# Effect of Seed Germination Periods on Protein Profile, Enzymatic Activity, and Chemical Composition of Some Fenugreek (*Trigonella foenum-graecum* L) Types

**By**

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**Abstract:** Fenugreek seeds (*Trigonella foenum-graecum)* have a high medicinal and nutritional value. The germination of leguminous seeds could reduce the bitter taste and improve their nutritional quality. These improvements would provide further benefits in its use as an ingredient in food applications. This study aimed to investigate the effects of soaking and different germination periods on chemical composition and nutritional values of three types of fenugreek seeds. Moisture, protein, fat, and ash content of dry fenugreek seeds varied significantly among three different types of fenugreek. Saudi Arabian seeds had higher amounts of globulin than did the other two types. SDS-PAGE showed minor differences in the protein profile between Egyptian fenugreek and the other two types. High molecular weight of main protein bands were decreased during germination process, in the same time, the moderate and low molecular weight bands were increased. The soluble globulin and albumin proteins content were decreased while, glutenin content was increased. The Egyptian type had the highest percentage of the proteinase activity, free fatty acids and the soluble carbohydrates after 48 h of germination whereas; the Saudi Arabian type had the highest amount of reducing sugars. The reducing sugar and soluble carbohydrates in Saudi Arabian type increased by 6.5- to 7-fold and 12.5-fold, respectively, after 48h of germination.

**Keywords**: Free fatty acids, nutritional value, protein profile, reducing sugars, soluble protein.

## INTRODUCTION

Fenugreek (*Trigonella foenum-graecum L.*) is an annual, bloat-free, forage legume and is currently grown in the continents of west, south and south-east Asia, North Africa, Mediterranean Europe, Australia, and North America ( Basu et al., 2014).

Fenugreek stems, leaves, and twigs are used for cooking while leaves and seeds have high medicinal and nutritional values (Kumar, 2015). These values are included hypoglycemic and hypolipidemic effects. Fenugreek seeds are considered rich sources of carbohydrates, proteins, fats, mucilaginous fibre, vitamins A and C, iron, calcium, and other minerals (Billaud and Adrian, 2001; Sauvaire, and Ribes, 2003). Moreover, the seeds are rich in bioactive compounds, such as coumarin, saponins, nicotinic acid, sapogenins, phytic acid, and trigonelline (Dahanukar et al., 2000). Fenugreek is reported to prevent constipation, improve digestion, stimulate the liver and spleen, increase the level of erythrocyte insulin receptors, purify the blood, enhance pancreatic function, and increase appetite (Randhir et al., 2004; Ahmadiani et al. 2001).

The germination of seeds reduces their bitter taste and improves the nutritional quality of leguminous seed, including fenugreek, to varying degrees (Hooda and Jood, 2005; Abd El-Aal and Rahma, 1986; El-Shimi and Damir, 1984; El-Mahdy and El-Sebaiy 1982). The effect of the germination period on the nutrient and chemical composition of Egyptian and Indian fenugreek seeds has been investigated. An evaluation of chemical, physical, and sensory properties revealed that biscuits containing 20% germinated fenugreek flour after 12h of seed soaking were nutritionally improved in comparison to biscuits with the same amount of raw seeds or soaked fenugreek seeds (Hussein et al., 2011). Hooda and Jooda (2005) concluded that wheat and fenugreek blends can be used at 15%, 10%, and 20% in the preparation of bread, biscuits, noodles, and macaroni, without affecting their overall sensory quality, to increase the total protein, lysine, mineral, and dietary fibre content.

Baus (2006) observed significant effects of environmental and agronomic treatments on both the forage and seed yield of many legumes. Grieshop and Fahey (2001) analysed soybeans from Brazil, China, and the United States and observed a significant variation in the dry and organic matter, crude protein, and lipid content of the seeds. The aim of the present work was to investigate the effect of germination on the chemical composition and nutritional value of three types of fenugreek seeds.

