# Effect of Gibberellic Acid on the Chemical Composition of Fenugreek Seeds

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## ABSTRACT

A field experiment was performed to assess the effect of foliar application of gibberellic acid concentrations on the chemical composition of fenugreek seeds "variety Giza 30".
Proximate composition, amino acids, lipid classes, fatty acids, essential oil and volatile compounds were determined in untreated and treated fenugreek seeds using recent techniques and up date equipments.The results indicated that fenugreek seeds were rich in protein, essential amino acids and different minerals. These compounds were increased by increasing foliar application of gibberellic acid concentrations on fenugreek plants. Total lipids extracted from fenugreek seeds showed the highest percentage of triglycerides. These total lipids were found to contain more than 75 % of unsaturated fatty acids. Essential polyunsaturated fatty acids increased by increasing gibberellic acid concentrations. Sixteen volatile compounds were identified by Headspace Gas Chromatography (HGC) while 13 compounds were identified after extraction of essential oil by Simultaneous Distillation Extraction (SDE). The most abundant volatile compounds in the essential oil of different fenugreek seed samples were sotolon, γ-nonalactone , δ- elemene, α- muurolene, Selinene, γ- cadinene and precursor of sotolon.

## INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) belongs to the family *Leguminosae*. The name of the genus, *Trigonella*, comes from Greek, meaning "three-angled," from the form of its corolla and its species name *foenum-graecum* means " Greek hay "(Grieve, 1979). It is one of the most widely used legume in the Mediterranean countries, the Middle East, India and China and now is cultivated in European countries and USA (Wichtl and Bisset, 1994; Bruneton, 1995; BHP, 1996; Budavari, 1996 and Leung and Foster, 1996). Fenugreek is a condiment-herb that has an ancient history which cultivated mainly for its seeds. Fenugreek seeds are used in India for preparation of curry powder after roasting (Nadkarni, 1976). In Egypt, seeds used as a supplement to flours for bread making and/or seeds are boiled and used as a hot drink (Hulba) and the boiled seeds are also eaten and/or seeds are germinated for 2 - 3 days and eaten ( Hidvegi *et al*, 1984 and Abdalla and Melton, 1991).

In general, seeds contain carbohydrates, protein, fixed and volatile oils, alkaloids, saponins, mucilage, minerals and vitamins. According to different factors such as the cultivation area and variety, fenugreek seeds contain 45-60% of carbohydrates, mainly mucilaginous fiber (galactomannans); 20-30 % of proteins of high in aliphatic, basic and acetic amino acids; 5-10 % of fixed oils (lipids) of high in unsaturated fatty acids; volatile oils, the most important aroma compound sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone), precursor of sotolon, elemene, selinene, n-alkanes, sesquiterpenes, alkanoles and lactones; pyridine-type alkaloids, mainly trigonelline, choline, gentianine and carpaine; the flavonoids, apigenin, luteolin, orientin, quercetin, vitexin and isovitexin; minerals, mainly potassium, phosphorus,calcium and iron; glycosides yielding steroidal sapogenins; cholesterol and sitosterol and vitamins A, B, C and nicotinic acid (Wichtl and Bisset, 1994; Bruneton, 1995; Budavari, 1996; Leung and Foster, 1996 and Newall *et al.*, 1996). According to the chemical composition of fenugreek seeds, its assumed to have nutritive and restorative properties and, also, stimulate the digestive process (Shang *et al*, 1997).

Fenugreek is recorded use dates back to ancient Egyptian medicine, first mentioned in the Ebers papyri (1500 *B.C.*) as an herb to induce childbirth. It has been used therapeutically for millennia in traditional Arabian, Greek, and Indian medicines (Thirunavukkarasu *et at.*, 2003). It was introduced into Chinese medicine in the eleventh century (Nadkarni, 1976; Grieve, 1979; Tu, 1992; Bown, 1995; Leung and Foster, 1996;). In the United States, it was a key ingredient in Lydia Pinkham's famous "Vegetable Compound," a popular nineteenth century patent medicine for menstrual pain and postmenopausal vaginal dryness (Duke, 1997). In Germany, fenugreek seed is usually used externally, prepared as an aqueous paste for poultices to reduce inflammation. Occasionally, it is used internally as a component of cholagogue and gastrointestinal remedy compounds (Wichtl and Bisset, 1994; Leung and Foster, 1996).

