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# Effect of Salinity on Seed Germination, Growth and Amino Acid Content in Fenugreek (*Trigonella faenum-graecum* L) Sprouts

CrossMark

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**Abstract:** This study was conducted to evaluate the effect of salt stress on amino acid profile and proximate analysis of fenugreek germination samples of two varieties: Giza 2 and Giza 30 for three days. The germination of sterilized fenugreek seeds was conducted using tap water and NaCl solution (2,000 ppm) where higher salt concentration lowered fenugreek germination quality. The germination of fenugreek seeds using tap water or brine increased the crude protein in both dry Giza 2 and Giza 30 seed varieties. Furthermore, sedative sprouts of Giza 30 recorded the highest crude protein (30.60%), fat (6.20%) and energy (333.13 kcal/g) while the use of sterilized Giza 2 seed variety recorded a higher percentage of carbohydrates (43.3%), fiber (8.10%) and ash (6.60%). Alternatively, although amino acid profiles indicated that aspartic acid and proline were most abundant in fenugreek sprouts, sulfur amino acids (cysteine and methionine) demonstrated the least values of the two varieties. Adopting saline water in germinating fenugreek seeds for the two Giza 2 and Giza 30 varieties decreased all amino acid contents compared with those of tap water treatment.

## 1 Introduction

The rapid increase in many countries' population is considered one major global obstacle, resulting in limited agricultural land, water, and food resources. Therefore, accurately defining the amount and quality of protein required to meet human nutritional needs is becoming increasingly important. As a result, from a nutritional viewpoint, studies considered the high quality of sprouts for health due to their rich nutritional profile (>50% protein) (Abdallah 2008). Fenugreek (*Trigonella foenum-graecum* L) is a small seed and a member of the legume family, growing in different world locations. The major countries that produce the seeds are India, Turkey, Ethiopia, and Egypt. Specifically, although the seed of fenugreek has been widely used in different countries worldwide, including medically or as human food, its use for other purposes is still under research. Besides, while this seed contains a low concentration of some steroids, its importance comes from its high protein content. Therefore, it is used during the manufacture of commercial steroids.

Fenugreek seeds were first cultivated thousands of years ago in Egypt before building the pyramid. Subsequently, Abdallah (2008) reported the germination and production of its sprouts as an old habit, which adapted to three days of sprouting as its optimum period for seed growth in the dark to produce etiolated sprouts. Since seed sprouts are the most common, usually eaten raw in salads, it has been recommended that before sprouting, seeds be disinfected by washing in a solution of 2% calcium hypochlorite ($CaCI_2O_2$) (Lang et al 2000, El-Gebaly et al 2018). Furthermore, although among the favors of this crop is its exclusive use as a food extra to improve seasoning and color, fenugreek additionally alters the surface of the food. Researchers also reported that while its green leaves and seeds are multipurpose, with 100 g seeds being reported to give more than 65% of dietary fiber because of its high fiber content, it can change food surfaces. Also, it is notable for gum. Additionally, it has been proposed as the most encouraging therapeutic spice, known to exhibit dietary benefits. For example, studies reported that it functions as an antidiuretic or anticarcinogenic agent in different therapeutic applications. Also, it could have hypocholesterolemic, hypoglycemic, cell reinforcement, antibacterial, gastric energizer activity, hepatoprotective, and antianorexic impact (Srinivasan 2006). Similarly, in present-day food innovation, it is used as a food stabilizer, cement, and emulsifying specialist because of its fiber, protein, and gum content. Interestingly, its protein is viewed as more dissolvable (91.3%) at an antacid pH of 11 (Meghwal and Goswami, 2012).

Seed germination is one of the most critical periods for determining the quality of crops subjected to salinity (Yildirim et al 2002). In dry and semi-dry cultivation areas, saltiness is the most genuine abiotic stress that influences pressure and changes biochemical reactions in a plant (Khan et al 2013). Therefore, plants can oppose osmotic pressure by expanding the size of their root framework or decreasing their leaf region (Guo et al 2002 as cited by El-Gebaly et al 2018, Wang 2005). Also, a study reported that a higher salinity level decreased seed germination, resulting in nonuniform emergence (Yang 2001). Nevertheless, germination also significantly increases the micronutrient and phytonutrient contents of all selected seeds, thus markedly increasing the nutritional value of seeds on sprouting. This finding ultimately signifies that sprouts should be enhanced to improve agricultural productivity, making them easy to use by low-income families (Wagner et al 2013). Based on the above background, our investigation determined the changes occurring in four amino acid fractions during the sprouting of fenugreek seeds using saline water. Then, we investigated the amino acid quality and chemical composition of fenugreek Giza 2 and Giza 30 sprouts. Subsequently, this study investigated the effect of sprouting using tap water and NaCl solution. The study's outcomes are proposed to be important for preventing protein-energy malnutrition in Egypt and for etiolated fenugreek sprouts potential application as a functional food ingredient.

