 h in the laboratory). Only distilled water was sprayed during germination periods at 9 a.m., 12 and 5 p.m. daily. The soaked seeds were dried in an air oven at 70°C for Lab analysis and 105°C to a constant weight for moisture determination, ground to pass through a 40 mesh sieve and stored for further analysis. The seeds were washed every 24 h with distilled water. Starting from the second day of germination, and every day thereafter, dishes of germinating seeds were removed, oven-dried, weighed and milled for proximate and chemical analysis.

### Chemical analysis

Moisture, protein, fat, fiber and nitrogen free extract were determined using standard methods of AOAC (1990). L-ascorbic acid was determined in the fresh sprouted chickpea samples according to the methods of Augustin et al., (1985). The sensitive method of Haug and Lantzsch (1983) was adapted for the determination of phytates in the samples.

## RESULTS AND DISCUSSION

### Proximate composition

The effects of sprouting time (germination), using distilled water under room conditions, on moisture, mineral matter (ash), crude protein, crude fat, crude fiber and nitrogen-free extract (NFE) is depicted in Figures 1 to 5.

### Moisture content

Initial moisture contents of the seeds of the two varieties (6.9 and 7.0%) did not differ significantly. However, the absorption pattern by each cultivar was significantly

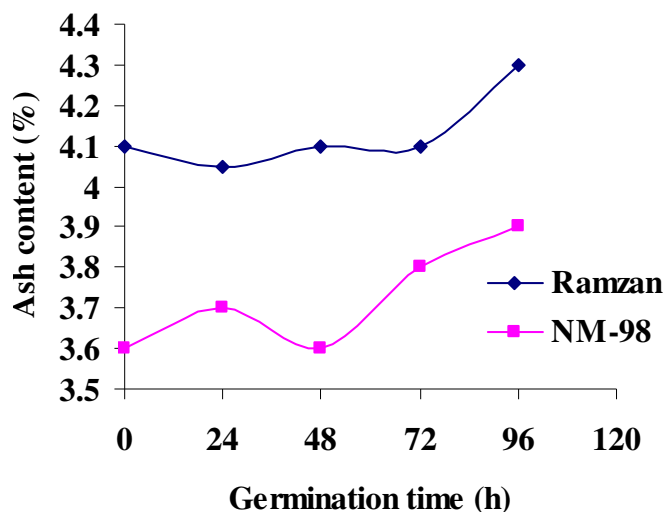


Figure 2. Effects of sprouting time on crude ash content of seed of Mungbean varieties.

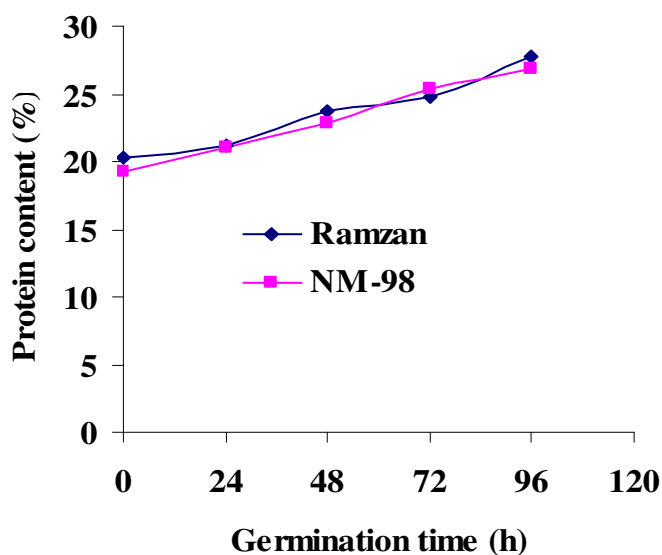


Figure 3. Effect of sprouting time on protein content seed of Mungbean varieties.

different ( $p \leq 0.05$ ). The moisture content of seeds of variety Ramzan rose to 58.1, 61.4, 80.2 and 87.2% with sprouting time of 24, 48, 72 and 96 h respectively. Water absorption of the seeds of variety NM-98 followed a similar pattern up to 72 h, and the corresponding values for 24, 48 and 72 h were 58.6, 61.7 and 80.7 but rose significantly higher to 89.5% at 96 h sprouting time. The overall mean values of moisture contents of the sprouts of two varieties were significantly different. Effect of soaking/germination was also significant on the moisture contents of the seeds, exhibiting an upward trend with increase in sprouting time (Figure 1).