## MATERIALS AND METHODS

### Samples collection

Three different samples of fenugreek seeds (Saudi Arabian, Indian, and Egyptian) were purchased from local markets in Riyadh, Saudi Arabia. The whole seeds were carefully cleaned and sieved to remove foreign materials before soaking in tap water at a ratio of 1:5 (w/v) for 12h at room temperature. The seeds were then germinated in Petri dishes between wet filter paper for 24, 36, and 48h at room temperature. At 12, 24, 36, and 48h, the seeds were milled in one batch by using an electric grinder and were mixed thoroughly; sub-samples were collected from different parts of each sample and stored in plastic cups at -20°C until use.

### Chemical composition

The moisture, total ash, protein and lipid content of the dry seeds were determined as described by the AOAC (1995). However, p*rotein fractions* (the albumin, globulin, glutenin, and prolamine) were estimated in the dry seeds and after 12, 24, 36, and 48h of germination following the method of Sauvaire et al. (1984).

### SDS-PAGE

For protein electrophoresis characterisation, the supernatants from the above extracts were dialysed against distilled water, precipitated overnight at 20°C by the addition of 9 volumes of cold acetone, and centrifuged at 16000 x g for 10 min at 4°C (Close et al., 1989). The air-dried protein pellets were dissolved in SDS-PAGE buffer, and equal quantities were fractionated by discontinuous SDS-PAGE using 12.5% polyacrylamide gels (Laemmli, 1970) and a PROTEN 11 electrophoresis unit (Bio-Rad) in accordance with the manufacturer's instructions.

**Proteinase activity**

Proteolytic activity was assayed by the azocasein method by measuring azocasein hydrolysis using sulphanilamide-azocasein (Sigma Chemical Co., St Louis, MO 63 178, USA) as a substrate according to Schokker and van Boekel (1997), using 2 g ground dry or germinated seeds (based on dry weight of the samples).

**Lipase activity**

The free fatty acid (FFA) content was measured as an indicator of the lipase activity in the fenugreek seeds. The native lipase activity was estimated using a modification of the titrimetric method (Khor et al., 1986). The assay mixture contained 5g olive oil/gum Arabic (Fisher Scientific Co. Fair Lawn, New Jersey, USA) emulsion substrate and 5g ground dry or germinated seeds (based on dry weight). The lipase activity was expressed as the percent FFAs liberated as oleic acid according to the equation: FFA% = (NaOH volume (sample – blank)*N*MW of oleic acid*100 / sample weight)*1000

**Soluble carbohydrates and reducing sugars**

The soluble carbohydrates were extracted from the dry and germinated seeds in accordance with Leprince et al. (1990) then the total soluble sugars were determined using the method of Jermyn (1975). The reducing sugars were assessed using DNS methods: the sample was mixed with 3 mL DNS reagent in boiling water for 5 minutes, and the final volume was adjusted to 10 mL after cooling to room temperature; the absorbance was then measured at 540 nm.

**Statistical analysis**

All of the chemical analyses were performed using three replicates. The results were subjected to an analysis of variance (ANOVA), and the means were compared using Tukey’s test ($p<0.05$) between pairs of data (Clewer and Scarisbrick, 2001) using Minitab 11 software.

## RESULTS AND DISCUSSION

**Chemical composition**

The moisture, fat, and ash content of the dry fenugreek seeds varied ($p<0.05$) among the three seed types. The Egyptian seeds had the highest moisture (7.1%) and fat (7.47%) content, but the ash content (2.91%) was significantly lower than in the Indian type (3.52%). The Saudi Arabian type exhibited the lowest moisture (3.86%), fat (6.47%), and ash (1.93%) content, with the differences being significant. The Saudi Arabian fenugreek seeds had ($p<0.05$) protein content 31.7% than the Egyptian seeds (29.39%); however, the Indian type exhibited a protein percentage (28.07%) that was similar to the Egyptian type. Our results for the composition of Egyptian and Indian fenugreek seeds were slightly different from those of previous reports (El-Shimi and Damir, 1984; Hooda and Jooda, 2005; Abd El-Aal and Rahma, 1986). Additionally, Hooda and Jooda (2005) reported that the seeds of an early-bunching Indian fenugreek variety had 13% moisture content, which we consider is too high for dry seeds.

