**Introduction**

Legumes are rich sources of proteins and carbohydrates for developing countries. Moreover, it is important source of proteins and carbohydrates, legumes were also reported to be good source of minerals (Meiners et al., 1976). Mung bean (Vigna radiata) is considered as an important legume due to its high level of protein and carbohydrate content. Its protein quality is equal or better than other legumes, like chickpea, black gram, peas, pigeon pea, etc (20,36). Mung bean has been consumed as a common food in China for more than 2,000 years. It is well known for its detoxification activities and is used to reduce swelling in the summer, refresh mentality and reduction heat strike (30). The seeds and sprouts of mung beans are also widely used as a fresh salad vegetable or common food in South East Asia, India and western countries (31). Mung beans contain balanced nutrients, including protein and dietary fiber, and significant amounts of bio-active phytochemicals. Because of high percentage of protein, polyphenols, oligosaccharides and amino acids on mung bean, this legume has high level of antioxidant, anti-inflammatory, antimicrobial activities and are involved in the regulation of lipid metabolism (4,16).

One of the most effective processes for increasing nutritional value of legume is germination. This process yield to improve free amino acids, dietary fiber, available carbohydrates and other compounds (43,44). Sprouting is affiliated with enhancement in the nutritive value of the seeds (19). In addition to this, bioavailability of trace elements, vitamin concentration and minerals increase during germination.

Phytic acid content is also reducing among germination. Consequently, flatulence decrease...
causing oligosaccharides, such as stachyose and raffinose, increasing protein digestibility and also improving sensory properties\(^{(19)}\). Biochemical reactions are done during germination. For instance, the synthesis of small active compounds from macromolecular substances, so it causes promotion in absorption and utilization. This progress cause to formation and accumulation of many types of active substances, such as polyphenols, saponins, vitamin C, etc (Tang et al., 2014).

The aim of this study was to optimize the steeping and germination conditions of mung bean and assess the seed composition changes. This project recommended the different biochemical modifications that developed in mung grain during steeping and germination and presented an improvement of nutritional properties of mung flour acceptable for snack and infant foods.

**Materials and methods**

**Materials**

Mungbean was obtained from Mashahad, Iran. Linoleic acid (99% pure) was purchased from Sigma. Acetonitrile sodium hypochlorite, NaOH, HNO\(_3\), CaCl\(_2\), hexane, isoctane, cupric acetate, soluble starch, maltose, 3,5-dinitrosalicylic acid (DNS), pyridine, Tween 20, KH\(_2\)PO\(_4\), borate buffered and NaOH were of analytical grade and purchased from Merck, and bovine serum albumin was purchased from Sigma. Deionized distilled water was used in all the experiments.

**Soaking**

The seeds were first surface sterilized by treating them with 1% sodium hypochlorite for 3 min. After rinsing, the sterilized seeds were soaked in distilled water (1:10; w/v) for 6, 27, and 48h at room temperature (28±2°C).

**Germination**

The soaked seeds were placed on moist filter paper bed in petri plates and incubated at three different temperatures (10, 22.5, and 35°C). The seeds were moistened with distilled water at regular intervals. The germinated seeds were sampled at intervals time of germination of 1, 2 and 3days. For further analysis, samples were stored in airtight containers.

**Chemical analysis**

Crude protein, ash, and fat were determined according to Association of Official Agricultural Chemists (AOAC) methods. The nitrogen content was estimated by Kjeldhal method, based on the assumption that plant proteins contain 16 g/100 g nitrogen, so protein content was calculated using the following formula:

\[
\text{Protein} = \text{Nitrogen} \times 6.25
\]

Minerals were determined using atomic absorption spectrophotometer model 3110 (Perkin Elmer, Norwalk, CT, USA) following the methods of AOAC(5).