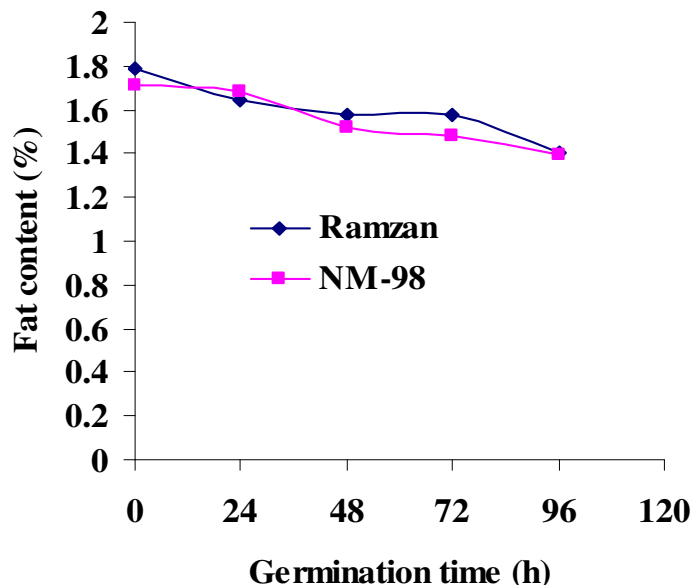


Figure 4. Effect of sprouting time on fat content seed of Mungbean varieties.

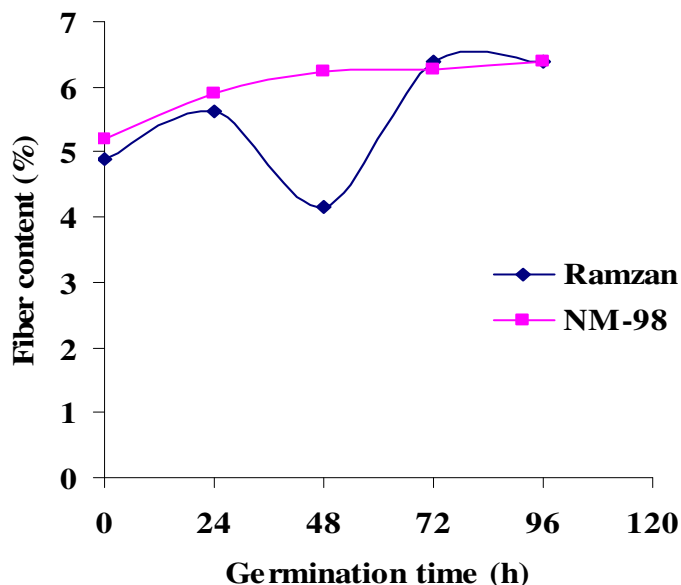


Figure 5. Effects of sprouting time on crude fiber content of seeds of Mungbean varieties.

Our results corroborates earlier reports of increased moisture content during germination of chick pea and other legumes seeds (Osman, 2007; Khattak et al., 2008; Khalil et al., 2007; Khatoon and Parkash, 2006). However, Ohtsubo et al. (2005) found contradictory results in the nutritional analysis of brown and germinated brown rice that contained lower moisture content in the germinated rice samples. As germination proceeds, seed

took up water from the surrounding in order for the metabolic process to start. Dry legumes absorb water rapidly, influenced by the structure of the legume. The increase in water uptake with time is due to the increasing number of cells within the seed becoming hydrated (Nonogaki et al., 2010).