## 2 Materials and Methods

This study was completed at the Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo, the Regional Center for Food and Feed (RCFF), and the Agricultural Research place (ARC), Giza, Egypt, in 2021.

### 2.1 Materials

Egyptian fenugreek seeds: Giza 2 and Giza 30 varieties (*Trigonella foenum-graecum* L) were obtained from the Agriculture Research Center Crops Research Institute, Giza, Egypt. Then, the seeds were cleaned of all impurities before sprouting. Also, calcium hypochlorite (70%) and sodium chloride (2,000 ppm) were obtained from the Ahram Company, Cairo, after which mixtures were prepared using tap water (water authorized for human drinking) (tap water + 2,000 ppm NaCl).

Treatments (12 groups) = (two fenugreek seed varieties: Giza 2 and Giza 30) × two saline-concentrated solutions (tap water + 2,000 ppm NaCl) × three determinants.

### 2.2 Methods

As described by El-Gebaly et al (2018), fenugreek seeds were sterilized in 2% calcium hypochlorite for 20 min. Then, the seeds (5 g) were placed in a 0.7 L capacity glass jar (household version) containing tap water (100 ml), which was then covered with cheesecloth and secured using a rubber band. Subsequently, the jar was stored in the dark for 12 h to allow soaking of seeds at room temperature, after which the soaking water was discarded and the seeds rinsed again with water in the jar (approximately 1.0 min.). Next, the rinse water was discarded, and the jar was inverted at a 45° angle and stored at room temperature in the dark for 12 h. The rinse-store procedure was repeated thrice until 72 h of cumulative time (harvest time) in tap water or 2,000 ppm NaCl solution.

### 2.3 Chemical composition

Fenugreek sprouts were dried at 60°C for 72 h and then ground and passed through a 40 mesh sieve. Samples were subsequently stored at 5°C until analysis. Sample contents were determined according to the previously established guidelines (AOAC 2012a, El-Gebaly et al 2018). All chemical composition analyses were conducted at the RCFF and the Agriculture Research Center (ARC).

### 2.4 Proximate analysis

The samples' total protein, ash, crude fiber, and lipid contents were determined according to the established guidelines (AOAC 2012a). Subsequently, while total carbohydrates were determined by subtracting, energy value was calculated using a formula by Chinma and Igyor (2007):

Energy value = [(9 × fat) + (4 × carbohydrate) + (4 × protein)]

### 2.5 Amino acid analysis

According to a previously established guideline, a total amino acid evaluation was performed (AOAC 2012b). Biochrom v.30 was used for the analysis.

### 2.6 Estimation of nutritional quality

Sprouts' nutritional quality was determined using amino acid profiles, and essential amino acid

$$EAAI = \sqrt[9]{\frac{[Lys\ x Threo\ x\ Val\ x\ Meth\ x\ Isoleu\ x\ leu\ x\ Phynylal\ x\ Histi\ x\ Trypt]a}{[Lys\ x Threo\ x\ Val\ x\ Meth\ x\ Isoleu\ x\ leu\ x\ Phynylal\ x\ Histi\ x\ Trypt]b}}$$

(EAAI) was calculated according to a method previously cited by Ijarotimi and Keshinro (2013), using the equation:

*100

where n = number of essential amino acids, $a$ = the concentration of essential amino acids [lysine, isoleucine, valine, threonine, leucine, phenylalanine, histidine, and methionine] in the test sample, and $b$ = the content of similar amino acids in a standard protein (%) (Ijarotimi and Keshinro 2013).

Subsequently, the biological value (BV) was calculated according to (Oser, 1959) using the equation:

BV= 1.09 * essential amino acid index (EAA) –11.7

However, the nutritional index (NI) percentage of sprout samples was determined using the equation by Ijarotimi and Keshinro (2013).

$$NI\ (\%) = \frac{EAAI \times \%protein}{100}$$

Finally, the protein efficiency ratio (PER) was estimated according to Alsmeyeret al 1974, using the equation given below:

PER = [−0.468+0.454 (LEU)−0.105 (TYR)]

The formula was also used to calculate the amino acid scores (%).