Different clinical studies have investigated its hypocholesterolemic and hypoglycemic actions in normal and diabetic humans (Ali *et al*, 1995; Bruneton, 1995; Newall *et al.*, 1996). Gel fiber present in fenugreek seeds, reduces the rate of glucose absorption and may also delay gastric emptying, thereby preventing the rise in blood sugar levels following a meal (Sharma et al., 1996; Abdel-Barry *et al*, 2000). Significant reductions in serum-cholesterol concentrations were, also, reported (Sharma, 1986; Stark and Madar, 1993; Sowmya and Rajyalakshmi, 1999). A subsequent study investigated the lipid-lowering activity of fenugreek seeds in 60 non-insulin dependent diabetic subjects (Anuradha and Ravikumar, 1998; Prasanna, 2000). The effect on lipid levels was sustained and lasting (Ravikumar and Anuradha, 1999).

Gibberellic acid ($GA_3$) received more attention to indicate its simulatory effect on the growth of different plants. A few numbers of publications have been published on the effect of gibberellic acid foliar application on plant growth and yield of fenugreek but no publication was found on the effect of $GA_3$ on chemical composition of fenugreek seeds. Shedeed *et al* (1991) reported that aster's plant content of total phenols was reduced by $GA_3$ treatments while some minerals as phosphorus and potassium were increased. Selim *et al* (1992) found that protein content of roselle increased by increasing $GA_3$. Eid *et al* (2002) indicated that unsaturated fatty acids of fenugreek seeds increased by foliar spraying by Sahara fertilizer at 3 gm/L.

The present study was conducted to investigate the effect of foliar application of gibberellic acid concentrations on the chemical composition of fenugreek seeds to evaluate their nutritive value.

## MATERIALS AND METHODS

Fenugreek cultivar "Giza 30" seeds were obtained from the legumes Department of the Agricultural Research Center, Ministry of Agriculture, Cairo, Egypt.

The field experiment was carried out during the two seasons of 2001/2002 and 2002/2003 at the Experimental Farm of Faculty of Agriculture, Saba Basha, Alexandria University at Abies (Adam, 2003).

Air-dried seeds of fenugreek cultivar "Giza 30" were used for the analysis. The seeds were ground to a fine powder (1 mm mesh) using Wiley Mill, Philadelphia.

### 1. Proximate analysis

Chemical constituents were determined using the appropriate methods as follows:

1.1. **Moisture, crude fiber, total sugars** and **ash contents** were determined in different samples of fenugreek seeds according to the standard methods AOAC (1990).

1.2. **Nitrogen content** was determined by Kjetec Auto 1030 Analyzer with a Tecator Digestion System at Alexandria Oil and Soap Company, Alex., Egypt. **Protein (%)** was calculated by multiplying total nitrogen by a factor of 6.25.

1.3. **Trigonelline alkaloid** (%) was determined by the method mentioned by Abdel Wahab and Selim (1986).

1.4. **Total lipids** (%) of fenugreek seeds were extracted by chloroform : methanol (2 : 1,v/v) as described by Folch *et al* (1955) and determined as described by Melton *et al.*(1979).

1.5. **Mineral contents;** representing calcium, iron, magnesium, phosphorus, potassium, sodium and zinc were determined in fenugreek seeds using Atomic Absorption Emission Spectrophotometer, Model 550 AA. Samples

were ashed at 500 °C, dissolved in 6N HCl and a proper dilution was used according to the mineral analyzer.

2. **Amino acids** of fenugreek seeds were analyzed using amino acid analyzer Model 710B (American Burdick and Jackson). Samples of the ground seeds were purified using the Sep-Pak $C_{18}$ cartridge (Water's Inc.), hydrolyzed with 6N HCL, evaporated in a desicator and dried by lyophilization. The dried residue was dissolved in a mixture of triethylamine(TEA) : methanol : water; 1 : 2 : 2, by volume, and then redried. The dried samples were derivatized with methanol : water : TEA : phenyllisothiocyanate(PITC), 7 : 1 : 1 : 1, by volume, diluted and analyzed using Model 510 HPLC pumps, Model 440 absorbance detector, Model 440 absorbance detector, Model 710 B automated injector and Model 730 data processor.