### Ash content

Ash contents are shown in Figure 2. The two varieties differed significantly ( $p \leq 0.05$ ) in ash content with Ramzan having higher (4.1%) value as compared to NM-98 (3.6%). Ash contents, calculated on moisture free basis, increased with increase in sprouting time. In variety Ramzan, the ash content slightly decreased with 24 h germination and thereafter with 48 and 72 h germination it become at par with control. After 96 h germination the ash content reaches maximum level in both the varieties. El-Adawy et al. (2003) reported significant increase in ash content during sprouting in mungbean, pea and lentil seed. The decrease in crude fat and carbohydrate contents during sprouting may have led to the apparent increase observed in ash and other chemical components.

### Crude protein content

The two varieties did not differ significantly in protein contents, which ranged from 19.25 to 20.3%. However effect of sprouting was highly significant ( $p \leq 0.05$ ) on protein content, which showed increasing trend with sprouting time (Figure 3). The protein content (reported on dry weight basis) in variety Ramzan increased from an initial value of 20.3 to 27.7% in 96 h sprouting. In case of variety NM-98 the protein contents increased from an initial value of 19.25 to 26.8% in 96 h sprouting. The pattern of increase in concentration of protein content in the seeds of the two varieties was similar throughout the sprouting and the two varieties ended on an almost similar value at 96 h sprouting. However, mean values of protein content of both the genotype differed significantly ( $p \leq 0.05$ ) from each other.

Increase in protein content was also noted by Camacho et al. (1992) during germination of beans, lentils, chick pea and pea's seeds. Ohtsubo et al. (2005) found an increment in crude protein of germinated brown rice. Our data, regarding the effect of sprouting on the proximate composition of mungbean seeds, agree with Obizoba (1991) who reported increase in % moisture, % crude protein and % ash. According to him, total nitrogen, total non-protein nitrogen; protein nitrogen, true protein nitrogen also increased with sprouting. Parameswaran and Sadasivan (1994), Khatoon and Prakash (2006), Urbano et al. (2005), Ghavidel and Prakash (2007), and Kaushik et al. (2010) also noted increase in the percent

protein in germinated grains. Bau et al. (1997) assumed that the increased was due to synthesis of enzyme proteins (for example, proteases) by germinating seed or a compositional change following the degradation of other constituents. A further explanation was done by Nonogaki et al. (2010) where they noted that protein synthesis occurred during imbibitions and that hormonal changes play an important role in achieving the completion of germination.

### Crude fat content

The ether extract values of the two varieties differed significantly ( $p \leq 0.05$ ). The crude fat concentration decreased with sprouting time. It decreased from 1.79 to 1.4% and 1.71 to 1.39% in Ramzan and NM-98, respectively (Figure 4).

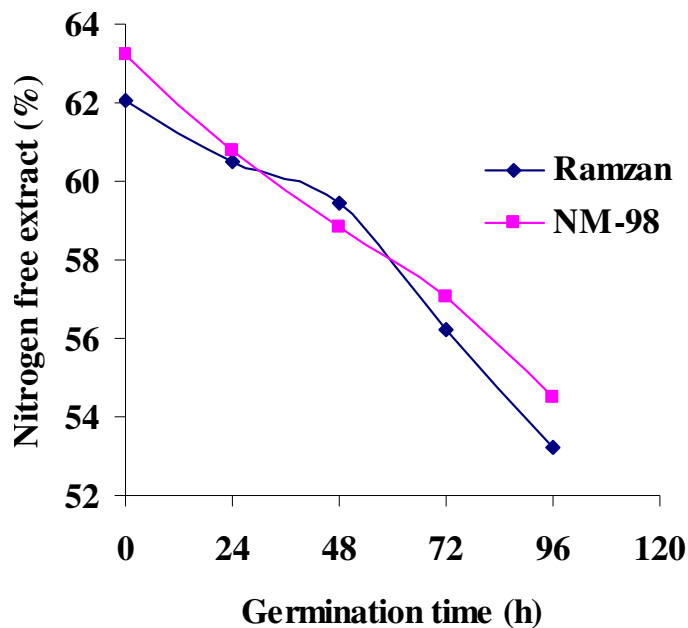
Our results were in line with Badshah et al. (1991) and Chung et al. (1998) who noted significant losses in lipid content during canola sprouting. The decrease in fat content of seed could be due to total solid loss during soaking prior to germination (Wang et al., 1997) or use of fat as an energy source in sprouting process (El-Adawy, 2002).

