



SOME CHEMICAL, NUTRITIONAL AND BIOLOGICAL PROPERTIES OF CHICKPEA (*Cicer arietinum* L.)

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ABSTRACT

This study was carried to evaluate some chemical, nutritional and biological properties of chickpea seeds. Raw seeds were soaked for 6 and 12 hr., and directly cooked under atmospheric pressure until swelled and become more tender. Although protein content increased by 3% -8%, while fat and ash decreased by 17%, 21% and 20%, 34% after soaking-cooking for 6 and 12 hr., respectively. Raw seeds possessed 1.89 mg/g phytic acid. However gradual decrease was observed by soaked-cooked treatments. Similar results were noticed in tannins and trypsin inhibitor. Eighteen amino acids were detected in either raw or soaking-cooking seeds, that included essential and non- essential amino acids. In raw seeds, arginine, lysine and leucine were the predominate essential amino acids followed by phenylalanine, isoleucine, valine and threonine. The concentration of these constituent was decreased after soaking-cooking for 12 hr., except leucine, isoleucine and threonine. Methionine was the most deficient amino acid and valine was the second limiting amino acid in raw and soaked 6 and 12 hr., In the biological experiment diabetic rats fed on raw chickpea exhibited a significant decrement in blood glucose by 41% compared with the diabetic control group, it was the most effective at the hypoglycemic agent.

Key word: Chickpea, chemical composition, antinutritional factor, cooking, serum glucose.

INTRODUCTION

Legumes play an important role in human nutrition since they are rich sources of plant protein, calories, certain minerals and vitamins. In African diets legumes are also, the major contributors of protein and calories for economic and cultural reasons. They are generally consumed after processing into various products, like milling into dehulling, puffing or roasting into snack food, grinding into flour for different food preparation or as germinated grains. Soaking is a domestic technological treatment that is often used by mothers to prepare complementary foods at home. It can be a simple prolongation of the obligatory washing of the seeds and can also have other advantages, such as facilitating dehulling or swelling of seeds. Traditional

methods of processing and cooking legumes have been evolved to produce acceptable, appetizing and nutrition products. Processing of legumes increases the digestibility and enhances the aroma, sensory qualities, nutritional attributes and inactivate antinutritional factors (Tharanathan and Mahadevamma, 2003; El-Maki *et al.*, 2007). Chickpea (*Cicer arietinum* L.) is one of the oldest and most widely consumed legume in the world and the most important grain- legume crops in the world with the Asia region contributing most to the production, 7.67 million tonnes (Mt) of the 10.38 Mt of the world chickpea production.

Chickpea seeds can play an important role as a low- glycaemic functional ingredient in a healthy diet (Bhatty *et al.*, 2000; FAO, 2004; Angulo-Bejarano *et al.*, 2008; Shirani and Ganesharane, 2009). Although, Bessar and El-

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Sayed (1997), suggested little or no changes in the essential amino acids after soaking of some legumes. Alajaji and El-Adawy (2006) reported that heat of cooking decreased the concentration of essential amino acids in chickpea.

Diabetes mellitus (DM) is the most significant chronic disease and it is one of the causes of death in the modern society. Diabetes mellitus (DM) is divided into two major categories: type 1 and type 2. Type 2 of diabetes have a distinct pathogenesis, but hyperglycemia and various life-threatening complications, resulting from long-term hyperglycemia, are their most common features (Hyen-Lee *et al.*, 2006). Legume, generally have low glycemic index values, low glycemic load diets have been associated with reduced risk of developing (T2DM) in several large prospective studies. It have been shown to decrease blood glucose responses compared to the other cereal based foods such as whole meal bread and are of very benefit in all the diabetes and hyperlipidemia patients (Madhusudhan and Tharanathan, 1995; Salmeron *et al.*, 1997). Legume is recommended for better glucose control in persons with diabetes (Nestel *et al.*, 2004; Liang *et al.*, 2008). It have been found to have potent antidiabetic effect (hypocholesterolemic and hypoglycemic effect) on account of their various constituents that comprise protein, fat, fibre and isoflavones (Kaushik *et al.*, 2010).

