Nutrients and anti-nutrients of high chlorophyll – mungbean sprouts as affected by different periods of germination and sprouting stages

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Abstract: The variation of nutrient and anti-nutrient compounds in commercial mungbean cultivars (Chinat 72, MS-1, Chinat 80, and L3-8) during seven periods of germination and sprouting was determined. The seeds were selected randomly at 6 h of soaking (1st stage), 23 h (2nd stage), 47 h (3rd stage), 71 h (4th stage), 77 h (5th stage) of sprouting, and 12 h, and 24 h of sunshine exposing (6th and 7th stage, respectively). It was found that nutrition compositions (including protein content, crude fiber content, vitamin C content, total minerals, and HCL-extractability of minerals) of all cultivars significantly increased with germination and sprouting. At the last stage, the total phenol was the highest amount which was not significantly different from all cultivars. The total antiradical capacity (%, DPPH inhibition) increased up to the maximum value in the last two stages of sprouting. The results showed that the phytic acid, the anti-nutrient component decreased with the consequence of germination, and reached the untraceable value at the last stage. In addition, the highest amount of chlorophyll (7.15-8.99 mg/100 g) was found in Chinat 72 and MS-1 cultivars at the last stage of sprouting, comparing to Chinat 80 and L3-8 cultivars. It is therefore recommended to consume high chlorophyll mungbean sprout with the benefits of high nutrient constituents and low price purchase comparing to other green vegetables.

Keywords: high chlorophyll, phytic acid, total phenolic content, radical scavenging activity, HCl extractability of minerals, mungbean sprouts

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1 Introduction

In Thailand, mungbean is considered as an economically beneficial crop, and its production and trade have been rather successful in terms of their volume and value of exports. Green gram [*Vigna radiata* (L.) Wilczek] and black gram [*Vigna mungo* (L.) Hepper] have been grown widely throughout the country for domestic use and export to other countries or areas such as Taiwan, India, and China. In perspective of cash crop of mungbean the Ministry of Agriculture has supported the improvement and releasing of new bred mungbean cultivars with the goals of higher yield, resistance to diseases and insects, and also producing good quality of mungbean seeds needed for the legume market demand.

A number of mungbean cultivars are cultivated. Chinat 72 (green gram) and Chinat 80 (black gram) are the ones commonly grown due to their tolerance to different stresses and produce high yield. However, as an attempt to meet the market increasing demand each year, MS-1 and L3-8 cultivars are now released to the farmers which are bred from Chinat 72 and Chinat 80, respectively, by Chi-Nat Field Crops Research Center, Department of Agriculture, Thailand. These two new bred cultivars are considered worthwhile in terms of high yield and tolerance to stresses.

Mungbean is an especially important source of dietary proteins and also rich in fiber, which aids with digestion and absorption of food. It plays a vital role in cholesterol metabolism, and thus controls blood

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cholesterol levels. Due to its nutritional benefits, mungbean is recommended to be consumed directly in different forms and ways, for example, to cook it with vegetables or meats as well as a dessert or process it to mungbean starch or flour used as an ingredient in many food products. However, anti-nutritional factors limit the food applications of mungbean[1,2]. Germination or sprouting is one of the traditional processes to overcome this problem[3-5]. Normally, mungbean is allowed to germinate for three to four days which is suitable for direct consumption. Mungbean sprouts can be eaten fresh or cooked as vegetable accompaniment to a meal. Even though many literatures have indicated the improvement of nutrients in mungbean sprout by germination[6-8], there are limits information on the nutrient alteration when the seeds are sprouting until the true leaves appear (called high chlorophyll mungbean sprout). With the increased interest in the exploitation of sprouted mungbean with true leaves in order to replace with other green vegetables usually eaten as a fresh dish or as a salad meal, the study is designed to evaluate the nutrients and anti-nutrients as affected by germination and exposure to sunshine of mungbean sprout. Patterns of changes in constituents taking place during germination and sprouting of different selected cultivars of green gram and black gram (Chinat 72 and Chinat 80) and their hybrids (MS-1 and L3-8) are also examined.

2 Materials and methods

2.1 Germination and sprouting of mungbean seeds

Clean-mungbean seeds (Chinat 72, MS-1, Chinat 80, and L3-8), obtained from Chi-Nat Field Crops Research Center, Department of Agriculture, Thailand, were determined for their germination rate. The germination rate of 97% - 100% was a proper rate for further study. Prior to germination and sprouting, the seeds were soaked for 6 h at room temperature. The imbibed seeds were germinated by spreading them on the 3-storey in the black plastic container without light (called "3-storey condominium") and watered three to four times a day. The seeds sprouted until the cotyledons appeared then the sprouts were exposed to the sunshine for 24 h. The seeds were taken randomly at 6 h of soaking (1^{st} stage), 23 h, 47 h, 71 h, and 77 h of sprouting in the black container (2^{nd}, 3^{rd}, 4^{th}, and 5^{th} stage, respectively), and at 12 h, and 24 h of sunshine exposing (6^{th} and 7^{th} stage, respectively). The sample seeds were freeze dried in preparation for quality determinations.