**Soluble proteins**

Legume seed proteins consist of storage proteins, structural proteins, and biologically active enzymes. Storage proteins can be categorised into globulins, albumins, prolamines, and glutenin (Burstin, 2011). These proteins were determined in the dry fenugreek seeds and during germination (29.1%-31.9%). Although the amount of total protein did not significantly differ during the germination of the three fenugreek types, there were significant differences in the globulin, albumin, and glutenin content. The percentage of these proteins based on the dry weight was significantly affected by germination, though there were no significant interactions between the seed type and germination with regard to these proteins. The albumins and globulins were ($p<0.05$) decreased by the germination process because the dry seeds had the highest percentages, but the lowest percentages were estimated in the 48h-germinated seeds (Fig. 1). Overall, the three seed types had similar albumin percentages (12.59-12.71%) after 48h of germination; the dry seeds showed 14.49-14.95% albumin. In contrast, the glutenins were increased ($p<0.05$) during germination for all the seed types. After 48h of germination, the Egyptian seeds had the lowest globulin content (6.55%), whereas, the Saudi Arabian seeds had ($p<0.05$) the highest glutenin and globulin content (9.99 and 8.33%, respectively). The Indian fenugreek seeds had the significantly lowest globulin content (6.54%). The variations in soluble proteins of the three fenugreek types during germination were correlated with the percentage of these proteins in the dry seeds (Fig. 1).

These results regarding total proteins, albumin, globulin, and glutenin are in agreement with the results of Abd El-Aal and Rahma (1986); however, Hooda and Jooda (2005) observed an increase in the fenugreek seed protein content after 60h of germination. The increased amount of glutenin soluble protein in germinated fenugreek seeds was in agreement with the results of Maneemegala and Nandakumar (2011) for *Vigna* spp. The reduction in the amount of albumin and globulin could indicate the mobilisation of reserved proteins and the synthesis of new proteins necessary for embryo growth. This change in the composition of proteins and the soluble fraction of germinated fenugreek seeds could affect the functional properties of the proteins isolated, as previously reported by James and Jayasena (2012). Nutritionally, albumins are important source for essential amino acids, however, glutenin, which are rich in glutamine, asparagine, arginine, and proline, are low in the nutritionally important amino acids lysine, tryptophan, and methionine (Žilić et al., 2011). The stability of the total protein content during germination and the reduction in albumins and globulins, considered approximately 22-25% of dry seeds, might reflect the hydrolysis of these proteins into their component amino acids. Graham (2008) emphasised that storage proteins are the major source of amino acids for embryo development, and the amino acids released are used to synthesise the necessary enzymes and components for germination. The increase in glutenin after 48h of germination would benefit fenugreek flour, with the significant reduction in albumins and globulins indicating a high content of essential amino acids in the flour.

