

PHYTOCHEMICAL COMPOUNDS AND ANTIOXIDANT ACTIVITY OF SOME LENTIL GENOTYPES GROWN IN EGYPT

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ABSTRACT: *The present study was carried out to compare and evaluate different varieties of Lentil seeds grown in Egypt for selecting the high quality varieties . The obtained results revealed that, lentil variety Giza 51 contains the highest crud protein content 25.55% followed by variety Sinai 1 which recorded 24.83% while the lowest value of crud protein was 23.10% for variety Giza 29 . potassium is the major element among all of the determined minerals content, Sinai1 had the highest potassium 420 mg/100g for all samples, Furthermore, Giza 370 contain the highest content of iron 11.30 mg/100g . Giza 51 variety had the highest content of total polyphenols which was 720 mg GAE /100g followed by variety Sinai 1 which 710 mg GAE /100g. In addition, Giza 51 variety presented the highest DPPH scavenging activity 63.50%, followed by Sinai 1 62.42%, while Giza 9 contain the highest content of folate which was 196. µg /100g followed by Sinai 1 which recorded 193.0 µg /100g. On the other hand, the phytic acid, content of the different lentil seed varieties were ranged from 0.49 to 0.56%. Furthermore, (H.C.A.) ranged from 106.70% for Giza 29 to 118.80% for Sinai 1. However, Giza 9 had the highest cookebility which was 96.50% followed by Sinai 1 which recorded 91.70%.*

Key words: *Lentil - Phytochemical compounds - Cooking quality.*

INTRODUCTION

Lentil (*Lens culinaris*) is an ancient crop of classical Mediterranean civilization and continues to play an important role in human health (Zohary and Hopf 2000). Legumes such as beans, chickpeas and lentils, are seen as staple foods and they are nutritious and improve health known as the 'meat of the poor people. Because of their high protein content, beans are now presented as a staple food for vegetarians and for people affected by nutrition related health problems, such as diabetes, obesity, and over-weight (Leterme and Munoz 2002). Lentil (*Lens culinaris* Medikus) is excellent source of proteins, carbohydrates and dietary fibers. Besides, legume flours provide many essential amino acids, vitamins, minerals, oligosaccharides and phenol compounds (Roy *et al.*, 2010). However, antinutritional factors such as phytic acid, trypsin inhibitors and tannins, which are present

in lentil was considered undesirable for obstructing the bioavailability of minerals (Wang and Daun 2006) and they compromise the protein digestibility, reducing the nutritional value of this food (Rathod and Annapure (2016). Therefore, it must be substantial reduction or complete elimination of these antinutrients before they can be safely consumed. The effects of processing on antinutritional factors vary notably, depending on the techniques and conditions, including time, temperature and moisture content, which in turn can enhance the bioavailability of proteins and minerals (Alonso, *et al.*, 2000). Lentil seeds have high levels of iron, potassium, folate, and niacin and are often recommended for vegetarians who need to supplement their diet with additional iron and protein. (Chung, *et al.*, 2008). Lentils possessed the highest concentrations of phenolic contents and antioxidant activity, and the higher

antioxidant activity was strongly correlated with the phenolic contents (Xu and Chang 2007). Dietary antioxidants may play an important role in protecting the cell against damage caused by free radicals. Consumption of foods containing antioxidants may prevent some diseases and therefore, it is very important to determine their antioxidant capacity in order to estimate the repercussion on oxidative stress in living beings (Doblado *et al.*, 2007).

Also, lentil seeds are bioactive dietary supplements and as an antioxidant protect the human body from free radicals as well as retard the progress of many chronic diseases including hypertension, diabetes mellitus, cardiovascular diseases and cancer (Ford *et al.*, 2007).

The present study was carried out to compare and evaluate some varieties of Lentil grown in Egypt for selecting the highest quality varieties.

MATERIALS AND METHODS

Materials:

Five Lentil seeds varieties namely Giza 9, Giza 29, Giza 51, Giza 370 and Sinai 1 were obtained from fields crops Research Institute, Agric. Research Center, Ministry of Agric. Giza. Egypt.