### Crude fiber content

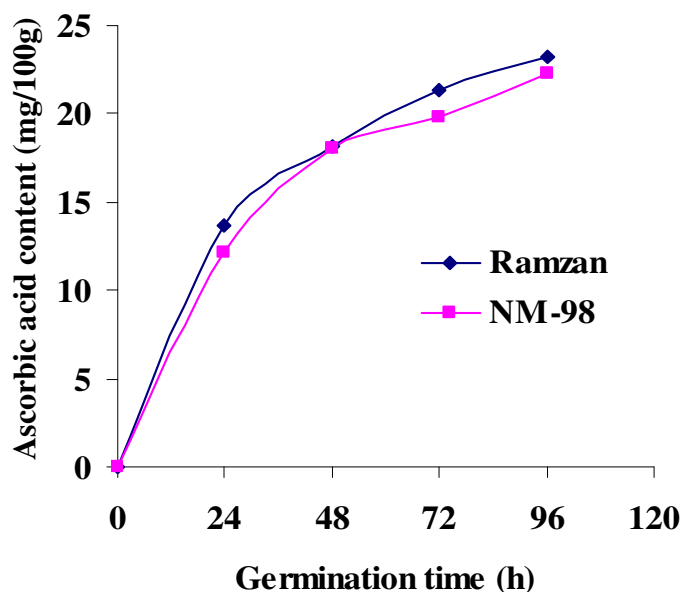
Genotypic differences in the fiber contents were significant ( $p \leq 0.05$ ). Sprouting significantly increased the crude fiber contents, with mean values of 4.88 in the control and 6.4% at 96 h sprouting. However it can be noted that the only significant reduction was recorded in the 48 h Ramzan sprouting, whereas further sprouting time increased the fiber content (Figure 5). Chung et al. (1998) reported that in barley (but not in canola) sprouting was associated with significant increase in crude fiber from 3.75% in unsprouted barley to 6% in 5 days sprouts due to synthesis of structural carbohydrates such as cellulose and hemicellulose, a major constituent of cell walls. In a similar study conducted on soybean, Jimenez et al. (1985) also noted an increase in the fiber and protein content of soybean seeds during sprouting.

### Nitrogen free extracts (NFE)

The NFE contents, which represent the digestible carbohydrates, differed significantly in the two varieties, with Ramzan having lower (62.03%) value and NM-98 having the higher (63.24%) contents. Sprouting resulted in almost linear reduction of this carbohydrate fraction (Figure 6). Our results were supported by Harmuth-Hoene et al. (1987) who reported losses, in dry matter and carbohydrates for wheat, mungbean and chickpea



**Figure 6.** Effect of sprouting time on NFE content seed of Mungbean varieties.



**Figure 7.** Effects of sprouting time on ascorbic acid content of seeds of Mungbean varieties.

seeds during sprouting. Khalil et al. (2007) while working on Kabuli and desi type chickpea varieties, reported significant increase in moisture, protein, ash content and decrease NFE content. Inyang and Zakari (2008) show significant decrease in carbohydrate levels of the instant fura samples. The decrease might be due to alpha amylase activity (Lasekan, 1996). This alpha-amylase

breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination.