This investigation was conducted to evaluate some chemical, nutritional and biological properties of chickpea.

MATERIALS AND METHODS

Materials

About 15 kg of chickpea (*Cicer aritinum* L.) seeds were purchased from the local market in Giza, Egypt during 2010-2011 season.

Methods

Preparation of raw chickpea (soaking and cooking)

Chickpea seeds were sorted carefully rinsed and drained, the clean seeds were divided into three equal portions of a known weight. The first portion was used as a raw seed, the second and third portions were soaked in tap water at room

temperature ($25\pm 1^\circ\text{C}$) at a ratio of 1:5 (W/V) for 6 or 12 hours, respectively. After soaking, seeds were directly cooked under atmospheric pressure. Cooking time of soaked seeds was expressed as the time required for splitting 70% of the whole seeds. Cooked chickpea seeds were drained and dried in a hot air oven at 55°C for 18-24 hours according to the method of Jood *et al.* (1988). The dried seeds were milled with laboratory mill (MLW, T Hype : SKI, watt 100, West Germany) and designed as T1 and T2 for chickpea soaked for 6 and 12 hr., and cooked, respectively.

Chemical analyses

Moisture content, ash, ether extract, total crude protein and crude fiber were determined according to the methods described by AOAC (1995). Total carbohydrates were calculated by difference.

Antinutritional factors

Pytate, trypsin inhibitor and tannins. Phytate content was extracted according to the method of Mohamed *et al.* (1986). Trypsin inhibitor content was assayed according to the method of Hamerstrand *et al.* (1981). Tannin content was evaluated according to the method reported by Burns (1971).

Amino acids determination

Amino acid contents were determined according to the methods described by Millipore Co-Operative (1987). Acid hydrolysis was carried out to evaluate the amino acids except cosine and tryptophan. Sample (100mg) was weighted in a burning tube containing 5ml of 6N HCL. The tube was sealed under vacuum and placed in 110°C oven for 24 hr. After evaporation, the dried residue was dissolved in citrate buffer (pH 2.2). The technique of high performance amino acid analyzer (Knauer Germany) was used. Tryptophan was chemically determined after alkali (Na OH) hydrolysis by the method of Blauth *et al.* (1963). The amount of essential amino acids of protein were calculated as a percentage of its content in a standard amino acid pattern which was the provisional amino acid scoring idea protein by the joint FAO/WHO Committee (1973).

$$\text{EAA score} = \frac{\text{Test amino acid}}{\text{Reference amino acid}} \times 100$$

Biological evaluation

Thirty albino male rats were purchased from the laboratory animal Department, Research, Institute of Ophthalmology, Giza, Egypt. Rats were housed in plastic cages under normally healthy conditions for two weeks and fed on basal diet. The animal room was maintained at (22°C±2°C) with timed lighting from 7 am to 19 pm and relative air humidity of 40% to 60%. The initial weights of rats were in the rang 100±10 g. (Arafa *et al.*, 2008).

Basal diet

The basal diet formula was prepared according to the method described by Reeves *et al.* (1993). It contains casein 14% (≥ 85% protein) cellulose (5%), vitamin mixture (1%), salt mixture (3.5%), sucrose (10%), choline chloride (0.2) and corn oil (10%). The basal diet was completed to 100g with corn starch (Table1).

Preparation of diabetic rats

Rats were injected with freshly prepared Alloxan (150 mg / kg). Three days after administration of Alloxan serum glucose levels were determined. Rats exhibiting blood glucose levels more than 300 mg /dl were included in the study .Six rats were used in each experiment (Ahmed *et al.*, 2005).