3. **Lipid classes** of extracted lipids were separated into different lipid fractions using a disposable silica cartridge (Bond-Elut, Analytichem, Inc., USA) as described by Abdalla and Melton (1991). Lipids in each fraction were determined as a weight percentage of the weight of sample applied to the silica cartridge. Lipid fractions were chromatographed on a 20 X 20 cm, 250µ thick silica gel plate (TLC; Analtech, Inc., USA) using different solvent systems as recommended by Abdalla and Melton (1991). Lipids separated by TLC were visualized and identified by comparison of Rf values of standards chromatographed at the same time as the lipid samples.

4. **Fatty acid composition** of the extracted fixed oil from different fenugreek seeds samples was determined. The extracted lipids were saponified and converted to methyl esters according to AOCS (1983). Fatty acid methyl esters were analyzed using Shimadzu Gas Chromatography GC 12A equipped with flam ionization detector (FID) on 3.1 m x 3.2 mm glass packed column DEGS 10 % (Shimadzu Company, Japan).

5. **The volatile oils** were extracted from fresh ground fenugreek seeds using two different procedures at Food Chemistry Department, Wagenningen University, The Netherlands. In the first procedure; A hundred grams of dried seeds were subjected to a three-hours hydrodistillation using Simultaneous Distillation Extraction (SDE) as described by Abdalla (1990). The extract was concentrated to approximately 1 ml under freezing condition. Volatiles were, qualitatively, analyzed by Gas Chromatography and quantitatively by GC-MS. In the second procedure; volatile compounds of samples were analyzed by static headspace capillary gas chromatography. One gram of each dried ground fenugreek seed sample was transferred into a special headspace bottle sealed with silicon teflon

cap. The bottles were inserted into the headspace sampler (HS 800 Fison instrument), incubated at 65°C for 5 min then volatiles in head-space of bottle was injected to gas chromatography (HRGC 5300 Carlo Erba Instrument). All operating conditions for headspace GC are described by Abdalla and Roozen (1999).

## RESULTS AND DISCUSSION

### 1. Proximate composition

The results of the chemical constituents of fenugreek seeds during two seasons (2001 and 2002) are presented in Table 1.

In general, fenugreek seeds (variety Giza 30) are rich in protein, crude fiber, carbohydrates and different minerals as potassium, phosphorus, calcium, magenisum and iron. The seeds contained about 8% of total lipids and 0.1 of trigonelline alkaloid.

The percentages of moisture, crude protein, total lipids, crude fiber and ash of untreated (control) seeds during two seasons are in close agreement with those reported by Abde-El Hamide *et al* (1984); Abd El-Aal and Rahma (1986); Abdalla and Melton (1991).

Treatments with two concentrations (50 and 100 ppm) of gibberellic acid during two seasons (2001 and 2002) had an effect on different constituents of fenugreek seeds. For instance, the levels of crude protein increased up to 30 % in dry weight with the increase of gibberellic acid level. Some other components such as total sugars, total lipids and trigonelline were slightly increased with the increase of aaplied gibberellic acid concentrations. However, reported results on protein are in compatible with those of Selim *et al* (1992); Mohamed and Naguib (2002).

Hidvegi *et al* (1984) concluded that the protein quality of fenugreek seeds is approximately equal to that of soybeans so that fenugreek could become a promising nutritional factor as a source of protein in Egypt due to the high cost of animal proteins.

This study indicated that the foliar application of gibberellic acid increased the protein level and improved the nutritional quality of fenugreek seeds.

Trigonelline, a nitrogen compound (alkaloid) is found in fenugreek seeds and present in many legumes (Mohamed and Naguib, 2002). When this compound comes in contact with acids or heated, it yields nicotinic acid (niacin) which lowers total cholesterol while increasing high density lipoprotein; HDL (Nakhla *et al*, 1991; Yoshikawa *et al*, 1997). Likely this compound increased up to 0.3% by increasing $GA_3$ during two seasons.

The results of minerals showed that untreated fenugreek seeds contained higher levels of potassium, phosphorus, calcium, magnesium and iron. These results are in parallel with Rajagopalan (1998) and Mohamed and Naguib (2002). Potassium, phosphorus and calcium were increased by increasing $GA_3$ concentration in both seasons. These results are in matching with those obtained by Shedeed *et al* (1991) on aster plants and Selim *et al* (1992) on roselle.

