Annals of Agric. Sc., Moshtohor,
Vol. 34(3): 995-1022, 1996.

BIOCHEMICAL CHANGES OF SOME CHEMICAL CONSTITUENTS IN CHICKPEA SEEDS DURING GERMINATION AND PROCESSING

BY

Zaki, A. M. ; Aboul Fetouh, S. A. ; Hanan Mohany. and El-Morsi, E. A.
Agricultural chemistry Dept. Fac. of Agric. , El-Minia Univ.

ABSTRACT

The effect of germination on the chemical composition such as protein constituents, total soluble sugars, trypsin inhibitor and polyphenolic compounds was studied in the seeds of two variety of chickpea. The effect of other processing such as, soaking, cooking, soaking plus cooking or the germinated seed on trypsin inhibitory activity (TIA) and the digestion of protein by trypsin was also investigated to evolve the most suitable methods of processing which produces a meal of good quality for human consumption from chickpea seeds. The results could be summarized as follows: (1) Globulins and albumins tend to decrease as the germination advanced whereas, glutelins and prolamins showed an opposite trend in the seeds of two variety of chickpea. (2) The solubility of albumins extracted from germinated seed was slightly decreased compared to the control indicated slight effect on the pH on minimum solubilities and the same is true for total globulins.(3) Giza-1 contained higher levels of TIA and polyphenolic compounds than Giza-531. (4) TIA sharp decrease during the first 24 hr of germination to reach 47.12 and 50.55 % of its amount in ungerminated seed samples. (5) Polyphenolic compounds were decreased during the first 24 hr of germination to reach 68.3 and 80.4 % of its amount in ungerminated seed samples. (6) Soaking in water for 12 hr reduced TIA to 69.2 and 74.3 % of its levels of unsoaked seeds of Giza-1 and Giza-531 respectively. Cooking was more effective than soaking in removing these antinutritional factors when preceded by soaking, cooking considerably decreased TIA to 35 and 36.7 % of its original amount in unprocessed samples. Cooking of the germinated seeds was the most effective processing in reduction of TIA. (7) The SDS-PAGE pattern of albumins indicated the dissociation of chickpea total albumins extracted from Giza-1 and Giza-531 into at least 16-19 subunits with molecular weight varied between 14.5-89 KD. Some of the subunits with large and medium molecular size tend to decrease as the germination advanced. Examination of the total globulins by SDS-PAGE indicated the presence of at least 14 to 17 subunits with estimated molecular weights of 15.1 to 141 KD. These subunits could be divided into four zones according to their molecular sizes. Zone 1, 2, 3 and 4 with indicated subunits with respective molecular weight of > 66, 44-66, 28-38 and 15.1 to 24 KD. (8) SDS-PAGE was used for examining the *in vitro* hydrolysis of chickpea

salt soluble proteins by trypsin. For control the subunits of MW greater than 45 KD was degraded rapidly during the first 10 min of trypsin digestion. Protein extracted from soaked seed, no remarkable change after incubation with trypsin for 10 or 15 min. Cooking of soaked seed produced different SDS-PAGE patterns compared to soaking samples.

INTRODUCTION

On a world-wide basis legume make an important contribution to human nutrition. Various legumes have long been the main staple food or are widely consumed by less affluent nations. When maximum nutrient for minimum cost's have dictated, these have been eaten whole.

Chickpea (*Cicer arietinum* L.) is a cool-season legume crop grown mainly for human consumption. In terms of area, it is the world 's third largest pulse crop (Kadam and Salunkhe, 1989).

Chickpea (*Cicer arietinum* L.) is a potential novel protein source which has not been fully explored. The seed of this legume has about 21-30 % protein (Khan *et al.*, 1979; Metry *et al.*, 1988; and Singh *et al.*, 1991). Chickpea like other legumes, contains trypsin inhibitor, polyphenolic compounds (Price *et al.*, 1980, Singh and Jambunathan 1981 and Saini *et al.*, 1992).

Trypsin inhibitors in legumes have been investigated in relation to effects on the pancreas (Sato and Herman 1990; Myers *et al.*, 1991) and influence on the pancreatic enzymes (Weder and Mueller, 1989, Arentoft *et al.*, 1991). As well as reduced animal weight gain (Peace *et al.*, 1991 and Herkelman *et al.*, 1992). The levels and types of trypsin inhibitors vary between different legumes, and this will complicate the evaluation of the role proteinase inhibitors may have as revealed from revious (Pusztai *et al.*, 1991; Arentoft *et al.*, 1993, El-Morsi 1996).

It is widely accepted that simple and inexpensive processing techniques such as soaking, cooking, autoclaving and germination are effective methods for reducing the levels of the antinutritional compounds in several legume seeds, which is essential to improve the nutritional quality of legumes and effectively utilize their full potential as human food.

Germination has been suggested as an inexpensive and effective method for improving the quality of legumes, by enhancing their digestibility (Reddy *et al.*, 1985), and reducing the content of antinutritional factors (El-Shakankery *et al.*, 1991; Vidal-valverde *et al.*, 1994; Ismail *et al.*, 1995, El-Morsi, 1996, Heba Aly 1996).