**Phytic acid**

High Performance Liquid Chromatography (HPLC) was used for analysing of Phytic acid, Shimadzu (lc-6a) system equipped with C18 (25 cm \(\times\) 0.6 mm) column and a florescence detector. Extraction and preparation of samples were done and the final injection into the HPLC column was performed on Dost and Tokul method\(^{(10)}\). Chromatographic determination of phytic acid was based on metal replacement reaction of phytic acid from colored complex of iron (III) thiocyanate and monitoring any reduction in concentration of colored complex\(^{(31)}\).

The mobile phase contain a mixture of 30% acetonitrile and water including 0.1M HNO\(_3\). 20 μl was injected. As a result of this action, retention time was about 3.5 min. The detection wavelength was set at 460 nm.

**Amylase activity measurement**

Preparation of substrate solution: 1% soluble starch was dissolved in 100 ml of phosphate buffer (pH=7), containing 0.01 M CaCl\(_2\).

Preparation DNS solution: The DNS reagent was obtained from 1g of 3,5-dinitrosalicylic acid dissolved in 20 ml NaOH 2N, adding 30g double tartrate of nitrium and potassium and was complet ed with distilled water to obtain 100ml solution.

An enzymatic extract of alpha amylase from mung flour was prepared by extracting 10 g mung flour in 100 ml distilled water for 30 min using a magnetic stirrer, then centrifuged at 6000 rpm and the obtained supernatant was diluted 10 times in distilled water.

Maltose was used as a standard solution in the concentration as 100 µg/ml\(^{(14)}\).

**Measurement method**

The reaction mixture was formed by soluble starch solution, the amylase extract and the
analysed factor was incubated for 5 min at 30°C for the enzymatic hydrolyse reaction and there after the reaction was stopped with DNS reagent and by boiling in reaction medium for 5 min. After cooling, the mixture was colorimtered at 546 nm in relation to distilled water (37, 41).

For observing the reducing sugar existing in the reaction medium at initial time, control samples were prepared, which were exactly same to the test samples with no enzyme existence (14, 32).

**Lipases activity measurement**

**Lipase assay:** At first, mung bean was ground and then 3 volume of hexane (1:10 v/v) for 30 min on an orbital shaker at 140 rpm. Remaining hexane about 10 min was allowed to evaporate at room temperature and 1 g of the ground defatted mung bean was weighed into each of the two test tubes: one sample (Af) and one blank. Olive oil (0.6 ml) and 0.15 ml distilled water were added to both tubes and then shaked. After that, 5 ml of hexane was added, and the tube was vortexed and then centrifuged at 1000 x g for 3 min. The hexane was pouring off into a 100-ml flask, and the extraction was repeated twice. The hexane extracts were pooled, and evaporation was performed on a rotary vacuum evaporator at 40°C, and the residue was dissolved in 4 ml of isoctane. The other test tube was capped and incubated for 4 hour at 40°C (6, 37).

**Measurement method:** 1 ml of 5% (w/v) cupric acetate, which had been adjusted to pH 6.1 with pyridine, was added and shaken vigorously for 1 min. The tubes were centrifuged at 1000 g for 1 min and the absorbance was read in a spectrophotometer at 715 nm. The sample absorbance was compared with the absorbance of oleic acid standard solutions prepared in isoctane (1 to 10 mM). Finally lipase activity was calculated according to Equation 1 (37):

\[
LA = \frac{1000 (4+V)(AF-AI)}{t \times s \times \varepsilon}
\]

where LA is the lipase activity (U/g), 1000 is the conversion factor from mol/L to Mequiv/ml, 4 is the volume of isoctane used to redissolve lipids (ml), v is the volume of olive oil added (ml), AF is the absorbance of sample after incubation at 715 nm, Ai is the absorbance of blank at 715 nm, \( \varepsilon \) is the molar absorptivity of oleic acid at 715 nm (M\(^{-1}\)cm\(^{-1}\)), \( t \) is the incubation time (h), \( l \) is the path length (1 cm for a standard cuvette), and \( s \) is the sample weight (g).