2.2 Crude protein

The crude protein content was calculated from nitrogen content, using the Kjeldahl method (Gerhardt, Germany). The factor of 6.25 was used in calculation[9].

2.3 Crude fiber

The crude fiber was determined by extraction with hot acid and base, using a crude fiber extractor (Velp Scientifica, Italy), according to the method of AOAC[9].

2.4 Reducing sugar

Reducing sugars was determined in the 50% ethanol extracts by the Somogyi-Nelson method using glucose, concentration between 10-100 μg/mL as a standard according to the method of Lai et al[10].

2.5 Vitamin C content

Quality determination of Vitamin C was measured by Indophenol Method[11].

2.6 Total phenolic content (TPC)

TPC was determined according to the Folin-Ciocalteu Spectrophotometric method[12].

2.7 Phytic acid content

Phytic acid contents were determined by the method of Wahab et al[13]. The defatted and finely ground sample (0.06 g) was extracted with 10 mL of 0.2 N HCl for 1 h. From this extract, 0.5 mL was put into a stopper test tube. Ferric solution (1 mL) was added to the tube and covered with the stopper. These tubes were heated in a boiling water bath for 30 min and allowed to cool at room temperature. 2,2, Bipyradine solution (2 mL) was added to the tube and mixed well. The absorbance was measured within 1 min at 519 nm. A standard calibration curve was prepared using the same procedure with the standard solutions of known concentration of sodium phytate range of 5-100 μg/mL. The concentration of phytic acid in the sample was calculated by the below formula:

Phytic acid (g/100 g) = Phosphorus phytate × 4.97

2.8 Radical scavenging activity: DPPH

The free radical activity was measured according to

the method of Katsube et al[14]. Dried mungbean seeds (3 g) were extracted by 10 mL of 80% ethanol solution in the 50 mL volumetric flask. The flask was shaken at 100 r/min for 2 h, at room temperature. The sample was filtered by using the Whatman paper No.1 to obtain the supernatant for determining DPPH. The supernatant (0.1 mL) was pipetted and added with 3.9 mL of 6×10^{-5} M DPPH in absolute ethanol solution. The sample was then left in dark room for 20 min and measured the absorbance at 520 nm by UV-vis spectrophotometer. The control sample was prepared by using 0.1 mL of ethanol added with 3.9 mL of 6×10^{-5} M DPPH in absolute ethanol. The results were expressed as inhibition (%) as follows:

inhibition (%) =

$$\frac{(Absorbance\ control - Absorbace\ sample)}{Absorbance\ control}\times100\%$$

2.9 Total mineral contents

Total mineral contents were determined by the atomic absorption spectrophtotometry[9]. The oven dried mungbean samples (2 g) were placed in a crucible and mineralized at 550°C for 3 h then cooled in a desiccator. Thereafter, ashes were transferred into a beaker and added with 20 mL of concentrated HNO_3, followed by 10 mL of H_2O_2. The mixture was heated at 100°C for 1 h and afterward, cooled and filtered. The filtrate was transferred into a 250 mL volumetric flask and distilled water was added to fill the flask to the mark. From this stock solution, 2 mL was pipetted into 50 mL flask and was made up to the required volume with distilled water. Mineral contents of these solutions were determined by atomic absorption spectrophotometer (AAS) for calcium, potassium, iron, and sodium (Spectr AA 220 Varian) having the hollow cathode lamp as a light source. The total concentration of minerals calculated from their standard curves.

2.10 HCl extractability of minerals

Hydrochloric acid extractability of minerals was determined following the method of El Maki et al[7]. The mungbean seeds (2 g) were continuously shaken with 100 mL of 0.03 N HCl at 150 r/min for 3 h at 37°C. The mixture was filtered through Whatman No.1 (ashless) filter paper and the clear supernatant was oven dried at 100°C. The procedure thereafter was similar to the determination of total mineral contents as described above. The HCl extractability of minerals was calculated as follows:

Mineral HCl extractability (%) =

$$\frac{Mineral\ extracted\ in\ 0.03\ N\ HCl}{Total\ mineral\ content}\times100\%$$