Nutritional experiments

Thirty male rats were used in the nutritional experiment after the adaptation period for two weeks. Rats were randomly divided into 5 groups, each contains 6 rats as follows: Group (1): was fed on the basal diet and considered as a control group negative (non diabetic rats) Group (2): fed on the basal diet and considered as control positive group (diabetic rats). Group (3): fed on raw chickpea untread). Group (4): fed on dried cooked seed of chickpea after 6 hr., of soaking. Group (5): fed on dried cooked seed of chickpea after 12 hr., of soaking (Table 1).

Blood sampling

Three days after administration of Alloxan. the blood samples were taken and after 15, 30 and 45 days of the experiments. The blood samples were obtained from orbital venous plexus by means of fine capillary glass tubes.

Each sample was allowed to clot for 1 min at 5°C centrifuged at 1500 rpm for 15 min, and the supernatant was frozen (-10°C) until analysis. Blood glucose was determined according to Trinder (1969).

Statistical analysis

Data were statistically analyzed by using SPSS software (SPSS, 2007). The results were expressed as ± SEM and the statistical analysis performed using one-way analysis of variance followed by Duncan's tests.

RESULTS AND DISCUSSION

Effect of Soaking and Cooking Treatments on Chemical Constituent of Chickpea Seeds

Raw chickpea seeds were soaked in tap water for 6 or 12 hr., and directly cooked after soaking, the seeds were swelled and became more tender by cooking treatment. Table 2 shows the chemical constituents of raw, soaked 6 and 12 hr., and cooked chickpea T₁ and T₂. Moisture content of raw seeds recorded 7.83% and increased to 8.43% and 9.28% after soaking-cooking for 6 and 12 hr., respectively. Cooking caused a significant increase in protein content by 3% and 8% after soaking for 6 and 12 hr., respectively. Significant reduction was also noted in fat and ash contents, they reached 17%, 21% and 20%, 34% after soaking- cooking for 6 and 12 hr., respectively. These results agree with (Wang, *et al.*, 2009) who reported that the increase of protein in cooked lentil may be due to the loss of soluble solids during cooking which would increase the concentration of protein in cooked seeds. While the decrease of ash content might be from diffusion of certain minerals into the cooking water. Meanwhile crude fiber of raw chickpea was 4.17%, cooking cause a significant increase in fiber content (4.88% and 5.37%) after soaking for 6 and 12 hr., respectively. Bressani (1993), reported that the increase in crude fiber of chickpea could have been due to protein-fiber complexes formed after possible chemical modification induced by the soaking and cooking of dry seeds. Moreover soaking and cooking treatments caused reducing chickpea carbohydrates, these results agree with (Almeida Costa *et al.*, 2006).

Table 1. Diet composition for experimental rats (g/100g)

Diet component	G ₁	G ₂	G ₃	G ₄	G ₅
Casein	14	14	-	-	-
Chickpea	-	-	49.93	47.98	45.74
Choline chloride	0.2	0.2	0.2	0.2	0.2
Sucrose	10	10	10	10	10
Corn oil	10	10	6.90	7.55	7.78
Cellulose	5	5	2.90	2.66	2.55
Salut mixture	3.5	3.5	1.58	2.03	2.35
Vitamin mixture	1	1	1	1	1
Starch	56.3	56.3	27.49	28.58	30.38

G₁ (normal rats), G₂ (diabetic rats), G₃ (diabetic rats were fed on raw chickpea)

G₄ (diabetic rats were fed on soaked – cooked chickpea 6 hr.)

G₅ (diabetic rats were fed on soaked – cooked chickpea 12 hr.)

Table 2. Chemical composition of chickpea seeds

Parameter (%)	Raw	T ₁	Increase	Decreases	T ₂	Increase	Decrease
Moisture	7.83	8.43	7.7%	-	9.28	18%	-
Protein	24.3	25.01	3%	-	26.23	8%	-
Fat	6.18	5.12	-	17%	4.86	-	21%
Fiber	4.17	4.88	17%	-	5.36	28%	-
Ash	3.84	3.07	-	20%	2.51	-	34%
Carbohydrates*	53.96	53.32	1%	-	51.74	4%	-

* Total carbohydrates were calculated by difference

Each value in a row followed by the same letter is not significantly different at P<0.05.