### Ascorbic acid

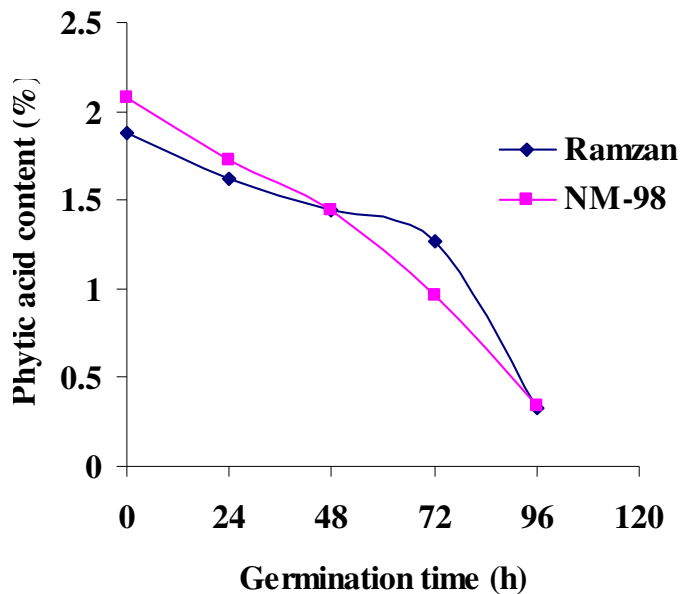
During Sprouting (germination) several enzyme systems become active and bring about profound changes in the nutritive value of pulses. Vitamin C, which is practically absent in dry legume seeds, increases in significant amounts after sprouting. The effect of sprouting time, under room conditions, on ascorbic acids content of both varieties of mungbeans namely Ramzan and NM-98.

Mungbean variety Ramzan contained more ascorbic acids as compared to NM-98. The ascorbic acid content of sprouted seeds (24-H samples) was  $13.7 \text{ mg}100\text{g}^{-1}$  in Ramzan and  $12.1 \text{ mg}100\text{g}^{-1}$  in NM-98. The effect of sprouting time on ascorbic acid content was found significant ( $P < 0.01$ ). Ascorbic acid contents increased from initial values of  $13.7$  to  $18.2$ ,  $21.3$ , and  $23.2 \text{ mg}100\text{g}^{-1}$  in 24, 48, 72 and 96 h sprouting of Ramzan seeds, respectively. Similarly in the case of NM-98 the initial ascorbic acid contents of  $12.1$  rose to  $18.0$ ,  $19.8$ , and  $22.3 \text{ mg}100\text{g}^{-1}$  after 24, 48, 72 and 96 h sprouting, respectively. It may, however, be noted that the initial differences in the two varieties in this regards were carried over during the entire sprouting time (Figure 7).

Our results are well in agreement with those of Fernandez et al. (1988) who reported a significant increase in ascorbic acid during germination. Riddoch et al. (1998) reported that many species of pulses produced significant quantities of vitamin C up to five days following germination in both light and dark although cooking caused a marked loss of ascorbate. Yang et al. (2001) monitored the changes in the concentration of vitamins C and E, beta-carotene, ferulic acid and vanillic acid in wheat seed over the germination period. Vitamins C and E and beta-carotene were barely detectable in the dry grains. However, upon germination the concentrations of these antioxidant vitamins steadily increased with increasing germination time, reaching their peaks after 7 days at  $550 \mu\text{g}$  for vitamin C,  $10.92 \mu\text{g}$  for alpha-tocopherol, and  $3.1 \mu\text{g}$  for beta-carotene. Similarly Deosthale and Barai (1949) reported that germination or malting increases the vitamin C and folic acid content of food legumes and also degrades the anti-nutrients present in these food grains. Significant increase in the content of ascorbic acid of different cereals and legumes seeds has also been reported by Harmuth-Hoene et al. (1987)

### Phytic acid

Fiber rich foods, including both cereals and legumes, contain high levels of phytate or phytic acid. Phytic acid is



**Figure 8.** Effects of sprouting time on phytic acid content of seed of Mungbean varieties.

the storage form of phosphorus in cereals and is released when the grain germinates. Phytates form insoluble complexes with many minerals, notably zinc, iron, magnesium and calcium at physiological pH. Soaking the grain or legume in water of optimum pH, cooking the soaked seeds and germination of the raw seeds are known to reduce phytate content. Both bread making and germination are frequently used to reduce the effects of phytates on mineral absorption. On the other hand, it has been reported that anti-nutritional factors such as phytate, trypsin inhibitor, and haemagglutinins are broken down on germination.