### 2.7 Estimation of other protein quality parameters

From the results obtained for the amino acid profile in seed sprouts, the total amino acids (TAA), total essential amino acids (TEAA), total nonessential amino acids (TNEAA) total acidic amino acids (TAAA), total aromatic amino acids (TATAA), total sulfur amino acids (TSAA), and total basic amino acids (TBAA) were estimated. Subsequently, the ratios (TEAA/TAA) and (Cys/TSAA) were also calculated.

### 2.8 Statistical analyses

Data were statistically analyzed by analysis of variance, using a completely randomized design, and the least significant difference at 0.05 levels, according to a method described previously by Snedecor and Cochran (1980).

## 3 Results and Discussion

### 3.1 Effect of sprouting using saline water on sprout characteristics.

**Table 1.** shows a significant difference (≤ 0.05) between sterilized three-day-old etiolated fenugreek sprout seeds.

The sprout length (6.79 cm), radical length (2.87 cm), and heavier fresh weight (0.89 and 0.121 mg) were observed when using tap water for sprouting, as compared with using 2,000 ppm NaCl. Interestingly, the interaction effect between seed sterilization and NaCl concentrations showed higher sprout length characteristics with sterilized seeds than with tap water. However, the sterilized seeds with tap water recorded heavier sprouts' fresh weight. Also, investigations revealed that increased NaCl concentrations

**Table 1.** Effect of NaCl concentration in sprouting solution of etiolated fenugreek sprouts sterilized seeds

| Variety (Sterilization) | Salinity (S) | Radical Length (cm) | Sprout length (cm) | 10 fresh sprout's weight | 10 dry sprout's weight |
|---|---|---|---|---|---|
| Giza 2 | Tap water | $3.05^{a}$ | $7.40^{a}$ | $0.92^{a}$ | $0.123^{a}$ |
| | 2000 ppm | $2.61^{ab}$ | $5.86^{b}$ | $0.86^{a}$ | $0.118^{a}$ |
| | Mean | $2.83^{a}$ | $6.63^{a}$ | $0.88^{a}$ | $0.122^{a}$ |
| Giza 30 | Tap water | $2.68^{ab}$ | $6.19^{a}$ | $0.86^{a}$ | $0.121^{a}$ |
| | 2000 ppm | $2.27^{b}$ | $5.36^{b}$ | $0.83^{a}$ | $0.117^{a}$ |
| | Mean | $2.48^{a}$ | $5.77^{b}$ | $0.84^{a}$ | $0.117^{a}$ |
| Average | Tap water | $2.87^{a}$ | $6.79^{a}$ | $0.89^{a}$ | $0.121^{a}$ |
| | 2000 ppm | $2.44^{b}$ | $5.61^{b}$ | $0.84^{a}$ | $0.119^{a}$ |
| L.S.D (0.05) | V | 0.39 | 0.78 | 0.15 | 0.018 |
| | S | 0.39 | 0.78 | 0.15 | 0.018 |
| | C x S | 0.55 | 1.10 | 0.21 | 0.026 |

On average, this experiment was conducted in duplicates.
Means in each column followed by the same letter are not significantly different at the 5% level ($p \leq 0.05$).

(2,000 ppm) recorded the lowest sprout characteristics. Similar results on the reduction effect of sprout characteristics with higher concentrations have been reported previously by Ahmed et al 2018.