Table 1. Influence of foliar application of gibberellic acid concentrations on the chemical constituents of fenugreek seeds during two consecutive seasons. Data expressed as mean of two or three samples.

| Character | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | 2001/2002 season | | | 2002/2003 season | | |
| | (Control) 0 ppm $GA_3$ | 50 ppm $GA_3$ | 100 ppm $GA_3$ | (Control) 0 ppm $GA_3$ | 50 ppm $GA_3$ | 100 ppm $GA_3$ |
| **Moisture** | 8.80 | 8.65 | 7.50 | 8.70 | 7.80 | 7.70 |
| **Chemical constituents ( % of dry weight)** | | | | | | |
| Crude protein | 26.90 | 29.15 | 31.05 | 26.70 | 28.95 | 31.25 |
| Total sugars | 41.05 | 41.85 | 42.50 | 40.30 | 41.75 | 42.15 |
| Total lipids | 7.20 | 7.75 | 8.15 | 7.20 | 7.55 | 8.65 |
| Crude fiber | 9.05 | 7.45 | 6.35 | 9.30 | 7.25 | 5.15 |
| Ash | 5.75 | 4.90 | 3.60 | 6.70 | 5.60 | 4.25 |
| Trigonellin | 0.11 | 0.22 | 0.31 | 0.10 | 0.19 | 0.28 |
| **Minerals (mg/100 g dry seeds)** | | | | | | |
| Calcium | 168 | 179 | 215 | 166 | 190 | 225 |
| Iron | 48 | 42 | 40 | 58 | 54 | 52 |
| Magnesium | 148 | 155 | 145 | 150 | 165 | 177 |
| Phosphorus | 289 | 325 | 395 | 289 | 338 | 398 |
| Potassium | 466 | 495 | 665 | 461 | 570 | 686 |

## 2. Amino acids

Due to the increase of protein in fenugreek seeds by increasing gibberellic acid concentrations applied during two seasons, amino acid composition of different untreated and treated fenugreek seed samples was determined and listed in Table 2.

The results of amino acid analysis, generally, indicated that fenugreek seeds contained high levels of aspartic and glutamic (acidic amino acids) and arginine (basic amino acid). These seeds are rich in the essential amino acids; leucine, isoleucine, lysine, phenylalanine, threonine and valine. Methionine is one of the essential amino acids is found to be in the lowest level. Most of these essential amino acids increased by increasing foliar application of gibberellic acid on the plants during the two seasons. These results indicated that the foliar application of $GA_3$ increased the nutritive value of fenugreek seeds according to the increase of protein and the most essential amino acids. These results are in agreement with different publications.

Previously, Hidvegi *et al* (1984) studied the nutritional characteristics of fenugreek seeds and they found that seeds were rich in many essential amino acids and poor in sulfur containing amino acids such as cystine, cystein and methionine. Abdalla and Melton (1991) found that fenugreek seeds contained high levels of glutamic, aspartic and arginine beside the most essential amino acids. Shang *et al* (1998) published that 17 amino acids in Chinese fenugreek seeds were analyzed by High Speed Amino Acid Analyzer. Seven of them are essential for human body so they referred that fenugreek seeds are useful in medicine and food.

However, no publication has been found, unless otherwise stated elsewhere, respecting the influence of foliar application of gibberellic acid on amino acids of fenugreek or legumes.

Table 2. Influence of foliar application of gibberellic acid concentrations on the amino acid composition of fenugreek seeds during two consecutive seasons.