Preparation of lentil samples:

Lentil seeds varieties were cleaned and divided into two parts the first one was used as Lentil seeds, where the second was milled to obtain the Lentil flours. The Lentil seeds and flours were kept in polyethylene bags and stored in freezer at -18°C until further analysis.

Proximate chemical composition of lentil seeds:

Moisture, ash, crude protein content (N x 6.25) and crude fat of the samples were determined according to the method described in A. O. A. C. (2005). Total carbohydrates content was calculated by

difference as follows: Total carbohydrates% = 100 – (crude protein% + ether extract% + ash%) on dry weight basis.

Determination of minerals content:

The sample was digested using concentrated HNO₃ for 2 hrs (till the solution become colorless) and diluted to 100 ml with distilled water. Calcium, manganese, magnesium, iron, zinc and copper contents of lentil samples were determined using the atomic absorption spectrophotometer Perkin Elmer Model 20180 following the method of Pearson (1976). Potassium and sodium contents of lentil samples were estimated using flame photometer as given by Pearson (1976). On the other hand, phosphorus was determined colorimetrically using ascorbic acid method as described by Murphy and Riley (1962).

Determination of phytochemical compounds :

Extraction of total phenolic compounds:

Total phenolic compounds were extracted according to the method described by Fernandez-Orozco *et al.* (2009). Flour of lentil seeds were subjected to phenolics extraction using methanol 95%. The extraction process was continued for 24 h at room temperature by stirring the flours in the solvent. The extracts were centrifuged at 4000 rpm for 15 min and filtered through filter paper (Whatman No. 41). The solvent was removed from the extracts using a rotary evaporator at 40°C under vacuum. The extracts were further dried using vacuum oven at 40°C and then kept in dry clean closed black glass bottle at 4°C for further analysis

Determination of total phenolic :

Total phenol compounds content was determined according to (Gutfinger, 1981). The method is based on the colour

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reaction of Folin-Ciocalteu reagent with hydroxyl groups. Reaction absorbance was measured at 760 nm using a spectrophotometer. The results were expressed as mg gallic acid per 100g of extract.

Antioxidant activity (DPPH) assay:

Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method described by Soler-Rivas, *et al.* (2000). Folate contents was determined according to the method of A. O. A. C. (2005).

Determination of antinutritional factors:

- 1- Phytic acid was measured using the method described by Fruehbeck *et al.*, (1995).
- 2- Tannins in the methanol extracts were measured according to the vanillin method of Price *et al.*, (1978).
- 3- Trypsin inhibitor was analyzed as described by Kakade *et al.*, (1974).

Determination of Cooking quality of lentil seed :

A- Hydration coefficient of seeds before cooking (H.C.B. %) 10 g dry seeds from each experimental varieties were soaked in tap water for 8 hr. at 25^oc then was calculated as

$$\text{(H.C.B. \%)} = \frac{\text{weight of soaked seeds} - \text{weight of dry seeds}}{\text{weight of dry seeds}} \times 100.$$

B - Hydration coefficient of seeds after cooking (H.C.A. %) was calculated by placing 10 g of dry seeds from each experimental varieties in placed glass tube 100 cm containing enough water . the tubes were put in oven for 2 hr. at 100c the Hydration coefficient of seeds after cooking (H.C.A.) was determined in cooked seeds according to the method of Selim, (2000) as follows

$$\text{(H.C.A. \%)} = \frac{\text{weight of cooked seeds} - \text{weight of dry seeds}}{\text{weight of dry seeds}} \times 100.$$

C- The total soluble solids (T.S.S. %) was determined in Hydration coefficient of seeds after cooking (H.C.A. %) above by drying at 60 c over night . (T.S.S. %) was calculated as Selim, (2000) .

$$\text{(T.S.S. \%)} = \frac{\text{weight drying} - \text{weight of empty pot}}{\text{initial weight}} \times 100.$$

D - The Seed cookebility% of lentil seeds was measured by using the normal press of fingers and comparing between the cooked seeds for their hardness, which means the maximum force throughout their test. This cooking test was made according to the method described by Selim, (2000).