T₁ (soaking and cooking 6 hr.), T₂ (soaking and cooking 12 hr.)

Effect of Soaking and Cooking Treatments on Antinutritional Substances of Chickpea Seeds

Soaking has been documented to be an effective treatment to remove antinutritional factor which can be eliminated with the discarded soaking solution. Additionally, legumes are mainly cooked after soaking to soften cotyledon, improve flavour and palatability, destroys antinutritional factors, enzyme inhibitors, increase nutritive value and

digestibility. The results in Table 3 show a significant decrease in phytic acid, tannins and trypsin inhibitor for soaked-cooked 6 and 12 hr., chickpea compared to raw seeds. Raw chickpea contain 1.89 mg/g phytic acid linearly reduction by 15% and 36% was recorded after 6 and 12 hr., of soaking and cooking, respectively (Table 3). Decreases in phytic acid during soaking may be due to the activation of phytase enzyme and catalyzes the hydrolysis of phytic acid into inositol phosphate and orthophosphate and/or migration of soluble phosphate from legume into

Table 3. Antinutritional factors phytic acid, tannins acid and trypsin inhibitor of chickpea

Chickpea	Phytic acid (mg/g)	(%) Reduction	Tannins (mg/g)	(%) Reduction	Trypsin inhibitor (TIU/mg)	(%) Reduction
Raw	1.89 ^a ±0.042	-	2.78 ^a ±0.142	-	12.33 ^a ±0.376	-
T ₁	1.60 ^b ±0.029	15%	2.25 ^b ±0.043	19%	8.01 ^b ±0.245	35%
T ₂	1.20 ^c ±0.057	36%	1.44 ^c ±0.033	48%	2.46 ^c ±0.073	80%

Each value in a column followed by the same letter is not significantly different at $P \leq 0.05$

T₁ (soaking and cooking 6 hr.), T₂ (soaking and cooking 12 hr.)

steeping medium. Additionally, cooking temperature caused completely inhibition for phytase activity (Bessar and El-Sayed, 1997). Tannin value of raw chickpea decreased to 19 and 48% after the soaking-cooking treatments (Table 3). Similar results were obtained by Mittal *et al.* (2012), who reported that various processing treatments, germination, boiling and pressure cooking caused reduction of tannins up to 93 percent in chickpea. The decrease may be attributed to the heat labile and water soluble nature of tannins. Raw chickpea seeds had 12.33 TIU/mg of trypsin inhibitor and increasing soaking-cooking time for 6 and 12 hr., caused elimination this substance by 35 and 80%, respectively (Table 3). The reduction of trypsin inhibitor may due to cooking treatment which stopped the activity of this component by denaturation and coagulation. Similar results were obtained by Hefnawy (2011), who reported that trypsin inhibitor activity was decreased by cooking treatments in lentil seeds. From the pervious data, it could be noticed that soaking for 12 hr., and directly cooking was the best treatment for increasing nutritive value and digestibility due to remove about 36% , 48%, and 80% of phytic acid, tannins and trypsin inhibitor, respectively.

Effect of Soaking and Cooking Treatments on Amino Acids of Chickpea Seed

The effect of soaking and cooking treatments on amino acid contents of chickpea seeds are shown in Table 4. Eighteen amino acids were detected either from raw or from soaking and cooking chickpea seeds. In raw seeds, arginine was the major essential amino acid (10.15/16g N) followed by lysine (7.51 g/16gN) and leucine

(7.30 g /16g N). Additionally, glutamic acid was the predominant non essential amino acid (17.25 g/16g N) followed by aspartic (11.24g /16gN) and serine (4.83g/16g N). These results agree with (Boye *et al.*, 2010). Generally, slight decrease was observed in the total concentration of essential and non essential amino acids of soaked- cooked (12 hr.) but individually there was increase in some amino acids as leucine, glutamic, glycine acids. These results were in accordance with Alajaji and El-Adawy (2006) and Hefnawy (2011), they reported that cooking treatment decreased the concentration of lysine (except microwave cooking) tryptophan, total aromatic and sulphur amino acids of chickpea and lentil.