| Amino acids (mg amino acid/g dry seed) | Treatments | | | | | |
|---|---|---|---|---|---|---|
| | 2001/2002 season | | | 2002/2003 season | | |
| | (Control) 0 ppm $GA_3$ | 50 ppm $GA_3$ | 100 ppm $GA_3$ | (Control) 0 ppm $GA_3$ | 50 ppm $GA_3$ | 100 ppm $GA_3$ |
| Aspartic | 20.40 | 20.65 | 21.95 | 20.15 | 21.90 | 21.15 |
| Arginine | 18.85 | 17.15 | 19.25 | 17.15 | 17.24 | 17.65 |
| Alanine | 9.85 | 10.12 | 10.54 | 10.20 | 11.14 | 11.85 |
| Glutamic | 21.20 | 22.55 | 21.65 | 21.50 | 22.65 | 21.55 |
| Glycine | 12.20 | 12.12 | 11.65 | 12.05 | 12.32 | 11.95 |
| Histidine | 7.25 | 6.55 | 7.20 | 6.45 | 6.65 | 6.30 |
| Leucine | 16.35 | 17.44 | 19.55 | 15.55 | 18.25 | 20.40 |
| Isoleucine | 13.30 | 14.52 | 16.35 | 12.54 | 15.25 | 17.05 |
| Lysine | 11.25 | 12.55 | 16.10 | 10.10 | 13.25 | 17.95 |
| Methionine | 2.25 | 2.22 | 2.10 | 2.10 | 2.55 | 2.20 |
| Proline | 9.70 | 8.90 | 9.10 | 9.50 | 8.65 | 9.25 |
| Phenylalanine | 11.80 | 13.14 | 15.25 | 10.65 | 13.75 | 15.85 |
| Serine | 10.45 | 10.10 | 9.95 | 10.40 | 10.25 | 9.80 |
| Tyrosine | 5.55 | 5.20 | 5.25 | 5.65 | 5.15 | 5.55 |
| Thereonine | 3.15 | 3.85 | 3.55 | 3.20 | 3.65 | 3.75 |
| Valine | 9.85 | 10.20 | 10.90 | 9.95 | 9.55 | 10.40 |

## 3. Lipids and fatty acids

Total lipids were extracted from different fenugreek seed samples then separated into lipid classes (fractions) using disposable silica cartridge eluted by chloroform, acetone and methanol, respectively.

However, a good recovery of lipids fractionated (A, B and C) on disposable silica cartridge was found for elution system, average of 99% of the total lipid. For example, fraction A, eluted by chloroform, showed the highest percentage (87 % of total lipid recovery) of triglycerides. Fraction B, eluted by acetone, showed the lowest percentage (3 % of total recovery) of glycolipids. Fraction C, eluted by methanol, was 10 % of total recovery lipid which mostly contained phospholipids.

These results are in compatible with those of Abdalla and Melton (1991) who found that lipids of fenugreek seeds consisted of 83 % of a fraction containing primarily triacylglycerols and 15 % of a fraction containing polar lipids. Recent data are, also, in close agreement with those of Rajagopalan (1998) who referred that every 100 gram of dry mature fenugreek seeds contained 7.5 lipids, classified to 6.3 gram (84 % of lipids) as neutral lipids; mainly triglycerides, 900 mg (12 % of lipids) as polar lipids containing phospholipids; mainly phosphatidyl choline and phosphatidyl ethanolamine and glycolipids.

The fatty acid composition of the total lipids extracted from fenugreek seeds samples during two seasons are given in Table 3. However, fenugreek seeds were found to contain more than 75 % of unsaturated fatty acids, mainly linoleic and linolenic acids.

Table 3. Influence of foliar application of gibberellic acid concentrations on the fatty acid composition of total lipids (fixed oil) extracted from fenugreek seeds during two consecutive seasons. Data expressed as mean of two determinations.

| Fatty acids (% of total fatty acids) | | Treatments | | | | | |
|---|---|---|---|---|---|---|---|
| | | 2001/2002 season | | | 2002/2003 season | | |
| | | (Control) 0 ppm $GA_3$ | 50 ppm $GA_3$ | 100 ppm $GA_3$ | (Control) 0 ppm $GA_3$ | 50 ppm $GA_3$ | 100 ppm $GA_3$ |
| Myristic | $C_{14:0}$ | 0.55 | 0.45 | 0.50 | 0.45 | 0.40 | 0.55 |
| Palmitic | $C_{16:0}$ | 18.88 | 14.05 | 12.20 | 19.60 | 14.24 | 11.15 |
| Palmitoleic | $C_{16:1}$ | 0.23 | 0.25 | 0.22 | 0.26 | 0.30 | 0.35 |
| Stearic | $C_{18:0}$ | 4.45 | 3.40 | 3.25 | 4.28 | 3.35 | 3.10 |
| Oleic | $C_{18:1}$ | 11.22 | 12.55 | 13.10 | 11.60 | 12.65 | 13.65 |
| Linoleic | $C_{18:2}$ | 35.32 | 37.05 | 38.28 | 35.19 | 37.55 | 38.65 |
| Linolenic | $C_{18:3}$ | 28.93 | 31.80 | 32.10 | 28.22 | 31.16 | 32.25 |
| Arachidic | $C_{20:0}$ | 0.42 | 0.45 | 0.35 | 0.40 | 0.35 | 0.30 |

Hence, the foliar application of gibberellic acid exerted an effect on the fatty acid composition of fenugreek seeds; whereas, unsaturated fatty acids increased by increasing $GA_3$ up to 83 % of total fatty acids.