2.11 Total chlorophyll

Total chlorophyll was determined by the method of Ghnaya et al[15]. Ground sample (0.5 g) was added with 50 mL of 80% acetone in the flask. The sample was continuously stirred for 30 min then centrifuged for 15 min at 10°C. The clear sample was measured about its absorbance at two wave lengths, 663 nm and 645 nm. The total chlorophyll was calculated as follow:

$$\text{Total chlorophyll (mg/100 g)} = 7.15\,A_{663nm} + 18.71\,A_{645nm}$$

3 Results and discussion

3.1 Effects of germination and sprouting on protein, crude fiber, reducing sugar, and vitamin C content

Results found an increase in crude protein content from 26% to 35% (dry matter) as germination advanced (Figure 1a). This was associated with a large increase in the non-protein nitrogen (NPN) and a smaller decrease in the protein nitrogen (PN). The increase in the NPN was found in three legumes; peas, beans, and lentils as germination was prolonged which could be caused by hydrolysis of storage proteins to yield amino acids for transfer to the growing sprouts and by an increase in nucleic acids[16]. This is due to the fact that, during the germination process, several enzymes are activated and some non-protein nitrogen substances, such as nucleic acids, are produced. The increase in the protein contents could be explained by the increased hydrolytic activities of the enzymes caused by sprouting resulted in improvements in the contents of the total protein due to the disappearance of starch[17]. A similar observation was found in germinated brown rice, and germinated rough rice as reported by Moongngarm and Saetung[18] and Kim et al.[19], as well as in germinated oat seeds by Tian et al[20]. Also, Sattar et al.[2] had earlier reported a slight increasing trend in the protein content of mungbean as affected by soaking and germination temperatures.