Essential amino acids composition of raw and soaked-cooked chickpea (T₁ and T₂) were compared with the FAO/WHO committee (1973) as show in Table 5. It was clear that the amount of isoleucine, leucine, phynilalanine and lysine of raw and soaked-cooked chickpea (T₁ and T₂) were higher than the FAO/WHO pattern, while the amount of other essential amino acid found in variation. Table 5 indicated that raw and soaked-cooked (T₁ and T₂) were deficient in methionine (first limiting amino acid), valine (second), meanwhile threonine and tryptophane were (third) limiting in raw and soaked-cooked (T₁ and T₂) of chickpea, respectively. It had been reported that methionine and cystine as limiting amino acid in chickpea (Zia-Ul-Haq *et al.*, 2007). These finding were reported by Cardoso-Santiago *et al.* (2001), who found that sulfur amino acids were higher than the provisional protein (OMS, 1998) for children between 2 and 5 years old in pure extruded chickpea.

Table 4. Effect of soaking and cooking treatments on amino acids content of chickpea seeds (g /16g N)

Amino acid g /16gN	Raw	Soaking –Cooking Treatments	
		T ₁	T ₂
1- Essential AA			
Isoleucine	4.30	4.00	4.30
Leucine	7.30	7.5	7.60
Lysine	7.51	6.89	6.10
Methionine	1.55	1.40	1.35
Histidine	3.43	3.34	3.23
Phenylalanine	5.74	3.60	5.64
Arginine	10.15	9.70	9.95
Threonine	3.55	3.80	4.25
Valine	3.90	3.91	3.80
Tryptophan	0.94	0.82	0.80
2- Non essential AA			
Aspartic	11.24	11.13	11.04
Serine	4.83	4.62	4.43
Glutamic	17.25	17.91	18.45
Proline	4.79	4.69	4.66
Glycine	4.30	4.40	4.60
Alanine	4.26	3.98	3.76
Cystine	1.30	1.20	1.10
Tyrosin	3.90	3.90	3.80

T₁ (soaking and cooking 6 hr.,)T₂ (soaking and cooking 12 hr.,)**Table 5. Effect of soaking and cooking treatments on chemical score and limiting amino acids of chickpea**

Chickpea	Threonine	Valine	Methionine	Isoleucine	Leucine	Phenyl- alanine	Lysine	Tryptophan
Raw	88.7	78.00	70.4 ^a	*	*	*	*	97.00
T ₁	95.00	78.00	63.00 ^a	*	*	*	*	85.00
T ₂	*	76.00	61.00 ^a	*	*	*	*	83.00
FAW/WHO, (1973)	4.00	5.00	2.20	4.00	7.00	2.80	5.44	0.96

* Composition supplies 100% or more of the requirement.

a First limiting amino acid

T₁ (soaking and cooking 6 hr.), T₂ (soaking and cooking 12 hr.,)

Effect of Raw and Treated Chickpea on Blood Glucose Levels of Diabetic Rats

Four groups of rats were injected by alloxan, these rats were fed on raw and soaked-cooked chickpea (T₁ and T₂). Blood glucose level was measured in all groups after 15, 30 and 45 days. Results in Table 6 shows that the control diabetic rats had 182.83±1.41 mg/dl at zero time and gradual decrease at the end of experiment (45 days). Meanwhile the diabetic rats feed on raw and soaked-cooked chickpea (T₁ and T₂) resulted significant decrease of serum glucose by 41%, 37% and 34%, respectively compared with the diabetic control group. This mean that raw chickpea was the most effective on the hypoglycemic agent. Fung *et al.* (2002) found that people consuming around 3 servings per day of legumes had a risk reduction T₂DM in the order of 20-30% compared with low consumers.