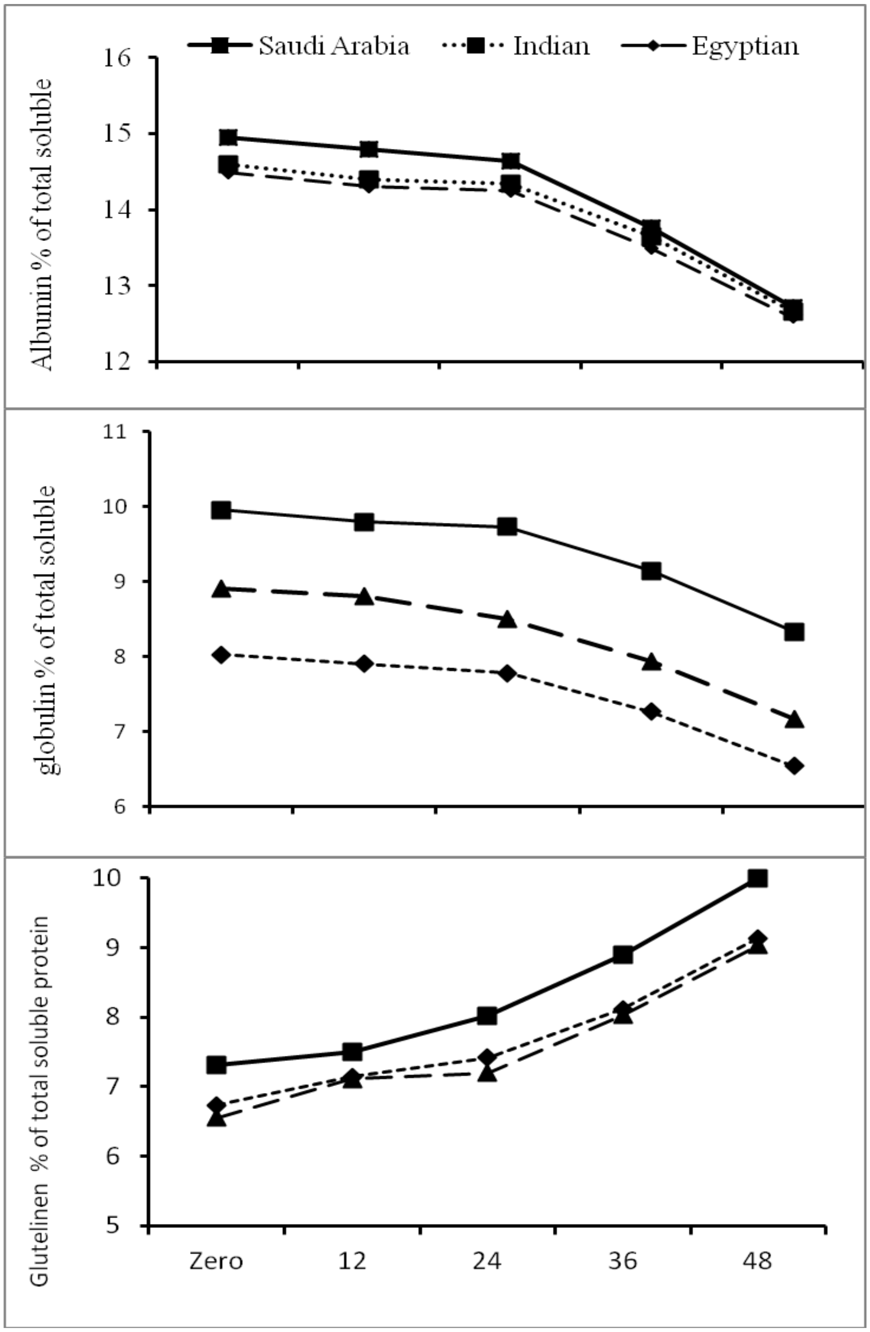


**Fig.1 :** Effect of germination period on the percentage of soluble albumin, glutenin and globulin of Saudi Arabian, Indian and Egyptian fenugreek seeds

**Electrophoretic analyses (SDS-PAGE)**

Although the total protein content showed little variation during the germination process, the SDS-PAGE protein profile showed significant alterations (Fig. 2). The three types of seeds showed more than 25 individual protein bands with molecular weights ranging from approximately 10 kD to more than 100 kD. Clear similarities were noted between the protein fractions of the dry seeds of the three types, particularly among the most main bands. Moreover, no differences were observed among the protein bands of the dry and 9- and 12h-germinated seeds. However, after 12h, minor qualitative differences in the protein composition between the Egyptian seeds and the other types were observed. During germination, the high-molecular weight protein bands decreased rapidly after 48h imbibition, a time corresponding to seedling establishment. Unlike the other two seed types, the Egyptian seeds exhibited high degradation in main protein bands after 36 or 48h of germination. The high molecular weight of protein bands decreased with increasing germination time, with a concomitant increase of the moderate and low molecular weight bands.