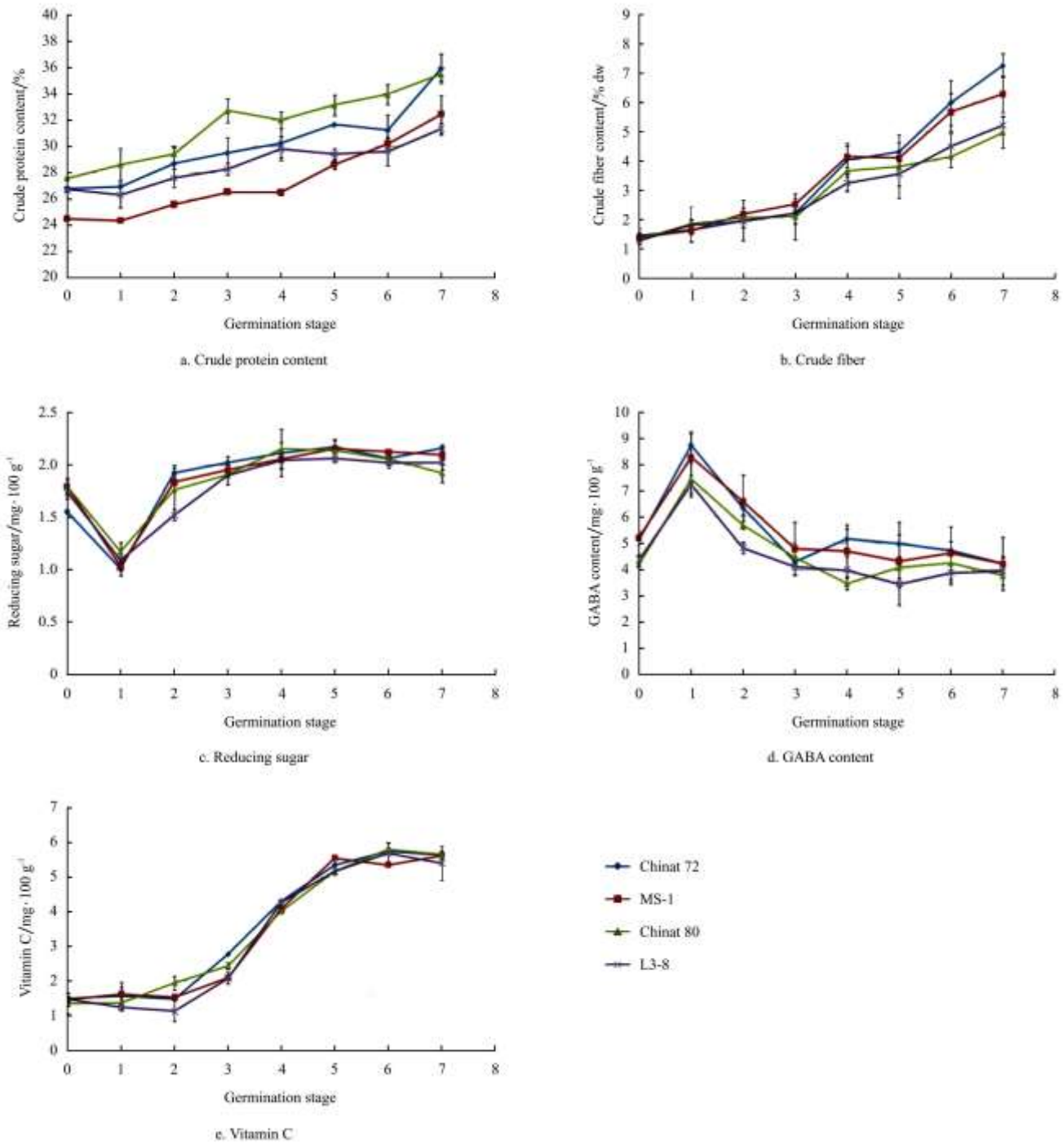

Figure 1 Effects of germination stage on crude protein content, crude fiber, reducing sugar, GABA content, and vitamin C. Germination stage includes: 0=before germination, 1=6 h of soaking, 2=23 h of sprouting, 3=47 h of sprouting, 4=71 h of sprouting, 5=77 h of sprouting, 6=12 h of sunshine exposing, 7=24 h of sunshine exposing.

Crude fiber increased from 1.3% to 3.5% - 4.0% when germination time allowed to 71 h for both green gram and black gram cultivars (Figure 1 b). However when germination was prolonged to 77 h and exposed to sunshine for 24 h, crude fiber in green gram (Chinat 72 and MS-1) was significantly higher than in black gram (Chinat 80 and L3-8). At this stage, crude fiber of Chinat 72 was the highest (7.2%). Results obtained in the present study were in agreement with other germinated legume seeds. Ghavidel et al.[21] reported an increase in soluble and total dietary fiber fractions but a decrease in insoluble dietary fiber fractions of green gram, cowpea, lentil and chickpea. El-Adawy et al.[6] found the increase in crude fiber in pea, lentil and mungbean at the 120 h of germination. The increase in crude fiber, a major constituent of cell wall may correlate with the

disappearance of starch and an increase both in the synthesis of structural carbohydrate such as cellulose and hemicelluloses during plant elongation.

The reducing sugar slightly decreased during soaking, then increased and reached constant at the 23-h germination. The decreases for green gram and black gram cultivars at the beginning of germination could be attributed to their use of reducing sugar as an energy source to start germination. When germination was prolonged, however, the hydrolysis of starch and non-starch polysaccharides to the monosaccharides was continued with the results of increasing in the reducing sugar needed for germination. Thus, exhausting and synthesis of reducing sugar occurred in balance amount during the germination which explained the slight changes of reducing sugars for all cultivars with the germination onwards (Figure 1c).

Vitamin C in both green gram and black gram cultivars increased from 1.3% at the 1st stage to 5.6% at the 7th stage, similarly in the study of Fernadez-Orozco et al.[22] who observed an increase in vitamin C in mungbean from 1.9% to 9% as affected by germination (Figure 1d).

3.2 Effects of germination and sprouting on total phenol compounds, DPPH inhibition, and phytic acid content

Phenolic compounds play a role in protecting the cells from free radical damage and involve in strengthening the plant cell walls during growth by polymerization into lignans and lignins[23]. Results found that total phenolic contents of green gram and black gram cultivars significantly increased from 0.1 g/100 g to 0.39 g/100 g at the beginning of germination to the end of 24-h sunshine exposure, respectively (Figures 2a and 2b). The increase in phenolic compounds was positively correlated with the increase in antioxidant activity as measured by % DPPH inhibition which increased from 22% to 67% at the last stage. The antioxidation activity of phenolic compounds is due to their ability to scavenge free radicals, donate hydrogen atoms or electron, or chelate metal cations. This is in agreement with Cevallos-Casals and Cisneros-Zevallos[24] who studied the impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Their study showed increase in phenolics from dormant seed to seven-day sprout differed among seeds and concluded that edible seeds were an excellent source of dietary phenolic antioxidants.