The objectives of this study were investigate the effects of germination on chickpea (variety Giza-1 and Giza-531) seed on chemical composition,

protein constituents, pH solubility profile, SDS-PAGE pattern, trypsin inhibitory activity (TIA) and polyphenolic compounds (tannins). Other processing treatments, soaking, cooking, soaking plus cooking and cooking the germinated seeds were examined as a method for inactivating or removing TIA.

Our ultimate goal in this work was to evolve the most suitable methods of processing which produce meal that has lower or no antinutritional factors which means producing meal of good quality for human nutrition from chickpea seeds.

MATERIAL AND METHODS

Seed samples:

The chickpea seeds (*Cicer arietinum* L.) variety Giza-1 and Giza-531 were purchased from Agricultural Research centre, Giza, Egypt.

Germination:

The seeds were sorted, cleaned, surface sterilized with 0.05N $HgCl_2$ solution (Gupta and Wagle, 1980). Seed germinated for 24, 48, 72, 96 and 120 hr. were immediately frozen and freeze-dried. Ungerminated seed served as a control.

Soaking:

Sample of chickpea seed Giza-1 and Giza-531 were soaked at room temperature in tap water (1:3 w/v) for 12 hr.

Cooking:

Some of the seeds from the soaking process, germination (72 hr.) and untreated samples were separately cooked for 30 min. in tap water (seed to water ratio 1:10 w/v).

Proximate analysis:

The chemical composition of the chickpea seed samples were determined according to the official methods of the Association of Official Analytical Chemists (AOAC, 1975). The minerals were determined by the service laboratory for soil, plant and water analysis at soil science department, Minia faculty of Agriculture, Minia university according to the method described in the AACC (1969).

Extraction and determination of soluble sugars:

Soluble sugars were extracted according to Macrae and Zand-Moghdlam (1978) method. Total soluble sugars were determined by the phenol-sulfuric acid method described by Dubois *et al.*, (1956).Total reducing sugars were determined by the modified neocuproine method described by Dygert *et al.*, (1965).

Extraction and determination of protein constituents:

The method of Tella and Ojehomon (1980) was used for extracting the various protein components of ungerminated and germinated chickpea seeds. The protein content of each fraction was determined by the biuret method (Gornall *et al.*, 1949).

Extraction of Trypsin Inhibitor (TI):

Finely, ground defatted sample was suspended in 0.5 M NaCl at ratio of 1:10 (w/v) and stirred for one hr at room temperature. The supernatant obtained after centrifugation at 6,000 r.p.m for 45 min was used for assaying TIA.

Extraction of Non-protein trypsin inhibitors (NPTI):

The method described by Hafez and Mohamed (1983) was used for preparing the NPTI. After the appropriate dilution, the NPTI activity was assayed by the same method used to assay the TIA.

Determinof trypsin inhibitor activity (TIA):

TIA were carried out as described by Hamerstrand *et al.*, (1981) modified with respect to the initiation of the TIA assay, i.e. trypsin was added the last component to the inhibitor substrate mixture (Stauffer, 1993). Benzoyl DL-arginine-*p*-nitroanilide hydrochloride (BAPA) as synthetic substrate for trypsin.

Determination of solubility:

Effect of pH on the solubility of salt-extractable protein was determined as described by El-Morsi (1982). The protein concentration in the supernatant was measured by recording the absorbance at 280 nm.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE):

The subunits structures of proteins were performed by SDS-PAGE as described by Laemmli (1970). A mixture of protein standards containing different proteins (66,000 - 14,200 KD) was treated and run under the same conditions and the relative mobility of each protein was plotted against its log molecular weight to obtain the calibration curve.

Determination of Polyphenolic compounds(tannin):

The polyphenolic compounds were extracted from each defatted sample (500 mg) by refluxing with 30 ml of methanol containing 1 % HCl for 20 min. The amount of phenolic compounds were estimated as tannic acid equivalent according to the Folin-Denis procedure (Swain and Hills, 1959).

Typsin Digestion:

The proteins of chickpea seeds subjected to some processing treatments as well as untreated seed, were extracted with 0.5M NaCl for 1 hr at room temperature [chickpea flour to extractant ratio of 1:5 (w/v)] with continuous stirring. After centrifugation at 6000 r.p.m. for 20 min, the clear supernatants

were made 50 mM in Tris-HCl, pH 8.1 prior to trypsin digestion using bovine pancreatic trypsin at a ratio of 1:10 (w/w) for trypsin to protein. Final protein concentration of 1.0 mg/ml, $CaCl_2$ concentration of 0.01M (Sathe *et al.*, 1983). Trypsin digestion was conducted at 37°C in water bath for 10 or 15 min. At the end of incubation period trypsin was inactivated by heating the sample in a boiling water bath (98°C) for 10 min., then subjected to SDS-PAGE.

Photography of gels:

Immediately after destaining had completed, the gels were photography by using a 36.mm camera with Kodak film at an ASA 100. The 36-mm camera was also at ASA

Scanning of gels:

Gels stained with coomassie Brilliant Blue R-250 were scanned at 580 nm, jonior 24 with PC spectrodensitometer (Helena, France) equipped for scanning a slab gel up to 10 x 10 cm in dimension.

RESULTS AND DISCUSSIONS

Effect of Germination on chemical composition of chickpea seeds:

Changes in some chemical constituents of chickpea seeds during germination are presented in Table (1), which showed increasing the crude protein from 23.63 to 26.59 and 21.60 to 23.59% in the seeds of Giza-1 and Giza-531 respectively during the first 72 hrs of germination.