**Lipoxygenase activity measurement**

Enzyme extraction was carried out by homogenizing 10 g of mung with 50 ml of water or potassium phosphate buffer. The slurry was readjusted to the required pH and then centrifuged at 2000 x g for 15 min. The supernatant containing lipoxygenase was used as the crude enzyme extract. Linoleic acid and purified soybean lipoxigenase were obtained from Nutritional Biochemicals Corporation. A standard curve was constructed using bovine serum albumin (13, 41).

Preparation of substrate solution: 0.5 ml of Tween 20 was dissolved in 10 ml of borate buffer of pH 9.0, and then 157 µl of linoleic acid was added. After that, 1 ml of NaOH (1 N) was added and the mixture was agitated until a clear transparent solution was observed. Subsequently, the borate buffer was added (90 ml) and the final volume made up to 200 ml with water.

**Measurement method:** 29 ml of the substrate solution was transferred into a 100 ml flask placed in a temperature controlled water bath set at 30°C. The substrate solution was aerated by a gentle stream of air for 2 min and the reaction was started by adding 1 ml of crude enzyme extract into the flask. Aliquots of 1 ml from the reaction medium were transferred into the glass tubes containing 4 ml of 0.1 N NaOH solution at time intervals (0.5 to 5 min). Changes were recorded for 5 minutes in wavelength of 234 nm. The blank solution was prepared by mixing 1 ml of the substrate solution with 4 ml of 0.1 N NaOH solution (13, 41).

**Experimental design**

Variation effects on soaking and germination time and also germination temperature were analyzed using the response surface methodology (RSM). The germination process was assumed to be a system affected by three independent variables, \( X_i \) (soaking time, germination time and temperature), that were controlled and measured. It was assumed that the dependent variables also referred to as responses, \( Y \) (protein, fat, fiber and ash content, phytic acid content, enzyme activity, and minerals), defined the system and were experimentally determined. Furthermore, a mathematical function was assumed to describe the relationship between responses, \( Y \), and factors, \( X_i \). The following second degree polynomial equation was fitted to the data:

\[
Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \sum_{j=1}^{3} \beta_{ij} X_i X_j
\]

\( \beta_0 \) is a constant, \( \beta_i \) and \( \beta_{ij} \) are model coefficients.
linked to linear effects, $\beta_{ii}$ are coefficients related to quadratic effects and $\beta_{ij}$ are interaction terms.

The levels of variables in coded and uncoded form are shown in Table 1.

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Symbol</th>
<th>Levels</th>
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<tr>
<td>Coded</td>
<td>Uncoded</td>
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<tr>
<td>Soaking time (h)</td>
<td>$X_1$</td>
<td>$t_1$</td>
</tr>
<tr>
<td>Germination time (h)</td>
<td>$X_2$</td>
<td>$t_2$</td>
</tr>
<tr>
<td>Germination temperature (°C)</td>
<td>$X_3$</td>
<td>T</td>
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Table 1: Values of independent process variables and their corresponding levels.

Statistical analysis
Design expert software was used to determine the effects of the independent variables. To calculate regression coefficients, analysis of variance (ANOVA) was carried out and the response surface was built at a 5% significant level. Experimental data were expressed as mean± standard deviation of triplicate measurements.

Results and discussion

Effect of soaking and germination conditions on crude protein content
The effect of different soaking time, germination time and temperature on some chemical properties of mung bean is as shown in Figure 1. Figure 1 shows the interactive effect of soaking time, germination time and temperature on protein content. The most crude protein content (30.98% dry weight) was found at soaking for 48h followed by germination at 35°C for 3h (Figure 1). The combination of soaking and germination times had a significant effect on crude protein content. The treatments significantly increased the protein content of mung bean (p<0.05). Increase in protein content could be due to alteration of other components (starch, lipids, ash, and crude fiber) which might have altered the proportion of the protein on dry weight basis during soaking and germination. Increase in dry matter content was probably as a result of the enzymatic activities that occurred during germination.