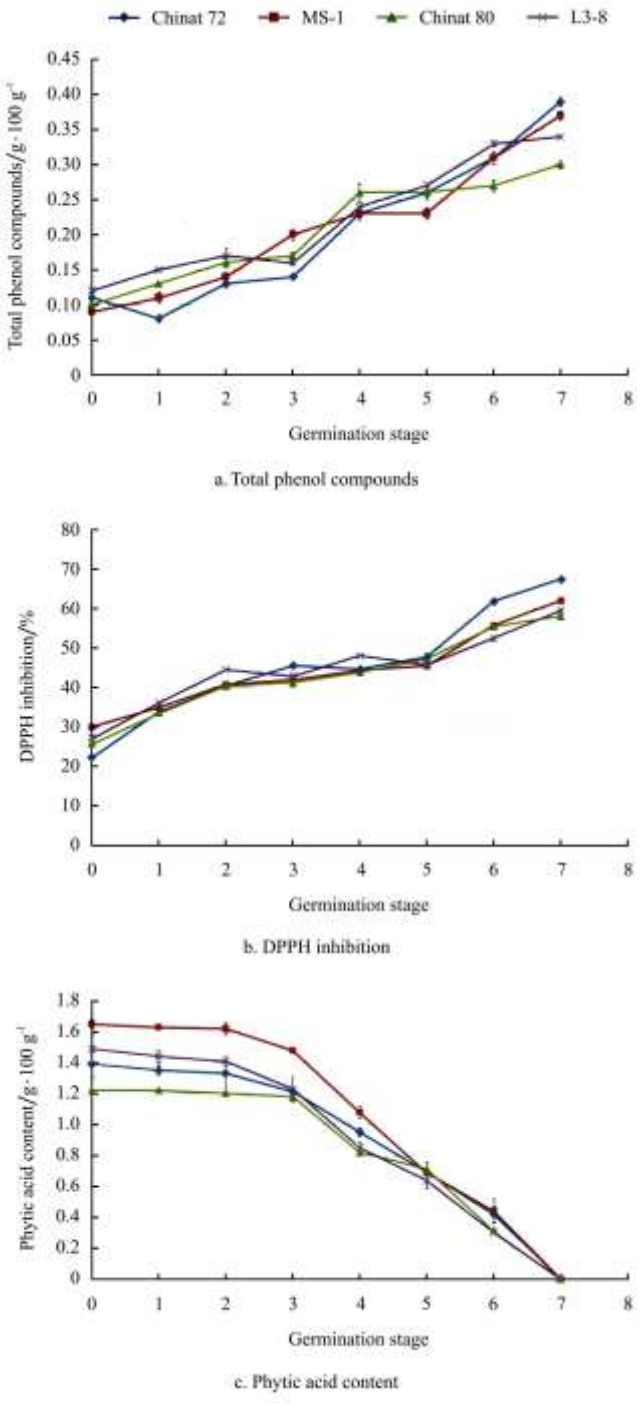

Figure 2 Effects of germination stage on total phenol compounds, DPPH inhibition, and phytic acid content. Germination stage includes: 0=before germination, 1=6 h of soaking, 2=23 h, 3=47 h, 4=71 h, and 5=77 h of sprouting, and 6=12 h, and 7=24 h of sunshine exposing

Phytic acid contents were dramatically decreased throughout the sprouting. Phytic acids decreased from

1.2%-1.6% to 0.7% after 77-h sprouting and dramatically decreased to 0.05% after being exposed to the sun for 24 h. Many studies proposed that the activity of endogenous phytase was the main factor leading to a reduction of phytic acid during soaking as a first step of germination[3,4,25] Phytase activity was found during sprouting of wheat, barley, rye, and oats because seeds need a lot of energy for their sprouting process[26] (Figure 2c).

3.3 Effects of germination and sprouting on total mineral contents and HCL extractability of mineral contents

The data indicated that Ca, K, and Na were the major minerals whereas Fe was a minor mineral constituent for both green gram and black gram cultivars. Before germination the Ca content varied from 28.26 mg/100 g to 32.25 mg/100 g, K content varied from 552.08 mg/100 g to 774.65 mg/100 g, Na content varied from 30.15 mg /100 g to 41.29 mg/100 g dry, and Fe content varied from 5.27 mg/100 g to 6.67 mg/100 g dry basis among four cultivars. It was found that after sprouting for 77 h and exposing to sunshine for 24 h, the total minerals significantly increased for all cultivars. It was noticed that increase of total iron content was higher than that of other minerals during sprouting and sunshine exposure (Table 1). The results were different from those of trace minerals of white bean cultivars which slightly increased with sprouting time as reported by Al-Numair et al[25].