Germination of chickpea seeds caused apparent increase in crude fiber and ash content (Table 1), on other hand, crude fat was decreased by 35.09 and 32.61% of the original amount in ungerminated seeds of Giza-1 and Giza-531 respectively after 120 hr germination.

Effect of germination on mineral content

The concentrations of iron and calcium were higher in ungerminated seeds of Giza-531 (8.2 and 200 mg/100 g) than Giza-1 (6.7 and 150 mg/100g) (Table 2). On the other hand phosphorus content of Giza-1 (540 mg/100 g) was higher than that recorded in Giza-531 (400 mg/100 g). The levels of calcium and iron determined in the present work are in a good agreement with those reported by Singh *et al.*, (1991) but lower than the values reported by Khan *et al.*, (1979) for these two elements in chickpea varieties studied by them except for iron content of chickpea 6560. The phosphorus content of chickpea samples studied here were higher than levels observed by Khan *et al.*, (1979).

Germination increased the levels of iron and phosphorus by 13.4 and 30% of its original amount of ungerminated seeds of Giza-531 after 48 and 72 hr of germination respectively and as the germination advanced both element tend to decrease to reach to 73.1 and 122.5% of its original levels for iron and phosphorus respectively by the end of germination period for Giza-531. .

Table (1): Effect of germination on some chemical constituents of chickpea seeds (% on dry weight basis)

G.P* (hr)	Crude protein		Crude fat		Crude fiber		Ash		Carbohydrate**	
	Giza-	Giza-531	Giza-1	Giza-531	Giza-1	Giza-531	Giza-1	Giza-531	Giza-1	Giza-531
Control	23.63±0.04	21.60±0.03	8.32±0.02	8.83±0.02	2.08±0.02	2.61±0.02	2.0±0.03	2.02±0.02	63.97±0.01	64.94±0.02
24	24.92±0.06	22.90±0.04	7.07±0.02	8.38±0.02	2.27±0.02	2.95±0.03	2.08±0.03	2.07±0.02	63.66±0.02	63.76±0.01
48	25.19±0.05	23.30±0.05	6.73±0.03	6.62±0.03	2.49±0.03	3.20±0.03	2.27±0.01	2.20±0.02	63.32±0.02	63.68±0.01
72	26.59±0.04	23.59±0.03	6.25±0.01	6.45±0.03	3.55±0.03	3.79±0.04	2.50±0.01	2.39±0.01	61.11±0.02	62.78±0.02
96	24.25±0.06	23.57±0.04	5.87±0.03	6.02±0.04	3.81±0.05	3.83±0.04	2.70±0.02	2.53±0.01	63.37±0.02	64.01±0.03
120	23.50±0.05	22.15±0.05	5.40±0.02	5.95±0.01	4.49±0.02	4.48±0.02	3.23±0.02	2.96±0.01	63.38±0.02	64.46±0.02

Values are the means of triplicates ± standard deviation of mean expressed on dry weight basis

* G.P = Germination Period (hr)

** Calculated by difference

Germination of Giza-1 decreased the levels of iron and phosphorus to account for 95.5 and 79.6% of its levels in ungerminated sample by the end of the germination period, also reduced the levels of calcium in the two chickpea varieties studied in this work to reach 45 and 73.3% of its original levels in the ungerminated seeds of Giza-531 and Giza-1 respectively after 120 hr of germination.

Table (2): Effect of germination on minerals content of chickpea seed (mg/100 sample).

G.P* (hr)	P**		Ca		Fe	
	Giza-1	Giza-531	Giza-1	Giza-531	Giza-1	Giza-531
0.0	540	400	150	200	6.7	8.2
24	440	430	90	70	5.4	9.0
48	440	490	70	90	5.7	9.3
72	430	520	70	90	7.5	8.3
96	340	430	110	90	6.0	7.5
120	430	490	110	90	6.4	6.0

* Germination period (hr) ; ** Phosphorus.

Effect of germination on carbohydrate contents of chickpea seeds

The total soluble sugars (TSS), total reducing sugars (TRS) and total non-reducing sug(TNRS) content of the two ungerminated chickpea seeds were determined to be, 6.65 , 7.50; 2.25 , 2.42 and 4.40 , 5.08 in Giza-1 and Giza-531, respectively. The total non-reducing sugars (TNRS) were calculated by differences. The TSS and TNRS declined sharply during the 24 hr of germination and then tend to increase as the germination progressed (Fig. 1 A and B).

It has been suggested by Abrahamsen and Sudia (1966) that a major portion of the soluble sugars in the dry seeds may be utilized for respiratory activity during the early stage of germination and this may explain the decrease of the TSS in the seeds at these stages (Fig. 1 A and B).

The effect of germination on the levels of total non-reducing sugars is presented in (Fig. 1 A and B), which indicated decreased of their amounts during the first 24 hr and in some cases 48 hr of germination and then tended to increase as the germination advanced. This may be attributed to the increase in sucrose content by the action of -galactosides, which cleaves selectively galactose from raffinose, stachyose and verbascose and leaves behind the non-reducing sugar sucrose.