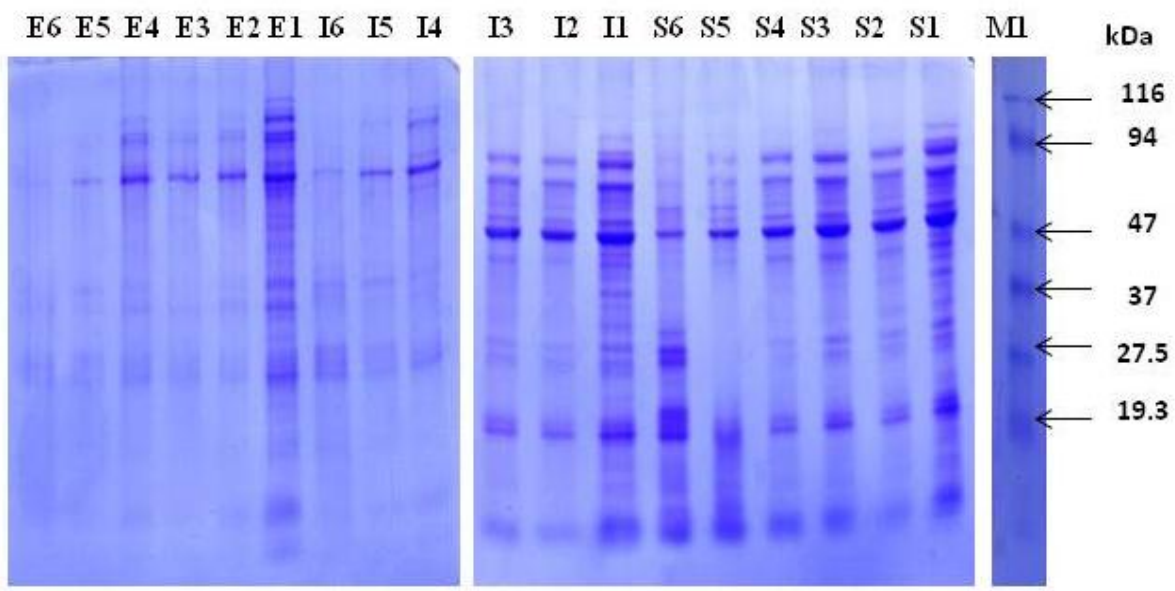


**Fig. 2:** SDS-PAGE of fenugreek proteins, M1, protein marker. S1-S6 are Saudi Arabian seeds after 0, 9, 12, 24, 36 and 48h of germination respectively, I1-I6 are Indian seeds after 0, 9, 12, 24, 36 and 48h of germination respectively, and . E1-E6 are Egyptian seeds after 0, 9, 12, 24, 36 and 48h of germination, respectively.

During the germination process, protein degradation usually occurs because the plant uses the storage proteins as a source of nitrogen and carbon for the synthesis of biomolecules (Rumiyati et al., 2012; Ahmad et al., 1995). The significant changes in the protein profile of the fenugreek seeds, particularly the Egyptian type, in the final stage of germination correlating with the significant reduction in albumins and globulins could be related to protein hydrolysis, synthesis, and mobilisation of proteins. Hsu et al. (1985) studied the protein profile during legume germination and observed a progressive decrease in large molecular weight of protein and the formation of new low molecular weight proteins. Cotyledons are the main storage protein in legume seeds, and an increase in the amino acid content, with transport to the growing seedling, has been observed following protein hydrolysis (Shimizu and Mazzafer, 2000).