Conclusions

Chickpea seed is characterized by high contents of basic nutritive compounds. The soaking process is common household processes

and it could be improve the nutritional value through increasing in protein content. So, soaking and cooked chickpea increased contents of crude protein and fiber, whereas reduced fat, ash, tannins, phytic acid and trypsin inhibitor. Also, it has sufficient amount of all essential amino acid except methionine, valine and tryptophan. Hence, chickpea has low glycemic index values and reduced risk of developing (T₂DM).

This study indicated that common household processes for legumes, in particular, soaking process could be improve the nutritional value through increasing in protein content. Also, soaking and cooked treatment considered as a cheap bio- process, therefore it is recommend that increasing its utilization in food products besidesas ingredient in normal food preparation. Hence, legumes have low glycemic index values and reduced risk of developing (T₂DM).

Table 6. Effect of raw and soaked and cooked chickpea on blood glucose level (mg/dl) of rats

Group	Zero time	15 days	30 days	45 days
G ₁	94.40 ^a ±0.36	88.00 ^a ± 1.80	90.73 ^a ±1.98	88.10 ^a ± 2.32
G ₂	182.83 ^c ±1.41	167.70 ^c ±0.91	161.93 ^d ± 0.99	158.23 ^d ±0.93
G ₃	149.06 ^b ±4.64	103.53 ^b ±3.38	98.80 ^{ab} ± 3.15	93.43 ^b ±1.18
G ₄	148.53 ^b ±4.56	106.96 ^b ±3.38	106.00 ^{bc} ±6.15	99.59 ^c ± 1.11
G ₅	153.80 ^b ±2.80	105.26 ^b ±2.91	111.1 ^c ± 3.38	104.38 ^c ±2.21
LSD at 5%	4.54	3.73	5.02	2.33

Each value in column followed by the same letter are not significantly different at P<0.05

G₁ Rats were fed on basal diet (Normal group) , G₂ Rats were fed on basal diet (Diabetic group)

G₃ Rats were fed on raw chickpea , G₄ Rats were fed on soaked-cooked chickpea(6hr.,)

G₅ Rats were fed on soaked-cooked chickpea (12hr.), Zero time (3 days after injection)

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بعض الخصائص الكيميائية والتغذوية والبيولوجية للحمص

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أجرى هذا البحث لتقدير بعض الخصائص الكيميائية والتغذوية والحيوية لبذور الحمص الخام التي تم نقعها لمدة ٦، ١٢ ساعة في ماء الصنبور ثم تم طهيها مباشرة تحت الضغط الجوي العادي ودرجة حرارة الغرفة. وقد أظهرت النتائج زيادة في البروتين بنسبة ٣%، ٨% ونقص في الدهون بنسبة ١٧%، ٢١% والرماد بنسبة ٢٠%، ٣٤% بعد النقع لمدة ٦، ١٢ ساعة على التوالي، بذور الحمص الخام تحتوى على ١.٨٩ ملجم/جم من حامض الفايترك ثم يحدث له انخفاض تدريجي بمعاملات النقع والطهي، وقد سجلت النتائج نفس الملاحظات بالنسبة للنتينات ومثبط التربسين. تم فصل ١٨ حامض أميني سواء كان في البذور الخام أو المطهية بعد النقع، حامض الأرجنين والليسين والليوسين كانت الأحماض الأمينية الأساسية السائدة في البذور الخام يليها الفينيل اللانين والايذوليوسين والفالين والثريونين، ولوحظ انخفاض في تركيز هذه الأحماض الأمينية بعد النقع لمدة ١٢ ساعة فيما عدا الليوسين والايذوليوسين والثريونين، الحامض الأميني الميثونين كان العامل المحدد الأول ويليه الحامض الأميني الفالين سواء في البذور الخام أو المطهية بعد ٦، ١٢ ساعة بعد النقع، الفئران المصابة التي تم تغديتها على الحمص الخام قد سجلت انخفاض ملحوظ في سكر الدم وصل إلى ٤١% بالمقارنة بمجموعة الكنترول المصابة.

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