### 3.2 Proximate analysis of sprouts

The proximate analysis results of etiolated fenugreek sprout from sterilized Giza 2 and Giza 30 variety seeds compared with their dry seeds are summarized in **Table 2**. Investigations revealed that while the Giza 30 sterilized seeds' mean recorded higher moisture content (10.27%), crude protein (30.60%), lipids (6.20%), ash (6.50%), and energy (333.13 kcal/g) values, sterilized seeds in the Giza 2 variety recorded higher carbohydrate (43.3 %) and fiber (8.10%) values. Alternatively, treatment with saline water (2,000 ppm NaCl) before sprouting decreased sprout carbohydrate percentage and data, which was more pronounced using sterilized seeds. However, using tap water or saline water increased the crude protein value compared with dry seeds, with more noticeable results observed when tap water was used for sprouting and recording more than an 84.92% increment in protein compared with dry seeds. Also, our investigations proposed that since no nitrogen source was added externally to the water and saline solution used for irrigation during sprouting, the highest crude protein values in three-day-old etiolated fenugreek sprouts could be because of a lessening in dry matter through the breathing of young sprouts. Therefore, this crude protein percentage increase was not a likely true increase (Chavan and Kadam 1989, Abdallah 2008, Dung et al 2010). Carbohydrate values showed opposite results with protein and showed a clear decreased value in sprouts compared with dry seeds, especially when using tap water for sprouting **(Table 2)**. Contrastively, while fiber and ash values increased in fenugreek Giza 30 and Giza 2 variety sprouts than in dry seeds, their lipid contents decreased during sprouting. Additionally, fenugreek-etiolated sprouts demonstrated lower energy values than dry seeds. This observation may be a clue to the decrease in carbohydrate and lipid content. Summarily, fenugreek contained 23%–26% protein, 6%–7% fat, and 58% starch, of which approximately 25% was dietary fiber. Also, a previous study noted that fenugreek is a rich wellspring of iron, containing 33 mg/100 g dry weight (US Department of Agriculture 2001).

### 3.3 Amino acid profile and quality of sprouts

**Table 3.** presents an overview of the essential and nonessential amino acids of the three days of fenugreek germination in Egypt (the two varieties: Giza 2 and Giza 30). The results showed that sterilizing seeds before germination and then germinating them with tap water or water with NaCl 2,000 ppm decreased essential amino acids and increased aspartic acid and

**Table 2.** Proximate analysis (g/100g) and energy (Kcal/g) of three-day-old etiolated fenugreek sprouts

| **Variety** | **Sterilized seed Giza 2** | | **NaCl, ppm** | | **Sterilized seed Giza 30** | | **NaCl, ppm** | | **Average** | **Giza 2 and Giza 30** | **NaCl, ppm** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **NaCl Concentrate** | **Dry seed** | **Tap water** | **2000** | **Mean** | **Dry seed** | **Tap water** | **2000** | **Mean** | **Dry seed** | **Tap water** | **2000** |
| **Moisture** | 9.3 | 10 | 11.1 | 10.13 | 9.2 | 10.7 | 10.9 | 10.27 | 13.9 | 15.35 | 16.55 |
| **Crude Protein** | 23.7 | 27.2 | 27.7 | 26.2 | 27.6 | 31.7 | 32.5 | 30.6 | 37.5 | 43.05 | 43.95 |
| **Carbohy-drates** | 48.6 | 42.6 | 38.7 | 43.3 | 43.6 | 36.2 | 36.4 | 38.73 | 70.4 | 60.7 | 56.9 |
| **Lipids** | 7.3 | 4.8 | 4.9 | 5.67 | 9.00 | 4.9 | 4.7 | 6.2 | 11.8 | 7.25 | 7.25 |
| **Fiber** | 6.1 | 8.7 | 9.5 | 8.1 | 5.8 | 8.6 | 8.7 | 7.7 | 9.00 | 13 | 13.85 |
| **Ash** | 5.00 | 6.7 | 8.1 | 6.6 | 4.8 | 7.9 | 6.8 | 6.5 | 7.4 | 10.65 | 11.5 |
| **Energy (Kcal/g)** | 354.9 | 322.4 | 309.7 | 329 | 365.8 | 315.7 | 317.9 | 333.1 | 537.8 | 480.25 | 468.65 |

prolines, two nonessential amino acids. Researchers previously reported that the germination of alfalfa seeds at three days decreased the essential amino acids, agreeing with our findings (El-Gebaly et al 2018). About the reasons for the concentrations of sodium chloride used in this study, data showed that sprouting of the two varieties, Giza 30 and Giza 2, in NaCl decreased essential amino acids compared with sprouting in tap water. Also, the interaction showed differences in the percentages of essential amino acids from the two varieties, Giza 30 and Giza 2. Additionally, investigations revealed that the variety in fenugreek seed was greater than the sprout in tap water and water treated with 2,000 ppm NaCl, indicating a lower proportion of essential amino acids than the seeds. Furthermore, the Giza 2 variety average recorded the highest results in isoleucine, threonine, valine, phenylalanine, leucine, methionine, and histidine, except for the amino acid (lysine), which recorded the best result in the Giza 30 variety class. Based on our findings, increased salinity concentrations often result in osmotic toxicity and/or specific toxicity, preventing water uptake and consequently reduced yield. Moreover, lysine is an essential amino acid, and its lack in diets can hinder development in youngsters and decrease resistance capacity (Zimmermann 2001).