Effect of germination on protein constituents in chickpea seeds:

The ungerminated chickpea seeds contained 21.6 and 23.63% crude protein on a dry weight basis for Giza-531 and Giza-1 respectively. More than 86.89% of the total extractable proteins were extracted with 0.5M NaCl. The salt-extractable proteins were composed of 21.80 and 23.49% albumins, 66.9 and 67.9% globulins in Giza 531 and Giza-1 seeds respectively. The protein contents of the samples were determined by the biuret procedure and expressed as the relative percentage of each protein fraction to the total extractable proteins. Glutelins accounted for 7.5 and 6.01%, and prolamins made of 3.76 and 2.60% of the total extractable proteins from ungerminated chickpea seeds variety Giza-531 and Giza-1 respectively. The effect of germination on the levels of various protein fractions in chickpea seeds was studied and the results are presented in Fig. 2 (A and B). The water soluble proteins (albumins) were decreased from 21.8% to 13.37% and from 23.49 to 13.79% in the seeds of variety Giza-1 and Giza-531 after 120 hr of germination (Fig. 2 A and B). The globulins were gradually decreased from 66.9% to 46.09% and from 67.9% to 51.43% in both chickpea samples (Fig. 2 A and B).

The glutelin showed an opposite trend to that of albumin and globulins. As shown in (Fig. 2 A and B), the relative percentage of glutelins increased from 6.54 to 37.99% and from 5.01 to 32.31% by the end of the 120 hr of germination seeds of Giza-531 and Giza-1 respectively.

Germination had no effect on the relative prolamins content during the first 24 hr of germination, but slight decrease was observed as the germination progressed (Fig. 2 A and B). The levels of albumin reported in this work are higher than the levels of 8.1 to 14.1% of the legume seed albumins (Bhatty, 1982). These differences could be attributed to the methods used for extracting and determining of various protein fractions. Moreover, Bhatty (1982) reported that the albumin may not be completely extracted or there may be co-precipitation of albumins with the globulins during their separation by dialysis. Alternately, the albumins may be contaminated with the globulins. Unlike the globulins which are storage proteins, the albumins are mostly enzymic or non-storage proteins. However, Murray (1979) showed that albumins of pea cotyledons were degraded during germination and thus behaved like the globulins or storage proteins. Similar changes in protein constituents during germinating fenugreek seeds were observed by Abdel-Salam *et al.*, (1991).

Effect of pH on the solubility of the chickpea seed albumins and globulins:

The pH solubility profiles of total albumins and total globulins extracted from ungerminated and germinated (for 24 and 120 hrs) chickpea seeds are plotted in Fig. (3A and B and 4A and B), which showed similarity of the six protein with only minor differences. The pH of minimum solubility occurred between pH 4.2 to 5.2 and 4.1 to 5.0 for total albumins of Giza-1 and Giza-531 .

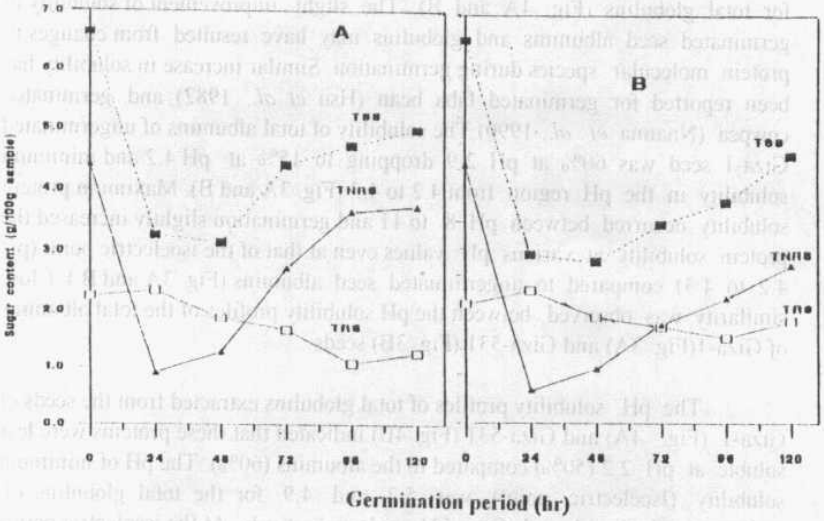

Fig. (1): Effect of germination on soluble sugars in chickpea seeds (A = Giza-1, B = Giza-531).

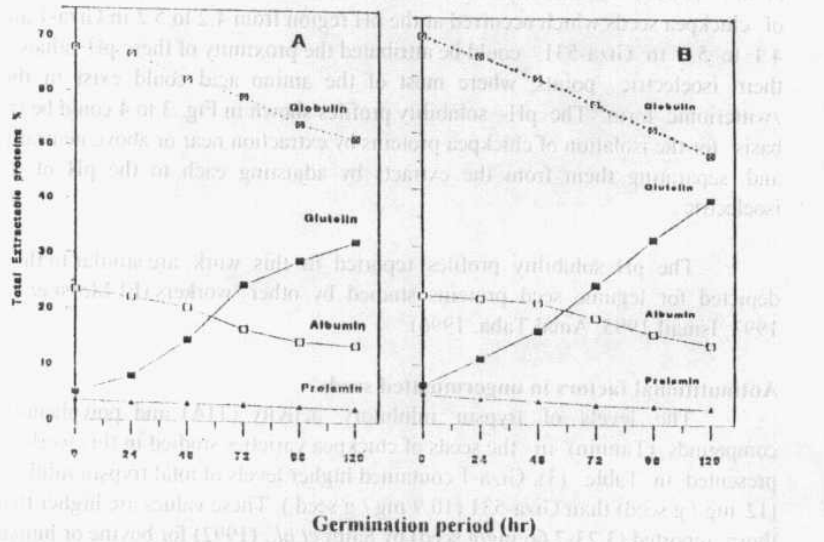

Fig. (2): Effect of germination on protein constituents of chickpea seeds (A = Giza-1, B = Giza-531).