Statistical analysis:

Most of the received data were analyzed statistically using the analysis of variance and the means were further tested using the least significant difference test (LSD) as outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Proximate chemical composition of different lentil seed varieties :

The chemical composition of five lentil seed varieties were determined and the results are recorded in Table (1). The moisture content of the five lentil seed varieties were ranged from 9.03 to 11.23%. However, lentil variety Giza 51 contains the highest content of crud protein 25.55% followed by lentil variety Sinai 1 24.83% while the lowest value of crud protein was 23.10% for lentil variety Giza 29 . These results are in line with those (Barbana and Boye 2011 and Rathod and Annapure 2016). They reported that, protein content can range from 20.6 to 31.4 (g/100 g) depending on lentil cultivar and soil or climate characteristics. Apparent also from the same table that, lentil seed varieties contain from 1.55 to 1.97% crud fat, 2.27 to 2.70% ash content, and 70. 02

to 72. 80% total carbohydrate. These results are in line with those (Ghavidel and Prakash, 2007 ; Dilis and Trichopoulou 2009; Silva-Cristobal *et al.*, 2010; Karaca *et al.* , 2011 and Ma *et al.*, 2011) . Furthermore, chemical composition of lentil is generally affected by environmental and genetic factors Eyaru, *et al.*, (2009) .

Minerals content of different lentil varieties:

Minerals or elements play an important role in human nutrition, some are essential for much component as hem for blood. magnesium and manganese for the activation of some enzymes and

stimulation insuline activity, calcium and phosphorus are essential for bones. Potassium is very important for cardiovascular diseases. (National Academy of Sciences, 2001). Minerals content of five lentil varieties were determined and the data were recorded in Table (2). The results indicate that potassium is the major element among all of the determined mineral contents. In addition, Giza 370 had the highest potassium compared to the other samples that recorded 955 mg k/100g, followed by Giza 51 940 mg k/100g. while, the lowest potassium content was 890 mg k/100g for Giza9 .

Table (1): Gross chemical composition of different lentil varieties .

lentil Varieties	Moisture%	Protein%	fat%	Ash%	Total carbohydrates%**
Giza 9	*10.68 b	*24.40 b	*1.97 a	*2.38ab	*71.25 c
Giza 29	9.03 d	23.10 d	1.83 ab	2.27 b	72. 80 a
Giza 51	11.23 a	25.55 a	1.73 b	2.70 a	70. 02 d
Giza 370	11.00 ab	23.70 c	1.67 b	2.40ab	72.23 b
Sinai 1	9.26 c	24.83 ab	1.55 c	2.31 b	71.32 c

* Each value is an average of three determinations.

+ Values followed by the same letter in Column are not significantly different at P ≤ 0.05

**Total carbohydrates was calculated by difference.

Table (2): Mineral contents (mg/100g) of different lentil varieties (on dry weight basis).

samples Mineral Elements	Giza 9	Giza 29	Giza 51	Giza 370	Sinai 1
P	410 ab	400 b	380 c	350 d	420 a
Na	11.22 b	8.90 e	10.10 c	12.10 a	9.50 d
K	890 e	900 d	940 b	955 a	920 c
Ca	71 ab	59 d	69 b	75 a	65 c
Mg	115 b	112 c	110 c	100 d	121 a
Fe	11.0 a	8.90 c	10.50 b	11.30 a	9.2 c
Zn	3.60 b	3.10c	3.50b	2.70 d	3.9 a

* Each value is an average of three determinations.