### 3.4 Nonessential amino acid composition

The nonessential amino acid compositions showed that aspartic acid and proline were the most abundant in sterilized buds **(Table 3)**. Notably, while high percentages of aspartic acid and proline were recorded when tap water was treated with Giza 2 variety sprouts (15.88 and 4.15 g/100g, respectively), low results were shown in the dry seeds: aspartic acid (8.90 g/100g) and proline (3.67 g/100g), whereas average results were recorded for the remaining amino acids of the variety Giza 2 (Alanine, Glutamic acid, Arginine, Glycine, Cysteine and Tyrosine) at ratios of 2.28, 7.42, 4.56, 2.37, 0.61, and 2.00 g/100 mg. Alternatively, we observed that the effects of all amino acids decreased for the two classes using sodium chloride solution treatments.

Increased salinity concentrations often result in osmotic toxicity and/or specific toxicity that prevents water uptake. Our investigations revealed that the effect on the amino acid concentration was relatively dependent on the sodium ions present in water. A similar trend has been reported previously (El-Gebaly et al 2018). Arginine demonstrates a huge impact on polyamine combinations, impacting cell division and improving health. The suggested portions for arginine supplementation range from 1.5 to 6.0 g/day (Zimmermann 2001).

In **Table 4**, the percentage of essential to TEAA/TAA of seeds in variety Giza 2 was 35.38%. However, while the best results were obtained upon germination using sodium chloride for variety Giza 30 (34.10%), the lowest results were obtained in variety

Giza 2 (31.19%). Also, the average germination percentage in the variety Giza 30 was recorded as the best (34.05%). These qualities were above the 26% ideal protein standards for youngsters and 11% for grown-ups (FAO/WHO/UNU 1985). For the TNEAA/TAA results, while the dry seeds recorded a lower germination percentage (64.62%) compared to tap water or water treated with NaCl 2,000 ppm, the best results

**Table 3.** Essential and nonessential amino acid composition (g/100 g protein) of three-day-old etiolated Egyptian fenugreek sprouts

| **Sterilize (ste)** | **Sterilized seed Giza 2** | | **NaCl, ppm** | | **Sterilized seed Giza 30** | | **NaCl, ppm** | | **Average** | **Giza 2 and Giza30** | **NaCl, ppm** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EAA** | **Dry Seed** | **Tap water** | **2000** | **Mean** | **Dry Seed** | **Tap water** | **2000** | **Mean** | **Dry seed** | **Tap water** | **2000** |
| **Iso** | 3.54 | 2.17 | 1.59 | 2.43 | 3.19 | 2.02 | 1.85 | 2.35 | 3.37 | 2.10 | 1.72 |
| **Ther** | 3.00 | 1.65 | 1.26 | 1.97 | 2.46 | 1.55 | 1.09 | 1.70 | 2.73 | 1.60 | 1.18 |
| **Val** | 3.04 | 2.39 | 1.81 | 2.41 | 2.61 | 2.17 | 1.89 | 2.22 | 2.82 | 2.28 | 1.85 |
| **Phe** | 3.65 | 2.87 | 2.13 | 2.88 | 3.01 | 2.78 | 2.51 | 2.77 | 3.32 | 2.82 | 2.32 |
| **Lys** | 5.19 | 3.23 | 2.35 | 3.58 | 4.53 | 3.18 | 3.13 | 3.61 | 4.86 | 3.19 | 2.74 |
| **Leu** | 5.40 | 3.42 | 2.49 | 3.77 | 4.64 | 3.29 | 3.05 | 3.66 | 5.02 | 3.35 | 2.77 |
| **Met** | 0.42 | 0.29 | 0.25 | 0.32 | 0.29 | 0.29 | 0.11 | 0.23 | 0.36 | 0.29 | 0.18 |
| **His** | 1.86 | 1.65 | 1.26 | 1.59 | 1.63 | 1.66 | 1.42 | 1.57 | 1.74 | 1.66 | 1.34 |
| **TEAA** | 26.08 | 17.65 | 13.14 | 18.95 | 22.36 | 16.93 | 15.05 | 18.11 | 24.22 | 17.29 | 14.10 |
| **Asp** | 8.90 | 15.88 | 11.41 | 12.06 | 7.72 | 14.01 | 13.64 | 11.79 | 8.31 | 14.94 | 12.52 |
| **Ala** | 2.95 | 2.06 | 1.84 | 2.28 | 2.64 | 2.06 | 1.64 | 2.11 | 2.80 | 2.06 | 1.74 |
| **Glu** | 13.00 | 5.00 | 4.26 | 7.42 | 11.30 | 5.09 | 3.96 | 6.79 | 12.15 | 5.05 | 4.11 |
| **Ser** | 3.59 | 2.17 | 1.55 | 2.44 | 3.48 | 2.13 | 1.71 | 2.44 | 3.53 | 2.15 | 1.63 |
| **Arg** | 7.55 | 3.27 | 2.85 | 4.56 | 6.49 | 3.14 | 2.25 | 3.96 | 7.02 | 3.21 | 2.55 |
| **Pro** | 3.67 | 4.15 | 3.75 | 3.86 | 3.37 | 3.86 | 2.98 | 3.40 | 3.52 | 4.01 | 3.37 |
| **Gly** | 4.14 | 1.54 | 1.44 | 2.37 | 3.59 | 1.62 | 1.27 | 2.16 | 3.86 | 1.58 | 1.36 |
| **Cys** | 1.10 | 0.37 | 0.36 | 0.61 | 0.69 | 0.40 | 0.29 | 0.46 | 0.89 | 0.38 | 0.33 |
| **Tyr** | 2.74 | 1.73 | 1.52 | 2.00 | 2.64 | 1.66 | 1.35 | 1.88 | 2.69 | 1.69 | 1.43 |
| **NEAA** | 47.64 | 36.18 | 28.99 | 37.60 | 41.92 | 33.97 | 29.09 | 34.99 | 44.78 | 35.07 | 29.04 |
| **TAA** | 73.72 | 53.83 | 42.13 | 56.55 | 64.28 | 50.90 | 44.14 | 53.10 | 69.00 | 52.36 | 43.14 |