**Proteinase activity (PA)**

Both the seed type and period of germination had a significant effect on PA, and there was a significant interaction between these two factors. The PA of the dry fenugreek seeds varied significantly at 0.353, 0.470, and 0.543 µmol/min for the Saudi Arabian, Indian, and Egyptian types, respectively (Table 1). The PA increased linearly with the germination period for all the fenugreek types. Overall, the highest PA was observed after 48h of germination for the Egyptian type (1.200 µmol/min), in comparison of the Saudi Arabian and Indian types (0.909 and 1.005 µmol/min, respectively) (Table 1). The gradual increase of PA up to 48h of germination could suggest higher PA after a longer germination period.

Table 1. Effect of germination period on the proteinase activity (µmol/min) of three different types of fenugreek seeds.

| Germination Period (h) | Seed type | | | |
|---|---|---|---|---|
| | Saudi Arabian | Indian | Egyptian | Mean ± SD |
| 0 | 0.353±0.003 | 0.470±0.007 | 0.543±0.009 | 0.455±0.083[e] |
| 12 | 0.498±0.043 | 0.595±0.002 | 0.825±0.026 | 0.639±0.147[d] |
| 24 | 0.511±0.018 | 0.673±0.044 | 0.850±0.038 | 0.678±0.149[c] |
| 36 | 0.762±0.023 | 0.698±0.003 | 0.954±0.080 | 0.805±0.122[b] |
| 48 | 0.909±0.043 | 1.005±0.005 | 1.200±0.032 | 1.038±0.131[a] |
| Mean ± SD | 0.607±0.209[c] | 0.688±0.184[b] | 0.874±0.222[a] | |

Values are the means of three replicates. The means within a row or a column followed by different letters are significantly different at a 5% level according to the LSD test.

These results are in agreement with those of Shipard (2005) who suggested that many enzyme inhibitors are effectively neutralised during germination while the activity of plant digestive enzymes is enhanced. The correlation between the high activity of PA and the reduction in albumins and globulins coinciding with protein depletion in the Egyptian fenugreek seeds revealed the involvement of proteinases in germinating fenugreek seeds. The mobilisation of storage proteins is initiated by endoproteinases, which convert water-insoluble storage proteins (globulins) into soluble peptides (albumins), which can be further hydrolysed to amino acids by exopeptidases (Shewry et al., 1995 and Ramakrishna and Rao, 2005).

**Lipase activity**

The FAA content was measured as an indicator of the lipase activity in the fenugreek seeds. The FFA content of Indian, Saudi Arabian and Egyptian types were 5.69, 6.04 and 8.12%, respectively. These results indicate partial lipase activity in the dry seeds, which was positively correlated with the seed moisture content of the three seed types. Both seed type and germination period significantly affected FFA content. The FFA content in all the seed types gradually increased following germination, with only the Egyptian seeds showing a significant increase in FFA content after 12h of germination (Table 2). A remarkable increase (more than 50%) in FAAs was observed after 48h of germination compared to 36h. Overall; the Egyptian seeds had the highest significant amount of FAAs (17.49%) after 48h of germination when compared with the two other types, which had similar values of 15.44 and 15.32%.

Table 2: Effect of germination period on the free fatty acid% of three different types of fenugreek seeds.

| Germination Period (h) | Seed type | | | |
|---|---|---|---|---|
| | Saudi Arabian | Indian | Egyptian | Mean ± SD |
| Zero | 6.04±0.01 | 5.69±0.30 | 8.12±0.25 | 6.61$^{d}$±1.16 |
| 12 | 7.14±1.03 | 6.79±0.47 | 10.43±0.81 | 8.12$^{c}$±1.87 |
| 24 | 7.33±0.63 | 6.98±0.33 | 10.76±0.06 | 8.36$^{c}$±1.85 |
| 36 | 8.75±0.23 | 9.92±0.58 | 11.41±0.44 | 10.03$^{b}$±1.21 |
| 48 | 15.44±0.44 | 15.32±0.93 | 17.49±1.15 | 16.08$^{a}$±1.31 |
| Mean ± SD | 8.94$^{b}$± 3.52 | 8.94 $^{b}$±3.64 | 11.64$^{a}$±3.29 | |

Values are the means of three replicates. The means within a row or a column followed by different letters are significantly different at a 5% level according to the LSD test.