Table 1 Effects of germination and sprouting for different periods on total minerals (mg/100 g, d.b.) of four mungbean cultivars

Cultivar	Stage of germination and sprouting	Total minerals (mg/100 g, d.b.)			
		Ca	K	Fe	Na
Chinat 72	Before germination	32.25±0.43*^{a}	774.65±1.21^{c}	5.75±0.81^{a}	41.29±1.24^{a}
	6 h-soaking	38.41±0.66^{b}	389.32±1.61^{a}	8.77±1.13^{b}	43.43±1.33^{b}
	23 h sprouting	42.17±0.27^{c}	553.20±3.94^{b}	10.82±1.66^{b,c}	42.77±0.28^{b,c}
	47 h sprouting	44.89±0.52^{d}	897.85±2.28^{d}	12.82±1.64^{c}	43.87±0.67^{c}
	71 h sprouting	50.23±0.52^{e}	1124.20±5.14^{f}	17.29±1.94^{d}	45.76±0.81^{d}
	77 h sprouting	57.32±0.26^{f}	1128.26±3.22^{f}	21.77±2.48^{e}	45.96±0.35^{d}
	12 h exposing to sunshine	67.50±0.34^{g}	1208.62±2.71^{e}	22.589±1.24^{e}	61.72±0.75^{e}
	24 h exposing to sunshine	70.06±0.51^{h}	1266.64±1.38^{g}	22.88±1.22^{e}	64.45±0.04^{f}
MS-1	Before germination	29.58±0.52^{a}	552.08±9.96^{a}	6.53±1.02^{a}	40.65±0.33^{a}
	6 h-soaking	32.37±0.48^{b}	647.76±0.22^{b}	7.84±0.65^{a,b}	42.34±1.29^{b}
	23 h sprouting	36.38±0.36^{c}	835.70±0.21^{d}	8.77±0.75^{b,c}	44.07±1.09^{b}
	47 h sprouting	40.21±0.37^{d}	866.03±3.56^{e}	9.669±1.38^{c}	47.97±2.24^{c}
	71 h sprouting	44.27±0.65^{e}	867.91±1.62^{e}	11.69±1.19^{d}	49.35±0.99^{c}
	77 h sprouting	48.26±0.37^{f}	903.16±6.15^{f}	13.76±1.04^{e}	54.21±5.30^{d}
	12 h exposing to sunshine	53.44±0.67^{g}	934.27±2.99^{g}	18.81±0.31^{f}	56.40±1.44^{d}
	24 h exposing to sunshine	54.29±0.58^{g}	1022.06±3.04^{h}	21.65±1.19^{g}	60.36±2.02^{e}
Chinat 80	Before germination	29.34±0.42^{a}	736.78±1.33^{a}	5.27±0.61^{a}	30.15±0.04^{a}
	6 h-soaking	35.23±0.23^{b}	745.46±3.42^{b}	6.06±0.88^{a,b}	34.34±3.34^{b}
	23 h sprouting	40.89±0.27^{c}	803.55±4.82^{c}	7.66±0.89^{b}	35.63±2.43^{b,c}
	47 h sprouting	43.21±0.37^{d}	850.23±2.56^{d}	11.56±1.29^{c}	35.88±1.42 ^{b,c}
	71 h sprouting	44.27±0.65^{e}	885.65±5.81^{e}	12.61±1.89^{c}	36.38±1.63^{c}
	77 h sprouting	48.00±0.75^{f}	895.24±3.70^{f}	14.54±0.54^{d}	38.61±1.17^{c}
	12 h exposing to sunshine	60.73±0.26^{g}	902.37±3.65^{g}	16.42±1.51^{d}	42.50±0.95^{d}
	24 h exposing to sunshine	61.42±0.35^{g}	921.55±2.87^{h}	18.48±1.95^{e}	47.19±0.88^{e}
LS	Before germination	28.26±0.79^{a}	632.01±0.44^{c}	6.67±0.97^{a}	35.04±0.41^{a}
	6 h-soaking	34.21±0.32^{b}	599.33±8.34^{b}	7.23±0.67^{a}	33.14±2.37^{a}
	23 h sprouting	38.14±0.61^{c}	549.47±3.08^{a}	10.45±1.65^{b}	39.26±1.14^{b}
	47 h sprouting	43.20±0.57^{d}	689.37±3.26^{d}	10.62±0.58^{b}	44.34±1.91^{c}
	71 h sprouting	53.35±0.54^{e}	690.34±1.14^{d}	11.78±2.19^{b,c}	47.95±1.22^{d}
	77 h sprouting	57.99±0.64^{f}	770.17±1.91^{e}	13.72±1.86^{c,d}	52.30±1.58^{e}
	12 h exposing to sunshine	70.10±0.37^{g}	796.55±3.35^{f}	14.65±1.27^{d}	53.02±1.92^{e}
	24 h exposing to sunshine	72.39±0.36^{h}	886.33±2.46^{g}	15.83±1.25^{d}	58.94±2.56^{f}

Note: *mean±SD. Values within a column with the same letter do not differ significantly (P=0.05).