**Ile** = Isoleucine, **Ther** = Therionine, **Val** = Valine, **Phe** = Phenylalanine, **Lys** = Lysine, **Leu** = Leucine, **Meth** = Methionine, **His** = Histidine, **TEAA** = Total Essential Amino Acids, **Asp** = aspartic acid, **Ala** = Alanine, **Glu** = glutamic acid, **SER** = serine, **ARG** = argenine, **Pro** = proline, **GLY** = glycine, **CYS** = cysteine, **TYR** = tyrosine, NEAA = Nonessential amino and **TAA** = Total amino acid

were during sprouting with salt water for the Giza 2 variety (68.81%), including the average germination in this variety (66.88%). Also, nutritional quality parameters are presented in **Table 4**. In TEAA/TNEAA, results showed that while the dry seeds recorded a higher germination percentage (54.75%) than tap water or water treated with NaCl 2,000 ppm, the best results were obtained during sprouting with salt water for the Giza 2 variety (45.33%). The average germination in the Giza 30 variety was the best (51.64%). Also, we observed that while sterilized seeds demonstrated better percentages of cysteine/methionine and TSAA (1.52%) than that when sprouting, whether using tap water or salt water (2,000 ppm) in the two varieties, the average germination in the Giza 2 variety was better than that of the Giza 30 variety. Accordingly, (Adeyeye 2004) reported that many sprouting vegetables contain substantially more cysteine than methionine. Also, similar data were retained previously with pea (Ahmed et al 2018).

The total aromatic amino acid (TArAA) of etiolated fenugreek in seed Giza 30 and Giza 2 varieties was great, with values from 7.28 to 8.23, respectively. The average sprout ranged from 6.22 g/100g using tap water to 6.46 using saline water (2,000 ppm NaCl). Also, Leucine/Isoleucine (Leu/Ile) content was investigated when germination was conducted using tap water or salt water with sodium chloride. The best results were obtained in etiolated fenugreek seeds compared with dry seeds. Additionally, while TAAA in fenugreek sprouts ranged from 19.02 to 21.9 seeds **(Table 4)**, almost close to the total essential amino acid in seeds, TBAA results ranged from 12.64 to 14.60 in Giza 30 and Giza 2 varieties. However, the average sprouts ranged from 9.14 to 9.73 in the Giza 2 and Giza 30 varieties.

Since the assay methods for estimating protein quality employed casein as the reference protein, the high quality protein used as a reference should be
determined simultaneously and under the same conditions as the assay procedure being used (FAO/WHO 1991). Therefore, using standard protein (casein) in this research for estimating protein and amino acid quality was recommended, even though it was not identical to the previously referenced protein (Ijarotimi and Keshinro, 2013).