Abd El-Aal and Rahma (1986) estimated that an approximately 4-fold concentration of FFAs coincided with a reduction in triacylglycerols after 5 days of germination in Egyptian fenugreek seeds. The increase in FFAs prior to seed germination is identical conditions reflects the metabolic changes associated with germination and early growth (Eze and Chila, 2010). Increasing lipolytic activity during germination results in the hydrolysis of triacylglycerols to glycerol and the constituent fatty acids (Chavan and Kadam, 1989; Akanni et al. 2005).

**Total soluble carbohydrates and reducing sugars**

Both the seed type and germination period had significant effects on the total soluble carbohydrates and reducing sugars of fenugreek seeds; however, there was no interaction ($p<0.05$) between the two factors. The Egyptian seeds had the highest total soluble carbohydrates, while the Saudi Arabian seeds had the highest amount of reducing sugars at the same germination time. The total soluble carbohydrates and reducing sugars increased linearly during germination to achieve the highest value after 48h, with the reducing sugars increasing by 6.5-7-fold depending on the type of seed (Table 3). Nevertheless, the increase in total soluble carbohydrates during the same period was more than 20% indicating the conversion of non-reducing sugars to reducing sugars during germination (Table 3).

These results are in agreement with El-Shimi et al. (1984), who found that the total sugar content of fenugreek seeds increased after 2 days of germination. In contrast, Abd El-Aal et al. (1986) and Hooda and Jooda (2004) estimated a reduction in total carbohydrates during germination. Seed germination is a series of steps that occur prior to sprout emergence, with a massive breakdown of the seed reserve components by different enzymes and transport of the products to support the growth and development of the seedling. The increase in total soluble sugars might be due to the mobilisation and hydrolysis of seed polysaccharides, which can be further hydrolysed by α-amylase to starch degradation products comprising a complex mixture of sugars that provide energy for the growth of roots and shoots (Veluppillai et al., 2009). Suda and Giorgini (2000) have suggested that the increase in soluble sugars in the rice embryo mostly originates from the catabolism of lipids. There was a reduction in some soluble proteins and increasing proteinase activity in the fenugreek seeds during germination that coincided with increasing total soluble carbohydrates and reducing sugars. Another source of sugars in the endosperm may be from amino acids derived from the breakdown of proteins occurring at the same time.

Table 3: Effect of germination period on the soluble carbohydrate and reducing sugar content of three different types of fenugreek seeds.

| Germination Period (h) | Total soluble carbohydrates | | | | Reducing sugars | | | |
|---|---|---|---|---|---|---|---|---|
| | Seed type | | | | | | | |
| | Saudi Arabian | Indian | Egyptian | Mean ± SD | Saudi Arabian | Indian | Egyptian | Mean ± SD |
| 0 | 40.9±1.8 | 41.6±2.0 | 42.4±1.8 | $41.6^{d}$ ± 1.7 | 0.71±0.09 | 0.69±0.02 | 0.59±0.06 | $0.66^{d}$ ± 0.80 |
| 12 | 47.5±0.3 | 48.6± .6 | 49.1±1.3 | $48.4^{c}$ ± 1.0 | 2.14±0.18 | 2.08±0.15 | 1.97±0.14 | $2.06^{c}$ ± 0.15 |
| 24 | 48.3±0.9 | 50.6±1.0 | 50.5±1.8 | $49.8^{bc}$ ±1.6 | 2.27±0.12 | 1.94±0.08 | 2.06±0.17 | $2.09^{c}$ ± 0.18 |
| 36 | 49.9±1.5 | 51.5±0.8 | 52.0±1.0 | $51.1^{ab}$ ± 1.4 | 3.76±0.18 | 3.51±0.27 | 3.35±0.31 | $3.55^{b}$± 0.29 |
| 48 | 51.1±2.2 | 52.7±0.6 | 53.1±1.2 | $52.3^{a}$± 1.6 | 4.68±0.25 | 4.47±0.56 | 4.13±0.48 | $4.43^{a}$ ± 0.46 |
| Mean ± SD | $47.5^{b}$±3.9 | $49.0^{a}$±4.2 | $49.4^{a}$±4 | | $2.7^{a}$±1.4 | $2.5^{a}$±1.4 | $2.4^{a}$±1.3 | |