respectively, and solubility was increased towards the acidic and basic sides (Fig. 3A and B). The solubility of albumins extracted from germinated seed slightly was decreased compared to the control and the germination indicated slight effect on the pH on minimum solubilities (isoelectric pH) and the same is true for total globulins (Fig. 4A and B). The slight improvement of solubility of germinated seed albumins and globulins may have resulted from changes in protein molecular species during germination. Similar increase in solubility has been reported for germinated faba bean (Hsu *et al.*, 1982) and germinated cowpea (Nnanna *et al.*, 1990).The solubility of total albumins of ungerminated Giza-1 seed was 60% at pH 2.9 dropping to 15% at pH 4.2 and minimum solubility in the pH region from 4.2 to 4.3 (Fig. 3A and B). Maximum protein solubility occurred between pH 8 to 11 and germination slightly increased the protein solubility at various pH values even at that of the isoelectric point (pH 4.2 to 4.3) compared to ungerminated seed albumins (Fig. 3A and B). Close similarity was observed between the pH solubility profiles of the total albumins of Giza-1(Fig. 3A) and Giza-531 (Fig. 3B) seeds.

The pH solubility profiles of total globulins extracted from the seeds of Giza-1 (Fig. 4A) and Giza-531 (Fig. 4B) indicated that these proteins were less soluble at pH 2.2 (50%) compared to the albumins (60%). The pH of minimum solubility (Isoelectric point) was 5.2 and 4.9 for the total globulins of ungerminated Giza-1 and Giza-531 seeds respectively. At the isoelectric points of the six proteins studied here about 10 to 25% of the proteins still soluble (Fig. 3 and 4), this may be due to the composition of the proteins since some proteins, albumins and some globulins may not precipitate at the isoelectric pH (El-Morsi *et al.*, 1984, 1993, Lasztity *et al.*, 1985). The minimum solubility of the protein of chickpea seeds which occurred at the pH region from 4.2 to 5.2 in Giza-1 and 4.1 to 5.0 in Giza-531, could be attributed the proximity of these pH values to their isoelectric points, where most of the amino acid could exist in the zwitterionic form. The pH- solubility profiles shown in Fig. 3 to 4 could be the basis for the isolation of chickpea proteins by extraction near or above neutrality and separating them from the extracts by adjusting each to the pH of its isoelectric .

The pH solubility profiles reported in this work are similar to those depicted for legume seed proteins studied by other workers (El-Morsi *et al.*, 1993; Ismail 1995, Amal Taha, 1996).

Antinutitional factors in ungerminated seeds:

The levels of trypsin inhibitory activity (TIA) and polyphenolic compounds (Tannin) in the seeds of chickpea varieties studied in this work are presented in Table (3). Giza-1 contained higher levels of total trypsin inhibitor (12 mg / g seed) than Giza-531 (10.9 mg / g seed). These values are higher than those reported (3.23-7.66 mg/g seed) by Saini *et al.*, (1992) for bovine or human or porcine trypsin in several chickpea varieties. They reported that the upper and

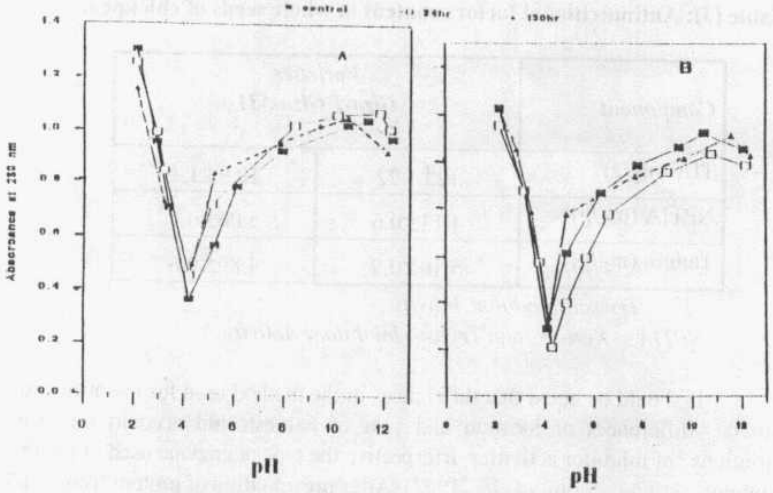

Fig. (3): Effect of pH on the solubility of total albumins extracted from chickpea seeds (A = Giza-1, B = Giza-531)