The most widely used method for measuring protein quality is the PER, which is the weight gain per weight of protein eaten. Results showed that the PER values of etiolated fenugreek seed samples were between 2.29 and 2.63 in Giza 30 and Giza 2 varieties, using tap water for sprouting, but was 1.84 using NaCl 2,000 ppm solution for sprouting compared with 1.71 in dry fenugreek seed sample **Table 3**.

These increments in the PER value can be explained by the increment in their leucine and tyrosine amino acid compositions **(Table 3)**. The PER value in fenugreek seeds using (2.63) was equal to that recorded in reference (2.5) (Oyarekua and Eleyinmi 2004). Also, our results showed that etiolated fenugreek sprouts in both tap and saline water decreased the essential amino acid index (EAAI) from 10.92 to 8.56 in Giza 2 and 11.17 to 8.63 in the Giza 30 variety compared with dry seeds, which was 13.75 and 14.12 in the two varieties, respectively.

Also, while the BV showed a similar decrease (2.29 to 0.48) in the tap water Giza 2 variety, the BV range in Giza 30 was from 2.37 to 0.20 compared with the seed, which was 3.69 and 3.29 in the two varieties, respectively.

These results showed that consuming etiolated fenugreek sprouts alone as food is inadequate, based on the content of its good nutritional quality (EAAI and BV less than 10%–100%) for food and base-protein compositions (Oser 1959).

Regarding its NI, data in **Table 4** showed that etiolated fenugreek sprouts in both tap and saline water decreased the nutritional loss index from 3.18 to 2.39 and 3.02 to 2.35 in Giza 2 and 30 varieties compared with the dry fenugreek seed sample (3.34 in Giza 2 and 3.79 in Giza 30). Moreover, regarding the NI, researchers also showed that etiolated clover sprouts in both saline and tap water increased the nutritional index to 26.98–28.31 compared with the dry clover seed sample (26.32) (El-Gebaly et al 2018).

**Table 4.** Nutritional quality parameters of three-day-old etiolated fenugreek sprouts' amino acids

| Sterilize (ste) | Sterilized seed Giza 2 | | NaCl, ppm | | Sterilized seed Giza 30 | | NaCl, ppm | | Average | Giza 2 and Giza30 | NaCl, ppm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EAA** | **Dry Seed** | **Tap water** | **2000** | **Mean** | **Dry Seed** | **Tap water** | **2000** | **Mean** | **Dry seed** | **Tap water** | **2000** |
| **TEAA/TAA%** | 35.38 | 32.79 | 31.19 | 33.12 | 34.79 | 33.26 | 34.1 | 34.05 | 35.1 | 33.02 | 32.68 |
| **TNEA/TAA%** | 64.62 | 67.21 | 68.81 | 66.88 | 65.21 | 66.74 | 65.9 | 65.95 | 64.9 | 66.98 | 67.32 |
| **TEAA/TNEAA%** | 54.75 | 48.79 | 45.33 | 49.62 | 53.34 | 49.84 | 51.73 | 51.64 | 54.09 | 49.3 | 48.55 |
| **TSAA** | 1.52 | 0.66 | 0.61 | 0.93 | 0.98 | 0.69 | 0.4 | 0.69 | 1.25 | 0.67 | 0.51 |
| **TArAA** | 8.23 | 6.25 | 4.91 | 6.46 | 7.28 | 6.1 | 5.27 | 6.22 | 7.76 | 6.18 | 5.09 |
| **LEU/ile** | 1.52 | 1.58 | 1.57 | 1.55 | 1.45 | 1.63 | 1.65 | 1.55 | 1.49 | 1.6 | 1.61 |
| **TAAA** | 21.9 | 20.88 | 15.67 | 19.48 | 19.02 | 19.1 | 17.6 | 18.57 | 20.46 | 19.99 | 16.63 |
| **TBAA** | 14.6 | 8.13 | 6.46 | 9.73 | 12.64 | 7.98 | 6.8 | 9.14 | 13.62 | 8.05 | 6.63 |
| **PER** | 2.63 | 1.84 | 1.43 | 1.97 | 2.29 | 1.78 | 1.71 | 1.93 | 2.46 | 1.81 | 1.57 |
| **EAAI** | 13.75 | 10.92 | 8.56 | 11.07 | 14.12 | 11.17 | 8.63 | 11.31 | 13.94 | 11.05 | 8.6 |
| **NI** | 3.34 | 3.18 | 2.39 | 2.97 | 3.79 | 3.02 | 2.35 | 3.05 | 3.57 | 3.1 | 2.37 |