Values are the means of three replicates. The means within a row or a column followed by different letters are significantly different at a 5% level according to the LSD test

## CONCLUSIONS

The chemical composition, total protein, fat, ash, and moisture content of fenugreek seeds varied among the three seed types analysed and were significantly altered during germination. The total soluble carbohydrates, reducing sugar content, and proteinase and lipase activities were increased, whereas albumins and globulins were markedly decreased during germination when compared to the dry seeds. The highest content of FAAs and total soluble carbohydrates was found in the Egyptian seeds after 48h of germination, and the highest content of reducing sugars was found in the Saudi Arabian seeds after the same germination period. The results of this study suggest that 48h of germination improves the nutritional profile of fenugreek seeds. Importantly, an increase in the total soluble carbohydrate and FFA content and a decrease in the albumin and globulin content would improve the nutritional profile of foods containing germinated fenugreek seeds. The changes in the protein profile possibly affect the functional properties of the seed proteins.

## ACKNOWLEDGEMENT

This research was partially funded by the Saudi Society for Food and Nutrition (SSFN) and the authors wish to thank the director of SSFN for this fund.

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# تأثير فترات الإنبات على صورة البروتين والنشاط الإنزيمي والتركيب الكميائي لبعض أصناف بذور الحلبة

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**الملخص:** لبذور نبات الحلبة (*Trigonella foenum-graecum*) قيمة طبية وتغذوية عالية. وتؤدي عملية إنبات بذور الحلبة إلى تقليل الطعم المر وتحسين الجودة الغذائية لها مما يزيد من تطبيقاتها في مجال الغذاء. تهدف هذه الدراسة إلى دراسة تأثير عملية النقع ومدة الإنبات على التركيب الكيميائي والقيمة التغذوية لثلاث أنواع مختلفة من بذور الحلبة. كان محتوى الرطوبة والبروتين والدهن والرماد مختلف اختلافاً معنوياً بين الأصناف الثلاثة من يذور الحلبة الجافة. واحتوت بذور الحلبة السعودية على كميات مرتفعة من الجلوبيولين أكثر من النوعين الآخرين. وأظهرت صورة البروتين بواسطة الهجرة في المجال الكهربي (SDS-PAGE) اختلافات طفيفة بين الحلبة المصرية والنوعين الآخرين. وقد انخفضت حزم البروتينات الرئيسة ذات الوزن الجزيئي المرتفع أثناء عملية الإنبات وفي نفس الوقت زادت حزم البروتينات ذات الوزن الجزيئي المتوسط والمنخفض. وانخفض محتوى الجلوبيولين والألبيومين بينما ارتفع محتوى الجلوتينين. وسجل صنف الحلبة المصرية بعد ٤٨ ساعة من الإنبات أعلى نسبة في نشاط الإنزيمات المحللة للبروتينات والأحماض الدهنية الحرة والكربوهيدرلت الذائبة. بينما سجل صنف الحلبة السعودية أعلى نسبة فى السكريات المختزلة. وقد زادت نسبة الكربوهيدرات الذائبة وكذلك نسبة السكريات المختزلة أثناء عملية الإنبات في صنف الحلبة السعودية بمقدار ٦,٥–٧ أضعاف و ١٢ ضعف على التوالي بعد ٤٨ ساعة من الإنبات.