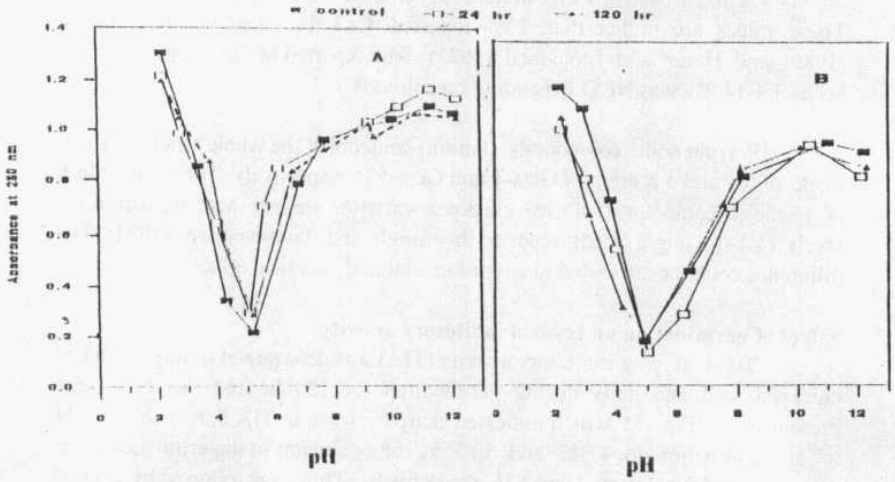

Fig. (4): Effect of pH on the solubility of total globulins extracted from chickpea seeds (A = Giza-1, B = Giza-531)

lower limits of their data on trypsin inhibitor activities in chickpea varieties are within a 2.1-2.5 fold variation in inhibitor activity against one particular enzyme except for bovine chymotrypsin (3.3 fold variation).

Table (3): Antinutritional factors content in whole seeds of chickpea.

Component	*Varieties*	
	Giza-1	*Giza-531*
TIA (mg/g)	12±1.22	10.9±1.1
NPTIA (mg/g)	4.13±0.6	5.06±0.7
Tannin (mg/g)	5.10±0.9	4.80±0.6

TIA = Trypsin Inhibitor Activity ;
NPTIA = Non-Protein Trypsin Inhibitory Activity

It should be noted that differences in the method used for quantification, varietal differences or location and year of harvestcould account for large variations of inhibitor activities, irrespective the type of enzyme used to monitor inhibitor activity (Saini *et al.*, 1992). After precipitation of protein from crude inhibitor extract of chickpea seeds by adding trichloroacetic acid, some of trypsin inhibitor activity was detected in the supernatant and referred to as non-protein trypsin inhibitory activity (NPTIA) which amounted 4.13 and 5.06 mg / g seed of Giza-1 and Giza-531 respectively. These data indicate that NPTIA accounted for 34.4% and 46.4% of TTIA in the seeds of Giza-1 and Giza-531 respectively. These values are higher than 25% reported for kidney bean by Abdel-Naem, (1989) and Hafez and Mohamed (1983) who reported of the TTIA in winged beans 3.4-14.2% was NPTI depending on cultivars.

Polyphenolic compounds (tannin) contents of the whole chickpea seeds made of 5.1 and 4.8 mg/g of Giza-1 and Giza-531 respectively. The mean values of phenolic compounds of two chickpea varieties studied here are within the levels (4.1-6.1 mg/g seed) reported by Singh and Jambunathan (1981). The difference could be attributed to environmental and genetical effects.

Effect of germination on trypsin inhibitory activity:

Total trypsin inhibitory activity (TIA) was determined in ungerminated chickpea seed and daily during germination for 120 hr and the results are presented in (Fig. 5) which indicated sharp decrease in TIA during the first 24 hr of germination to 47.12 and 50.55% of its amount in ungerminated seeds (control) of Giza-1 and Giza-531 respectively. This was followed by gradual decline as germination progressed to reach to about 30% of the control after 72 hr of germination and increasing the germination period to 120 hr produced only slight decrease of TIA to reach about 27.9 and 27.5% of the control by the end of the germination period (120 hr).

NPTIA in the seeds of Giza-1 declined sharply during the first 24 hr of germination and tend to show some increase following 72 hr of germination (Fig. 6). Different pattern was observed as result of germination on NPTIA in Giza-531.

Several investigators have reported different results regarding the effect of germination on TIA in legume seeds. During germination, TIA decreased in chickpea (Savage and Thomposon 1993), kidney bean and *Vicia faba* beans (Ismail *et al.*, 1995), faba bean (Rahma *et al.*, 1987), cowpea (El-Shakankery *et al.*, 1991, Issa *et al.*, 1994, Ismail *et al.*; 1995) and lentils (Vidal-vaverde *et al.*, 1994). However, other workers observed little or no change in TIA after germination of mungbean (Noor *et al.*, 1980), winged bean (King and Puwastein 1987) and lentils (Weder and Link 1993). Chang and Harrold (1988) found that germination of navy beans (Pindak cultivar) increased TIA by 46.2% and 39.2% after 3 and 6 day of germination respectively.

The decrease in TIA in the germinated seeds may result from the proteolytic degradation of the inhibitors during germination (Gupta and Wagle, 1980 and Hamza *et al.*, 1986).