**TEAA/TAA%** = Total Essential Amino Acid/Total Amino Acid, **TNAA/TAA%** = Total Nonessential Amino Acid/Total Amino Acid, **TEAA/TNAA%** = Total Essential Amino Acid/Total Nonessential Amino Acid, **TSAA=** Cysteine + Methionine, **TArAA** = Total Aromatic Amino Acids, **LEU/Ile** = Leucine/Isoleucine, **TAAA** = Total Acidic Amino Acids, **TBAA** = Total Basic Amino Acids, **PER** = Protein Efficiency Ratio, **EAAI%** = Essential Amino Acid index, **BV** = Biological Value and **NI** = Nutritional index.

**Table 5.** Effect of sprouting on the element content of Egyptian three-day-old etiolated fenugreek sprout characters *vs.* dry seeds (mg/kg)

| Variety | Sterilized seed Giza 2 | | NaCl, ppm | | Sterilized seed Giza 30 | | NaCl, ppm | | Average | Giza 2 and giza30 | NaCl, ppm |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Concentrate** | **Dry Seed** | **Tap water** | **2000** | **Mean** | **Dry Seed** | **Tap water** | **2000** | **Mean** | **Dry seed** | **Tap water** | **2000** |
| **Calcium** | 1105.6 | 2861.2 | 1727.8 | 1898.2 | 1770 | 3076.6 | 2184.3 | 2343.63 | 1437.8 | 2968.9 | 1956.05 |
| **Potassium** | 6307.8 | 9420.7 | 7102.4 | 7610.3 | 13855 | 10874 | 8733 | 11154 | 10081.4 | 10147.4 | 7917.7 |
| **Magnesium** | 785.3 | 1869.6 | 1517.4 | 1390.8 | 1634.7 | 2154.6 | 1434.2 | 1741.17 | 1210 | 2012.1 | 1475.8 |
| **Sodium** | 433.6 | 988.2 | 7575.5 | 2999.1 | 772.2 | 1046.2 | 5132.8 | 2317.07 | 602.9 | 1017.2 | 6354.15 |
| **Phosphorus** | 2321.2 | 6121.9 | 5172.1 | 4538.4 | 4859.9 | 6947.4 | 4409.4 | 5405.57 | 3590.55 | 6534.65 | 4790.75 |
| **Iron** | 57.4 | 82.5 | 89.15 | 76.35 | 75.6 | 85.7 | 88.46 | 83.25 | 66.5 | 84.1 | 88.805 |

### 3.5 Effect of sprouting using saline water and seed sterilization on the element content of Egyptian fenugreek seeds

The calcium, potassium, magnesium, sodium, phosphorus, and iron content of dry fenugreek seeds of the Giza 2 and Giza 30 varieties were the following: 1,105.6–1,770 mg/Kg; 6,307.8–13,855 mg/Kg; 785.3–1,634.7 mg/Kg; 433.6–772.2 mg/Kg; 2,321.2–4,859.9 mg/Kg; and 57.4–175.6 mg/Kg for, respectively **(Table 5)**. However, the sprouting of fenugreek seeds after three days old increased the element content of the seeds, except for magnesium, sodium, and phosphorus, when treated with tap water. Nonetheless, the highest calcium, potassium, and magnesium contents (2,968.9, 10,147.35, and 2,012.1 mg/kg, respectively) were achieved using saline water at 2,000 ppm. Also, results showed that although the potassium and magnesium contents decreased after sprouting, they were increased significantly with treatment using 2,000 ppm saline water over tap water. Contrastively, sodium and phosphorous contents in fenugreek sprouts were significantly increased to reach 10,17.2 and 6,534.65 mg/Kg, respectively, using tap water for sprouting fenugreek seeds, while it was 6,354.15 and 4,790.75 mg/Kg, respectively, for 2,000 ppm saline water.

**Table 5** also shows that sterilized Giza 30 seeds demonstrated higher values of potassium (11,154.00 mg/Kg), magnesium (1,741.17 mg/Kg), iron (83.25 mg/Kg), phosphorus (4,538.4 mg/Kg), and sodium contents (2,317.07 mg/Kg). Based on our findings, we propose that spouting is a good practice to enhance the element content of fenugreek seeds either using tap or saline water. However, to improve the mineral content of sprouts, seed sterilization is necessary.

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