Table 2 Effects of germination and sprouting for different periods on HCl extractability of the minerals (mg/100 g, d.b.) of four mungbean cultivars

Cultivar	Stage of germination and sprouting	HCl extractability of the minerals (mg/100 g, d.b.)			
		Ca	K	Fe	Na
Chinat 72	Before germination	32.27[(a)] ±0.43b	36.12 ±0.23[a]	24.37 ±0.38[a]	47.43 ±0.10[a]
	6 h-soaking	38.41 ±0.66[b]	32.26 ±0.54[b]	28.12 ±0.79[b]	50.66 ±0.22[b]
	23 h- sprouting	42.17 ±0.27[c]	34.27 ±0.66[c]	30.13 ±0.67[c]	63.35 ±0.22[c]
	47 h- sprouting	44.89 ±0.52[d]	46.36 ±0.59[d]	35.17 ±0.43[d]	69.47 ±0.24[d]
	71 h- sprouting	50.23 ±0.52[e]	58.34 ±0.44[e]	44.29 ±0.61[e]	75.64 ±0.22[e]
	77 h- sprouting	57.32 ±0.26[f]	59.02 ±0.31[f]	58.35 ±0.63[f]	80.53 ±0.35[f]
	12 h- exposing to sunshine	67.50 ±0.34[g]	65.42 ±0.41[g]	60.37 ±0.60[g]	86.41 ±0.33[g]
	24 h- exposing to sunshine	70.06[(h)] ±0.51[h]	68.22 ±0.44[h]	76.29 ±0.41[h]	95.66 ±0.05[h]
MS-1	Before germination	29.58 ±0.52[a]	34.05 ±0.70[a]	23.25 ±0.65[a]	33.53 ±0.35[a]
	6 h-soaking	32.37 ±0.48[b]	36.31 ±0.51[b]	25.21 ±0.43[b]	35.57 ±0.39[b]
	23 h- sprouting	36.38 ±0.36[c]	40.36 ±0.54[c]	29.12 ±0.23[c]	47.55 ±0.38[c]
	47 h- sprouting	40.21 ±0.37[d]	45.06 ±0.37[d]	31.90 ±0.64[d]	57.25 ±0.10[d]
	71 h- sprouting	44.28 ±0.65[e]	47.01 ±0.30[e]	37.14 ±0.23[e]	67.66 ±0.15[e]
	77 h- sprouting	48.26 ±0.37[f]	57.49 ±0.37[f]	44.11 ±1.39[f]	75.40 ±0.08[f]
	12 h- exposing to sunshine	53.44 ±0.67[g]	58.66 ±0.18[g]	46.29 ±0.49[g]	80.28 ±0.24[g]
	24 h- exposing to sunshine	54.29 ±0.58[g]	61.98 ±0.55[h]	68.04 ±0.32[h]	97.26 ±0.13[h]
Chinat 80	Before germination	29.34 ±0.42[a]	22.92 ±0.56[a]	24.23 ±0.66[a]	44.14 ±0.37[a]
	6 h-soaking	35.23 ±0.33[b]	37.50 ±0.35[b]	25.79 ±0.52[b]	46.97 ±0.28[b]
	23 h- sprouting	40.89 ±0.26[c]	41.78 ±0.56[c]	29.24 ±0.50[c]	51.24 ±0.46[c]
	47 h- sprouting	43.21 ±0.58[d]	44.67 ±0.35[d]	33.32[)] ±0.43[d]	52.93 ±0.42[d]
	71 h- sprouting	48.00 ±0.75[e]	47.88 ±0.32[e]	34.14 ±0.35[e]	59.25 ±0.48[e]
	77 h- sprouting	52.30 ±0.27[f]	57.49 ±0.37[f]	55.14 ±0.90[f]	63.96 ±0.53[f]
	12 h- exposing to sunshine	60.73 ±0.26[g]	58.20 ±0.22[f]	57.25 ±0.49[g]	69.07 ±0.37[g]
	24 h- exposing to sunshine	61.42 ±0.35[g]	61.61 ±0.47[g]	63.20 ±0.64[g]	77.12 ±0.34[h]
L3-8	Before germination	28.26 ±0.79[a]	35.49 ±0.54[a]	24.17 ±0.86[a]	47.24 ±0.91[a]
	6 h-soaking	34.21 ±0.32[b]	37.60 ±0.64[b]	27.40 ±0.57[b]	52.87 ±0.69[b]
	23 h- sprouting	38.14 ±0.61[c]	40.61 ±0.58[c]	29.41 ±0.41[c]	61.75 ±0.02[c]
	47 h- sprouting	43.20 ±0.57[d]	41.86 ±0.44[d]	30.32 ±0.38[c]	71.17 ±0.65[d]
	71 h- sprouting	53.35 ±0.54[e]	45.74 ±0.21[e]	35.34 ±0.44[d]	71.36 ±0.27[d]
	77 h- sprouting	57.99 ±0.64[f]	55.43 ±0.29[f]	47.08 ±0.19[e]	79.98 ±0.75[e]
	12 h- exposing to sunshine	70.10 ±0.37[g]	57.92 ±0.66[g]	64.03 ±0.78[f]	83.28 ±0.75[f]
	24 h- exposing to sunshine	72.39 ±0.37[h]	58.71 ±0.62[g]	64.47 ±0.24[g]	85.86 ±0.91[g]

Note: *mean ±SD. Values within a column with the same letter do not differ significantly (P=0.05).