These results are similar with those reported by Luo and Xie (2013) for faba bean which declared that protein concentration in soaked seeds was higher than in raw ones(29).

Ohtsubo et al. (2005) found an increasing rate of crude protein content of germinated brown rice. Increase in protein content was also reported by Adil Shah during germination of Mungbean varieties(30). Sprouting had an increasing effect on protein content of mung bean. This issue is similar to that in Obizoba (1991) finding(30), who reported that the total nitrogen, total-non-protein nitrogen and protein nitrogen is increased by sprouting. Khatoon and Prakash (2006), Urbano et al. (2005), Ghavidel and Prakash (2007) and Kaushik et al. (2010) also noted an increased rate in protein content of germinated grains(31,32,33,42). Adil Shah assumed that some compositional changes during mung bean seeds germination cause degradation of other constituents, such as synthesis of enzymatic proteins like proteases and consequently, protein content increased(32). A further explanation was done by Nonogaki et al. (2010) that protein synthesis occurred during imbibition and hormonal changes play an important role in achieving the completion of germination(34).

Effect of soaking and germination conditions on ash content

Result shows the interactive effect of soaking time, germination time and temperature on ash content. All factors had a significant effect (p<0.05) on mung bean seed ash content. Mung beanseed were soaked for 48 h, followed by germination for 3 days at 35°C, and they had higher amounts of ash content. El-Adawy reported significant increase in the ash content during sprouting of mungbean, pea and lentil seed(11). In spite of deluding rate of crude fat and carbohydrates content during sprouting time, ash and other chemical components increased. Akpapunam and Achinewhu observed similar results for ash content in different germinated pulses and legumes(30).
Effect of soaking and germination conditions on crude fat

Maximum contents of crude fat (1.01% dry weight) was detected during soaking for 48 h, and germination for 3 days at 35°C. Increase in germination time led the fat content of mung bean seeds to increase significantly (p≤0.05), this effect was observed by varying in soaking time and germination temperature. Lee et al. (2007) reported that the crude fat, dietary fiber and protein content increased significantly after germination in brown rice(22). Increase in these components has also been reported by Kim et al. (1993) for soybean, Park et al. (1986) for mungbean, and Jung et al. (2005) for germinated brown rice(15,20,36). However, in some report, the decrease in fat content of seed could be due to total solid loss during soaking prior to germination or use of fat as an energy source in sprouting process(2,11).

Effect of soaking and germination conditions on crude fiber

Although Chung reported that sprouting had a significant effect on barley crude fibre, the findings of this study showed that soaking and germination conditions had no effect on mung bean crude fibre. They observed that crude fiber increased and then it decreased during germination temperature due to synthesis of structural carbohydrates, such as cellulose and hemicellulose, a major constituent of cell walls(9). Range crude fibre reported between 4.11% and 8.7%.

Retention of minerals (Fe, Zn and P)

Germination time increase significantly (p<0.05) enhanced the contents of iron and zinc(Figures 1 and 2), Germination temperature increase significantly (p<0.05) enhanced the amount of phosphorus(Figure 3).

Similar observations were observed for the total content of minerals changes during germination as reported by Kumari et al. (2014) for soybean(21), Mubarak (2005) reported that soaking resulted in the greatest retention of all minerals in mung bean(33). Luo et al. (2014) demonstrated that germination brought about an enormous improvement of iron availability in faba bean, soybean, and rice(26). Germination period had no significant effect on the loss of total content of phosphorus. Hence, the loss observed may be due to soaking and washing during germination(21).

Phytate, the complex of phytic acid and mineral elements, is known as a chelating agent that reduces the bioavailability of minerals. The decrease in phytate content as a result of soaking and germination had beneficial effect on iron and zinc availability(27).