In general, the percentages of the fatty acids in untreated seeds are in agreement with Mohamed *et al* (1983), Abd El-Hamid *et al* (1984), Abdalla and Melton (1991); Rajagopalan (1998). For instance, Abdalla and Melton (1991) extracted total lipids from fenugreek seeds by different methods. They found that fatty acid composition of different total lipid extracts contained palmitic acid ranged from 9 -11 % and linoleic acid ranged from 41-46 % of total fatty acid composition. They referred that fatty acid composition of total lipids was significantly affected by the lipid extraction procedures. Eid *et al* (2002) indicated that unsaturated fatty acids of fenugreek seeds increased by foliar spraying by Sahara fertilizer at 3 gm/L. No data was found on the effect of plant hormones as $GA_3$ on fatty acid composition of seeds. The results of this study indicated that the foliar application of gibberellic acid increased the unsaturated fatty acids, especially the most important essential fatty acids; linoleic and linolenic acids. Linolenic acid is one of the polyunsaturated fatty acids which is responsible for the auto-oxidation and rancidity of oils, so that extracts of some spices as sage and oregano are added/combined with extracted lipids of fenugreek seeds to extend the shelf life of fenugreek seed oil.

## 4. Essential oil and volatile compounds

Essential oil of fenugreek seeds was extracted by first procedure (Simultaneous Distillation Extraction; SDE) as described in materials and methods. The amount of essential oil extracted from untreated and treated fenugreek seeds was ranged from 0.09 – 0.10 % ( 900 – 1000 mg extract from 100 gram dry ground seeds). Volatile compounds of this extracts were analyzed by gas chromatography and some unknown peaks were identified by Mass Spectrometry. On the other hand, in the second procedure, one gram of each

dried ground seed sample was incubated at 65°C in headspace sampler, then volatile compounds in head-space of bottle was analyzed using Headspace Gas Chromatography (HGC).

The results of volatile compounds by two different procedures (see Materials and Methods) are calculated as percentages of total compounds are listed in Table 4.

However, sixteen compounds were identified by HGC, while 13 identified after extraction of essential oil by SDE meaning that extraction of volatile oil prior volatile compounds analysis could be loose or decompose some compounds due to heating for long time or using different solvent systems. So that headspace GC is a good tool since volatile compounds analyzed directly from raw material.

The volatile oils of different fenugreek seed samples during two seasons consists, mainly, of sotolon, γ-nonalactone , δ- elemene, α- muurolene, Selinene, γ- cadinene, precursor of sotolon and some other minor constituents in addition to unknown compounds. Sotolon was the most abundant compound in the volatile oil of fenugreek seeds and seem to be the most important compound responsible for fenugreek seed aroma. These results are in close agreement with Mazza *et al* (2002) who analyzed volatile compounds of fenugreek seeds using headspace analysis and showed prominent presence of carbonyl compounds such as hexanal, 2-methyl-2-butenal, 3-octen-2-one, sesquiterpene hydrocarbons such as δ- elemene, α- muurolene and, γ-cadinene, alcohols such as pentanol and hexanol, heterocycle compounds such as sotolon, precursor of sotolon, γ-nonalactone and γ-caprolactone and other furan compounds.