In this study phytic acid was determined in the unsoaked control seeds and subsequently at each soaking interval in the seeds of the two mungbean varieties to study the genotypic and soaking effect on this antinutritional factor (Figure 8). Genotypic differences in the contents of phytic acid were non significant and the phytic acid contents in varieties Ramzan and NM-98 were 1.88 and 2.08% respectively. Soaking resulted in significant reduction in the phytic acid value. Reduction was noted right in the first soaking interval when values for the two varieties fell to 1.62 and 1.73%, respectively. It can however be noted that the two varieties exhibited different patterns for the hydrolysis of phytic acid during sprouting. This resulted in significantly ( $P \leq 0.05$ ) different values for the two varieties at the end of soaking time (96 h) with values of 0.33% for variety Ramzan and 0.34% for NM-98.

Chitra et al. (1995) reported that phytic acid content (mg/g) was the highest in soybean (36.4) followed by urd bean (13.7), pigeon pea (12.7), mungbean (12.0) and chickpea (9.6). On an average, phytic acid constituted

78.2% of the total phosphorus content and this percentage figure was the highest in soybean and the lowest in mungbean, and noted that there was a significant negative correlation between phytic acid and *in-vitro* protein digestibility of these genotypes. It was suggested that the genotypes of pulses with low phytic acid content could be identified and used in breeding programmes to improve their nutritive value and utilization.

Reductions in phytic acid contents of cereals and legume seeds have been frequently reported. Camacho et al. (1992) studied the changes promoted by germination on phytates, oligosaccharides, crude protein, amino acids and riboflavin contents of black and white cultivars of beans, lentils, chickpea and peas. Germination promoted a significant reduction in phytates levels. These changes were attributed to an increase of phytase activities. In fact, this enzyme would make a solubilization of phytates and would release soluble protein and minerals. El-Adawy, (2002) compared the effects of cooking treatments and germination and observed that germination was less effective than cooking treatments in reducing trypsin inhibitor, hemagglutinin activity, tannins and saponins; it was more effective in reducing phytic acid, stachyose and raffinose. Bakr (1996) carried out series of experiments in order to study the effects of main technological pretreatments practiced for preparing Egyptian faba bean products, that is, decortication as well as soaking and germination followed by dehulling on the nutritional value. All pretreatments caused a significant decrease in the antinutritional factors, especially soaking followed by dehulling, whereas decortication led to a significant increase in phytic acid content.

According to Chitra et al. (1996) germination reduced the phytic acid content of chickpea and pigeon pea seeds by over 60% and that of mungbean, urd bean, and soybean by about 40%. It has also been reported that *germination or malting degrades the anti-nutrients present in these food grains. Consequently, availability of iron in germinated grains improves significantly, especially in malted bajra and ragi* (Deosthale and Barai, 1949). Harmuth-Hoene et al. (1987) studied the influence of sprouting on biochemical properties of different cereals and legumes seeds. They observed that in wheat and mungbean, phytic acid was partially hydrolyzed.

Nestares et al. (1999) studied the effect of heating (120°C at 1 atm for 15 min), soaking (in distilled water, citric acid solution or sodium bicarbonate, at room temperature for 9 h) or cooking (boiling for 35 min) on the nutritive utilization of Ca and P and on phytic acid of chickpea. Soaking in acid solution followed by cooking decreased phytic acid content, suggesting that processing made part of the phytic acid P available. It was concluded that soaking before cooking is a simple and cheap technique that can be used both in the home and by industries that produce food products for nutritionally vulnerable persons with high Ca and P



requirements. Ibrahim et al. (2000) also noted that long-time soaking (16 h) in bicarbonate solution caused remarkable reduction in the antinutritional factors including phytic acid. Cooking pre-germinated cowpeas was most effective.

## Conclusion

It can be inferred from the present experiment that sprouting improved the nutritional worth of the mungbean in terms of higher concentration of nutrients, reduced phytic acid, improved protein content and ascorbic acid.

## ACKNOWLEDGEMENT

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