Effect of soaking and germination conditions on phytic acid

Cereals and legumes contain high levels of phytate or phytic acid. Phytic acid is 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. Both bread making and germination are frequently used to diminish the effects of phytates on mineral absorption. On the other hand, it has been reported that anti-nutritional factors, such as phytate and trypsin inhibitor are broken down during germination (Adil Shah et al., 2011).
Figure 4 shows the effect of process variables on phytic acid content. The process of germination profoundly decreased the phytic acid content and there was a steady decrease in phytic acid content as the germination temperature increased. The phytic acid content decreased to 6 mg/100 g in sample of 3 h germination at 35°C. There was about 55% reduction in the phytic acid content after soaking for 48 h, then germination for 3 h at 35°C.

Decomposition of phytic acid in relation to processing has been invariably observed in several legumes, including mung bean(38).

According to Adil Shah germination reduced the phytic acid content of Mung bean varieties(2). The hydrolysis of phytic acid in germinating mung bean seeds was found to be associated with increased hydrolytic activity of phytase. As the phytase activity increased, the phytic acid content decreased.

Liang et al. (2008) found out that soaking and germination had different effects on reducing phytic acid content in brown rice(23). These researchers have proposed that the activity of endogenous phytase was the main factor leading to the reduction of phytic acid during soaking.

**Alpha-amylase**

As shown in Figure 5, it was found out that temperature and germination time has great influence on alpha-amylase activity. The activity of alpha-amylase increases by increasing temperature and germination time. The greatest increase was observed in treatment with 3 days of germination at temperature of 35°C. Singh et al. (2001) found out that germination temperature has a greater impact on enzyme activity as compared to soaking time and germination time(39,40). Other studies showed that alpha-amylase activity is increased during germination and this increase depends on the varieties, measurement methods and other factors. The results of Lukow and Bushuk (1984) indicated 1600-fold and 3000-fold increase for two wheat varieties during germination temperature of 21°C for 54 h(24). Marsh et al. (1988) reported 20-fold increase in the activity of alpha amylase in germinated wheat seeds(29).

**Lipase**

According to Figure 6, it was found out that the lipase activity increases by increasing germination time. The maximum value was observed in treatment of 48 h soaking and 3 days germination at 35°C. Germination temperature has more influence on lipase activity compared two other factors. For the first time, some researchers showed that lipase activity severely increased during germination of wheat. Kubicka et al. (2000) in their study about changes in lipase activity during germination of wheat and barley found that the specific activity of lipase has increased. When germination started, the lipase activity is intensified, because the fat stored in the grain becomes activated and consumed as food. The amount of lipase increase during germi-
nation is related to elevated lipase activity and suitable interactions conditions.

**Lipoxygenase**

In Figure 7, it is indicated that lipoxygenase activity does follow a regular increase trend by increase in soaking time, germination time and temperature. Mosavi et al. (2014) reported that increasing rate of lipoxygenase activity in serminated wheat flour becomes slower after 36 h until 72 h.

**Figure 6:** Response Surface plot for Zn content in mungbean seed.

**Figure 7:** Response Surface plot for P content in mung bean seed.

Lipoxygenase has an important role in the oxidation of lipids which led to the oxidation of stored lipids and consumption of lipids in carbon form as an energy source to grow grain. Although, the amount of lipids in cereals is low, lipase and lipoxygenase have a significant effect on the quality of nutrition and nutritional value of wheat and grown barley as food. Kubicka et al. (2000) found out that some changes occur in the specific activity of lipoxygenase during germination of wheat and barley and these changes do not follow a clear trend. Their results are consistent with the results of this research.

**Conclusions**

RSM was used to establish the optimum process variables (soaking time, germination time and temperature) to reach the desired value of selected nutrients and antinutrients in mung bean. By using RSM, the optimum set of operating variables can be obtained graphically, in order to achieve the desired pretreatment levels for protein content and phytic acid. The process conditions yield an optimum process (maximum reduction in phytic acid and minimum nutrient components loss) which was soaking for 48 h followed by germination at 35°C for 3 day. These results suggest that soaked and germinated food grains contain significantly higher amounts of nutrients and lower values of antinutrients than their untreated samples and hence they can be used to make acceptable food products.

**References**


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