Table 4. Influence of foliar application of gibberellic acid concentrations on the volatile compounds of fenugreek seeds during two consecutive seasons.

| Volatile compounds (% of total compounds) | Treatments | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2001/2002 season | | | | | | 2002/2003 season | | | | | |
| | (Control) 0 ppm $GA_3$ | | 50 ppm $GA_3$ | | 100 ppm $GA_3$ | | (Control) 0 ppm $GA_3$ | | 50 ppm $GA_3$ | | 100 ppm $GA_3$ | |
| | 1st M | 2nd M | 1st M | 2nd M | 1st M | 2nd M | 1st M | 2nd M | 1st M | 2nd M | 1st M | 2nd M |
| 3-hydroxy-4,5-dimethyl-2 (5H)-furanone (Sotolon) | 19.8 | 21.5 | 19.1 | 19.6 | 18.8 | 20.5 | 18.8 | 20.5 | 17.3 | 18.5 | 17.2 | 18.4 |
| 3-amino-4,5-dimethyl-3,4-dihydro-2(5H)-furanone (precursor of sotolon) | 8.6 | 5.8 | 7.8 | 7.8 | 8.5 | 7.8 | 8.5 | 7.8 | 7.5 | 7.6 | 7.5 | 5.5 |
| Dihydroxy-5-pentyl-2 (3H)-furanone (δ-nonalactone) | 11.9 | 8.3 | 10.1 | 7.1 | 11.9 | 10.5 | 11.9 | 10.5 | 9.0 | 9.5 | 9.4 | 9.7 |
| Dihydroxy-5-ethyl-2(3H)-furanone (δ-caprolactone) | ND | 5.8 | ND | 6.9 | 7.2 | 7.6 | 7.2 | 7.6 | 6.2 | 6.9 | 6.7 | 5.8 |
| δ- elemene | 11.6 | 13.9 | 13.6 | 15.8 | 11.6 | 10.9 | 11.6 | 10.9 | 15.6 | 14.7 | 15.0 | 15.9 |
| γ- cadinene | 8.1 | 8.5 | 9.0 | 8.1 | 9.1 | 10.5 | 9.1 | 10.5 | 8.1 | 8.5 | 8.8 | 8.5 |
| α- muurolene | 11.2 | 13.8 | 13.0 | 13.1 | 10.2 | 12.4 | 10.2 | 12.4 | 14.2 | 14.8 | 14.0 | 15.8 |
| Selinene | 10.8 | 11.2 | 11.6 | 11.8 | 9.5 | 10.2 | 9.5 | 10.2 | 11.8 | 10.0 | 10.8 | 10.5 |
| Hexanal | 4.0 | 1.5 | 5.0 | 1.5 | 3.2 | 1.5 | 3.2 | 1.5 | 2.2 | 1.7 | 2.4 | 1.8 |
| 2-methyl-2-butanal | 2.3 | 1.5 | 2.2 | 1.5 | 1.4 | 1.5 | 1.4 | 1.5 | 1.3 | 0.7 | 1.5 | 0.9 |
| 3-Octen-2-one | 1.8 | 1.1 | 0.7 | 1.1 | 0.8 | 1.1 | 0.8 | 1.1 | 0.8 | 1.2 | 0.8 | 1.5 |
| Trans-cis-3,5-octadien-2-one | 1.8 | 1.8 | 2.0 | 1.3 | 1.1 | 1.8 | 1.1 | 1.8 | 1.1 | 0.9 | 1.3 | 1.0 |
| Pentanol | 2.5 | 1.2 | 2.4 | 1.1 | 1.5 | 1.2 | 1.5 | 1.2 | 0.5 | 1.6 | 0.9 | 1.4 |
| Hexanol | ND | 0.8 | ND | 0.6 | 0.6 | 0.8 | 0.6 | 0.8 | 0.6 | 0.7 | 0.6 | 0.8 |
| 2-methyl-2-buten-1-ol | 0.8 | 0.9 | 0.7 | 0.6 | 0.8 | 0.9 | 0.8 | 0.9 | 0.8 | 0.9 | 0.4 | 1.0 |
| 1-octen-3-ol | ND | 0.6 | 0.4 | 0.7 | 0.5 | 0.6 | 0.5 | 0.6 | 0.5 | 0.7 | 0.5 | 0.5 |
| Unknown compounds | 4.8 | 1.8 | 2.4 | 1.4 | 3.3 | 0.2 | 3.3 | 0.2 | 2.5 | 1.1 | 2.2 | 1.0 |

The foliar application of gibberellic acid of various levels during two seasons had no clear effect on the composition of volatiles. No publications was found respecting the influence of such hormones on the volatile compounds of legume seeds.