+ Values followed by the same letter in row are not significantly different at P ≤ 0.05

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However, the data in the same table revealed that, Giza 370 contain the highest content of iron which was 11.30 mg fe /100g followed by Giza 9 which recorded 11.0 mg fe /100g . while the lowest value of iron was (9.2 mg fe /100g) for Sinai 1. The iron is important for the schoolchildren, which mostly needs more iron to avoid the anemia especially in developing countries. The results indicated that lentil varieties are a good source for the minerals. Furthermore, Sinai1 contain the highest content of magnesium which was 121.0 mg/100g while the lowest value of iron was (100 mg fe /100g) for Giza 370. The results are in agreement with that reported by(Chung, *et al.* , 2008).In addition, Dilis and Trichopoulou (2009) reported that, Legumes are generally characterized by its high mineral levels, a feature that depends on the species, the agronomic cultivar and certain characteristics of the soil where its grow.

Total Phenolics Content, Antioxidant activity and folate contents of different lentil varieties:

Legumes contain several antioxidant compounds such as vitamins C and E,

phenolic compounds and reduced glutathione (Fernandez-Orozco *et al.*, 2009), which are considered to be natural antioxidants, representing an important group of bioactive compounds in foods, and may prevent the development of many diseases. Phenolic compounds do not only effectively prevent oxidation in foods; they also act as protective factors against oxidative damage in the human body. The antioxidant activity of phenolics is related to their chemical structure (Lopez-Amoros *et al.*, 2006). Total phenols extracted from different lentil seed varieties were determined and the results are recorded in Table (3). lentil variety Giza 51 contained the highest content of total phenols which was 720 mg GAE /100g followed by lentil variety Sinai 1 which recorded 710 mg GAE /100g, while the lowest value of total phenols was 625 mg GAE /100g for lentil variety Giza 29. The difference might be attributed to the genotype of lentils. The obtained results were in same trend with (Amarowicz *et al.*, 2009; Dilis and Trichopoulou 2009; Silva-Cristobal *et al.*, 2010; Zou *et al.*, 2011 ; Gharachorloo *et al.*, 2012 and Rathod, and Annapure 2016).

Table (3): Total Phenols Contents (mg GAE/100 g) , DPPH (%) radical scavenging activity and folate Contents (µg /100 g) of different lentil varieties (on dry weight basis):

Varieties	TPC(mg GAE /100 g)	DPPH%	folate (µg/100 g)
Giza 9	700.20 c	61.22 c	196 a
Giza 29	625.33 e	60.11 d	184 d
Giza 51	720.50 a	63.50 a	189 c
Giza 370	650.73 d	60.65 d	186 d
Sinai 1	710.55 b	62.42 b	193 b

* Each value is an average of three determinations.

+ Values followed by the same letter in Column are not significantly different at P ≤ 0.05

TPC : Total polyphenols content(mg galic acid /100g)

Antioxidant activity (DPPH%)

The results were shown in Table (3), all sample extracts possessed good DPPH radical scavenging activity. lentil variety Giza 51 presented highest DPPH scavenging activity (63.50%), followed by Sinai 1 (62.42%), Giza 9 (61.22%), Giza 370 (60.65%), and finally by Giza 29 (60.11%). Our results were in agreement with. (Amarowicz *et al.*, 2009 ; Zou *et al.* 2011 and Gharachorloo, *et al.*, 2012). In addition, Atienza *et al.* (1999) found that, legumes contain, beside phenolics, other bioactive compounds such as vitamins and carotenoids in different concentrations that can also affect the antioxidant activity of the samples. These compounds may exert a synergetic effect with phenolic compounds, which could be the reason for the observed differences in the antioxidant activity. It is also clear from the same Table that, Giza 9 contain the highest content of folate which was (196.0µg/100g) followed by Sinai 1 which recorded (193.0µg/100g) while the lowest value of folate was (184.0µg/100g) for Giza 29. These results are in the same trend of those reported by (Messina . 1999 and Chung, *et al.*, 2008). They reported that, lentil are an excellent source of folate, which in addition to being an essential nutrient is thought to reduce the risk of neural tube defects . One serving of beans provides more than half of the current RDA folate .

Antinutritional factors content in lentil seeds:

Antinutritional factors such as phytic acid, trypsin inhibitors and tannins, which are present in lentil was considered undesirable for obstructing the bioavailability of minerals and they compromise the protein digestibility, harming the nutritional value of this food (Rathod, and Annapure 2016). Phytic acid, tannins content and trypsin inhibitors activity were determined in five lentil seed varieties and results are recorded in Table (4). The phytic acid, content of different lentil seed varieties were ranged from (0.49 to 0.56%).