The effect of germination on polyphenolic compounds:

The effect of germination on polyphenolic compounds content (tannin) of the two chickpea varieties are presented in (Fig. 7) which indicated rapid decreases in tannin content during the first 24 hr of germination to reach 68.3 and 80.4% of its amount in ungerminated seeds of Giza-531 and Giza-1 respectively. As the germination advanced the tannin content tend to increase gradually to account for about two fold of that recorded in ungerminated seeds.

Our data are in accordance with that reported for lentils (increase of tannin content by 152% after 6 days of germination) (Vidal-valverde *et al.*, 1994). Moreover , Heba Aly (1996) observed reduction in tannin content during the first 24 hr of germination by 41.5% and 48.5% of its amount in ungerminated seeds of cowpea and kidney bean respectively but the tannin content tend to increase as the germination progressed to reach to 139, 146 and 246% of its levels in the ungerminated samples of kidney bean variety Giza-3 and Giza-6 and cowpea variety cream 7 respectively by the end of germination period (120 hr). Ismail *et al.*, (1995) reported different results regarding the effect of germination on tannin content in three legume samples. They indicated reduction in cowpea tannin during the first 24 hr of germination , then decreased at faster rate to reach to 52.5% of its amount in the ungerminated seed and the tannin content decreased to 70.7% and 58.2% of its levels in ungerminated kidney bean and field bean, respectively.

Effect of processing treatments on TIA:

The effect of several processing treatments, namely soaking, cooking, soaking plus cooking or cooking the seed germinated for 72 hr on TIA were

studied and the results are presented in (Fig. 8). In general, all processing treatments decreased the TIA by varying degrees and non of the processing treatments resulted in complete removal of TIA but the greatest decrease compared to unprocessed sample (control) was observed for germinated- cooked samples.

Soaking in water for 12 hr resulted in decline of TIA to 69.2 and 74.3% of its levels of unsoaked seeds of Giza-1 and Giza-531 respectively and the cooking was more effective than soaking in removing these antinutritional factors. Soaking and cooking considerably decreased TIA to 35 and 36.7% of its original amounts in unprocessed seeds of Giza-1 and Giza-531 respectively. This indicated that soaking plus cooking inactivated TIA more than applying one of two treatments alone. Similar observations were observed by other workers (Salunkhe and Kadam,1989; Barampama and Simard, 1994; Ismail *et al.*, 1995) in legume seeds subjected to soaking or cooking treatments.

It is evident from the data show in (Fig. 8) that cooking the germinated seeds was the most effective processing in reduction of TIA . As mentioned before the TIA was decreased to about 30% of its level after 72 hr of germinating chickpea seeds compared to raw samples. Higher reduction in TIA was observed in germinated cooked chickpea seeds (Fig. 8). According to Salunkhe and Kadam (1989) cooking improved the protein digestibility which attributed to protein denaturation and inactivation of TIA by heat treatment. Several investigator (Baramparma and Simard, 1994; Ismail *et al.*, 1995) observed higher values for protein digestibility when legume seeds cooked after soaking or germination.

Effect of germination on subunit structures of total albumins and total globulins:

The SDS-PAG electrophoretic patterns of the total albumins and globulins separated from the seeds of Giza-1 and Giza-531 together with protein standard are presented in (Fig. 9).

The electrophoretic patterns presented in Fig. 9a, indicated the dissociation of the total albumins extracted from Giza-1 and Giza-531 into at least 16 and 19 subunits with molecular weights (MW) varied from 14.5 to 89 (KD).

Densitomertic scanning of gels shown in (Fig. 10), which were used for quantitating the concentration from the area ratio of a peak to the total peaks which means analyzing all subunits of the protein in one measurements.

The most abundant subunits of total albumins isolated from ungerminated seed (control) of variety Giza-1 was that of 26.3 KD which made more than 30% of the total subunits followed by the subunit of 20.9 KD. .