## CONCLUSION

Fenugreek seeds "variety Giza 30" are rich in protein, essential amino acids, crude fiber, carbohydrates and different minerals as potassium, phosphorus, calcium, magnesium and iron. The levels of crude protein, essential amino acids, trigonelline, potassium, phosphorus and calcium were increased by increasing foliar application of gibberellic acid concentrations on fenugreek plants.

Total lipids extracted from fenugreek seeds eluted by chloroform showed the highest percentage of triglycerides. These total lipids were found to contain more than 75 % of unsaturated fatty acids, mainly linoleic and linolenic acids. These essential fatty acids were increased by increasing foliar application of $GA_3$ on fenugreek plants.

Sixteen volatile compounds were identified by HGC while 13 compounds were identified after extraction of essential oil by SDE meaning that extraction of volatile oil prior volatile compounds analysis could be loose or decompose some compounds due to heating for long time or using different solvent systems. The volatile oil of different fenugreek seed samples consists mainly of sotolon, γ-nonalactone , δ- elemene, α- muurolene, Selinene, γ- cadinene, precursor of sotolon and some other minor constituents in addition to unknown compounds. Sotolon was the most abundant compound in the volatile oil of fenugreek seeds and seem to be the most important compound responsible for fenugreek seed aroma.

Generally, the foliar application of gibberellic acid improved the nutritional value of fenugreek seeds due to the increase in protein level, essential amino acids and essential fatty acids.

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## الملخص العربى

## تأثير حمض الجبريليك على التركيب الكيماوى لبذور الحلبة

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تم إجراء تجربة حقلية بمزرعة الكلية خلال موسمى ٢٠٠١/٢٠٠٢ ، ٢٠٠٢/٢٠٠٣ ورش تركيزات ٥٠ و ١٠٠ جزء فى المليون من حمض الجبريليك على نبات الحلبة "صنف جيزة ٣٠ " ودراسة تأثيرة على التركيب الكيمـــاوى لبذور الحلبة. وفى بذور الحلبة غير المعاملة والبذور المعاملة بحمض الجبريليك خلال الموسمين تم تقديـــر proximate analysis والـــتى شـــملت الرطوبة والبروتين الخام والسكريات والليبيدات والألياف الخام والرماد والترايجونيلين وبعض المعادن. كما تم تقدير كل من الأحماض الأمينية بجهاز amino acid analyzer ودراسة أقسام الليبيدات بواسطة silica cartridge وكذلك TLC وتم تقدير الأحماض الدهنية بواسطة Gas

Chromatography وإستخلاص الزيوت العطرية ( الطيارة) بواسطة Simultaneous Distillation Extraction (SDE) وتقدير المركبات الطيارة فى الزيوت العطرية المستخلصة بطريقة SDE أو تقديرها مباشرة فى البذور المطحونة بواسطة Headspace Gas Chromatography( HGC) .

أوضحت النتائج أن بذور الحلبة تحتوى على نسبة مرتفعة من البروتينات والكربوهيدرات والألياف كما أنها تحتوى على كل من الأحماض الأمينية والدهنية الضرورية لجسم الإنسان. وقد إزداد محتوى البذور المعاملة من البروتينات والأحماض الأمينية والدهنية الضرورية وبعض المعادن وكذا الترايجونيلين بزيادة تركيزات حمض الجبريليك. وأوضحت دراسة الليبيدات أن زيت بذرة الحلبة يحتوى على ٨٧% جليسريدات ثلاثية وتمثل الأحماض الدهنية عالية عدم التشبع والضرورية لجسم الإنسان (اللينولييك واللينولينيك) أكثر من ٧٥% من تركيب الليبيدات. كما أوضحت دراسة الزيوت العطرية أن إستخدام جهاز HGC كان الأفضل فى تقدير المركبات الطيارة مباشرة من البذور المطحونة وقد وجد أن أهم المركبات الطيارة فى بذور الحلبة هى sotolon يليها γ-nonalactone , δ- elemene, α- muurolene, Selinene وبناء على هذة النتائج يمكن القول أن رش نبات الحلبة بحمض الجبريليك يكون له تأثير إيجابى على القيمة الغذائية لبذور الحلبة نظرا لزيادة محتواها من البروتين وبعض المعادن والأحماض الأمينية والدهنية الضرورية لجسم الإنسان.