On the other hand, lentil seed Giza 9 contains the highest content of tannins, which was 0.70% followed by lentil seeds Giza 370 which recorded (0.69%) while the lowest value of tannins was (0.64%) for lentil seeds Sinai 1. Results also in the same Table indicate that, trypsin inhibitors activity of different lentil seed varieties were ranged from 2.82 (IU/mg dry mater) for Giza 29 to 3.12 (IU/mg dry mater) for Giza 9. In addition, no significant differences between the different studied types of lentil seed varieties for their trypsin inhibitors activity. These results are in line with those (Ghavidel and Prakash 2007; Dilis and Trichopoulou 2009 and Rathod and Annapure 2016) .

Cooking quality of different lentil seed varieties (on dry weight basis):

The cooking quality of five lentil seed varieties were determined and the results are recorded in Table (5). The values of Hydration coefficient of seeds before cooking (H.C.B.) for different lentil seed varieties were ranged from 116.60% for Giza 29 to 129.70% for Giza 370. Same results were found by Selim (2000). Who reported that, high capacity to absorb water indicate good seed quality.

The percentage amount of water uptake by cooked seeds as represented by Hydration coefficient after cooking (H.C.A.) ranged from 106.70% for Giza 29 to 118.80% for Sinai 1. However, lentil variety Giza 9 contain the highest content of cookebility % which was (96.50%) followed by lentil variety Sinai 1 which recorded (91.70%) while the lowest value of cookebility % was (88.40%) for lentil variety Giza 29 . These results are in the same trend of those reported by (Ezzat, Zakia, *et al.*, 2005 and Selim, 2000).

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Table (4): Phytic acid (%), tannins content (%) and trypsin inhibitors content (IU/mg dry mater) of different lentil seed varieties (on dry weight basis).

Varieties	Phytic acid (%)	Tannins (%)	Trypsin inhibitor (IU/mg dry mater)
Giza 9	0.53 ab	0.70 a	3.12 a
Giza 29	0.49 b	0.67 a	2.82 a
Giza 51	0.55 a	0.65 ab	2.96 a
Giza 370	0.50 b	0.69 a	2.90 a
Sinai 1	0.56 a	0.64 b	3.03 a

* Each value is an average of three determinations.

+ Values followed by the same letter in Column are not significantly different at $P \leq 0$.

Table (5): Cooking quality of different lentil seed varieties .

Varieties	(H.C.B.)	(H.C.A.)	T.S.S. %	(coc.%)
Giza 9	120.40 c	110.50 b	1.50 b	96.50 a
Giza 29	116.60 d	106.70 c	1.14 c	88.40 c
Giza 51	123.50 b	110.00 b	1.97 a	89.30 c
Giza 370	129.70 a	108.40 c	0.80 d	89.50 c
Sinai 1	117.50 d	118.80 a	1.16 c	91.70 b

* Each value is an average of three determinations.

+ Values followed by the same letter in Column are not significantly different at $P \leq 0.05$

Hydration coefficient after cooking (H.C.A.)

Hydration coefficient before cooking (H.C.B.)

cookebility (coc.%)

For the total soluble solids (T.S.S. %) data in 5 table clearly showed significant variation among the tested samples. Giza 51 gave the highest value of T.S.S., 1.97, while Giza 370 recorded the lowest value of T.S.S., 0.97 . (Ezzat, Zakia, *et al.*, 2005 and Selim, 2000).

Conclusion:

From the obtained results, it can be concluded that: lentil variety Giza 51 contain the highest content of crud protein , minerals , total Phenolics content and antioxidant activity followed by variety Sinai 1 furthermore, Giza 370 contain the highest content of iron and potassium on the other hand, Giza 9 variety contain the highest content of folate and fat .Based on the above, it is recommended to use lentil seeds as a source of minerals, proteins and antioxidants,

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