HCL extractability of the minerals revealed that contents of Ca, K, Na, and Fe increased with the germination and sprouting for all cultivars (Table 2). There were slight variations of HCL extractability of Ca, K, Fe, and Na contents among green gram and black gram cultivars ranged from 28.26% to 32.27%, 22.92% to 36.12%, 23.25% to 24.37%, and 33.53% to 47.43% before germination, respectively. Among minerals observed in this study, calcium is considered to have a significant role in nutrition. Calcium helps in bone development and its deficiency can lead to improper development of bone in growing children leading to various deformities of the skeletal system. After sprouting and sunshine exposure, Ca extractable content of all cultivars significantly increased to the range of 54.29%-2.39%. Potassium is another significant mineral found in mungbean. It has various roles in metabolism and body functions and is essential for the proper function of all cells, tissues, and organs. The HCl extractable content of all cultivars significantly increased from the range of 22.92%-36.12%, before germination to 58.71%-68.22% after sprouting and sunshine exposure.

Mungbean seed is also a good source of iron and sodium. Iron, a trace mineral, is necessary for the

prevention of anemia while sodium, a macro mineral, is actually necessary to regulate your blood pressure and blood volume. Similar trend was found in the HCl extractability of Fe content in green gram and black gram cultivars. The Fe HCl extractable contents increased from the range of 23.25%-24.37% to 63.20%-76.29% as from 0 h of germination onwards. At the stage of 24 h sunshine exposure, the maximum value of Na HCl extractable content was found in Chinat 72 and MS-1 ranged from 95.66% to 97.26%, respectively whereas the Na HCL extractable content in Chinat 80 and L3-8 ranged from 77.12% to 85.86%, respectively.

3.4 Effects of exposing to sunshine on the chlorophyll contents of mungbean varieties

After exposing to sunshine for 12-24 h, the chlorophyll contents of Chinat 72, MS-1, Chinat 80, and L3-8 significantly increased to 8.99 mg/100 g, 7.15 mg/100 g, 2.69 mg/100 g, and 3.99 mg/100 g, respectively. It was noticed that the chlorophyll contents of green mungbean (Chinat 72 and MS-1) was higher than those of black mungbean cultivars (Chinat 80 and L2-8) which was related to the early development of cotyledons and the more vigor sprouts of those two green gram cultivars than the two black gram cultivars. Chlorophyll pigment concentration like other pigments; carotenoid and anthocyanins is being of interests in health promotion. Many efforts have been made to increase these phytochemicals in leafy green vegetables. Supplemental light of selected wavelengths could be strategically used to enhance nutritional value including phytochemical pigments and growth of baby lettuce grown under white light[27]. Lefsrud et al.[28] investigated chlorophyll and carotenoid pigments accumulations within kale leaves of differing maturity stages and concluded that chlorophyll pigments reached the maximum levels in the kale leaves between one and three weeks in age, and then decreased in response to leaf age.

4 Conclusions

It was concluded that sprouting periods had significant effects on nutrients and anti-nutrients of green gram and black gram cultivars. Results are in agreement with other sprouted grains and legume seeds, for example, wheat grain[29], Alfalfa, lentils, mungbeans and soybeans[30], which showed the improvement to various extents of their nutrients and essential minerals and vitamin during germination and sprouting. Our results demonstrated that the sprouted mungbean showed higher nutrients included vitamin C content, antioxidant activity, and important necessary mineral contents whereas phytic acid, anti-nutrient factor was untraceable when the seeds were sprouted until the true leaves appeared. High chlorophyll content was the additional nutrient value obtained by this cultural practice management. The health benefits from vegetables with chlorophyll have value and considered as blood builders. Thus, high chlorophyll mungbean sprout is the new alternative recommended to consumers for high nutrient components and inexpensive alternative to other green vegetables and can be considered as a valuable addition to the diet. Further studies are needed to investigate the new intentionally practice, for instance the supplemental light of selected wavelength during sprouting to enhance nutritional value of mungbean cultivars.

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