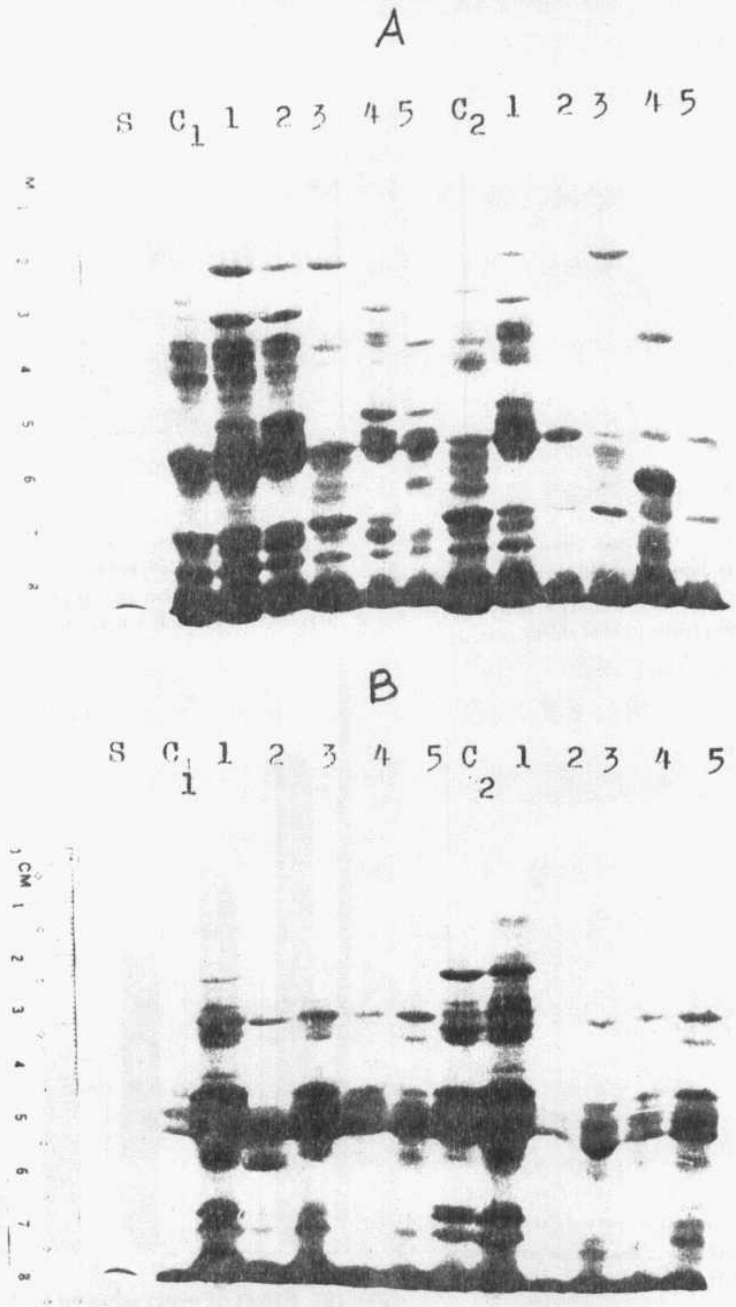

Fig. (9): SDS-PAGE patterns of chickpea albumins (a) and globulins (b). S: Standard proteins; C_1: Control of Giza-1; C_2 Control of Giza-531 and 1, 2, 3, 4 and 5 germination periods.

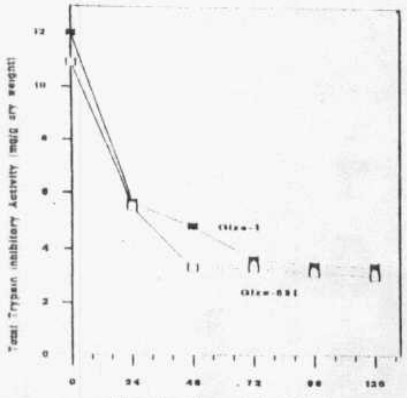

Fig. (5): Changes in trypsin inhibitory activity (TIA) during the germination of chickpea seeds.

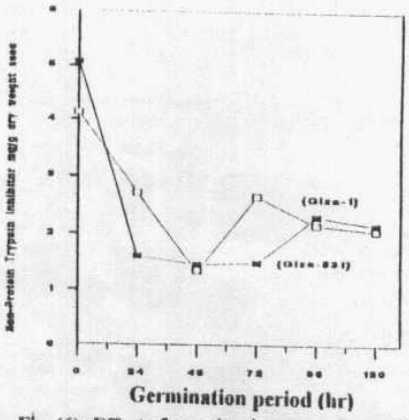

Fig. (6): Effect of germination on non-protein trypsin NPTI inhibitor in chickpea seeds.

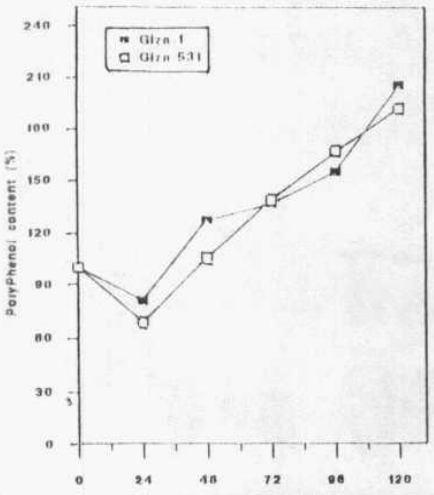

Fig. (7): Effect of germination on polyphenol content in chickpea seeds.

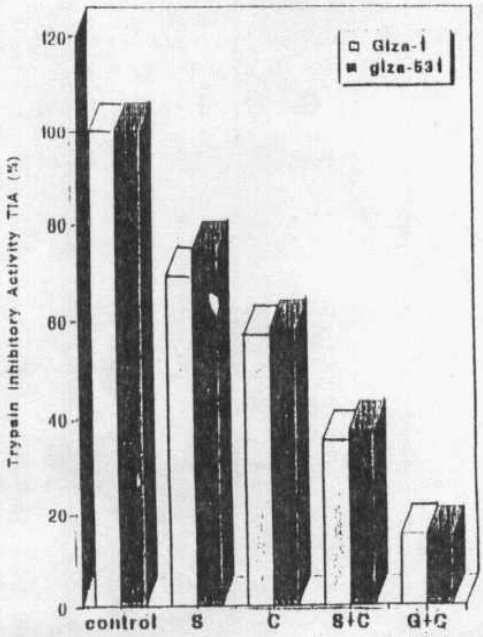

Fig. (8): Effect of processing on total trypsin inhibitory activity in chickpea seeds.
Control = Unprocessed seeds; S = Soaking; C = Cooking S+C = Soaking + Cooking; G+S = Germination + Soaking

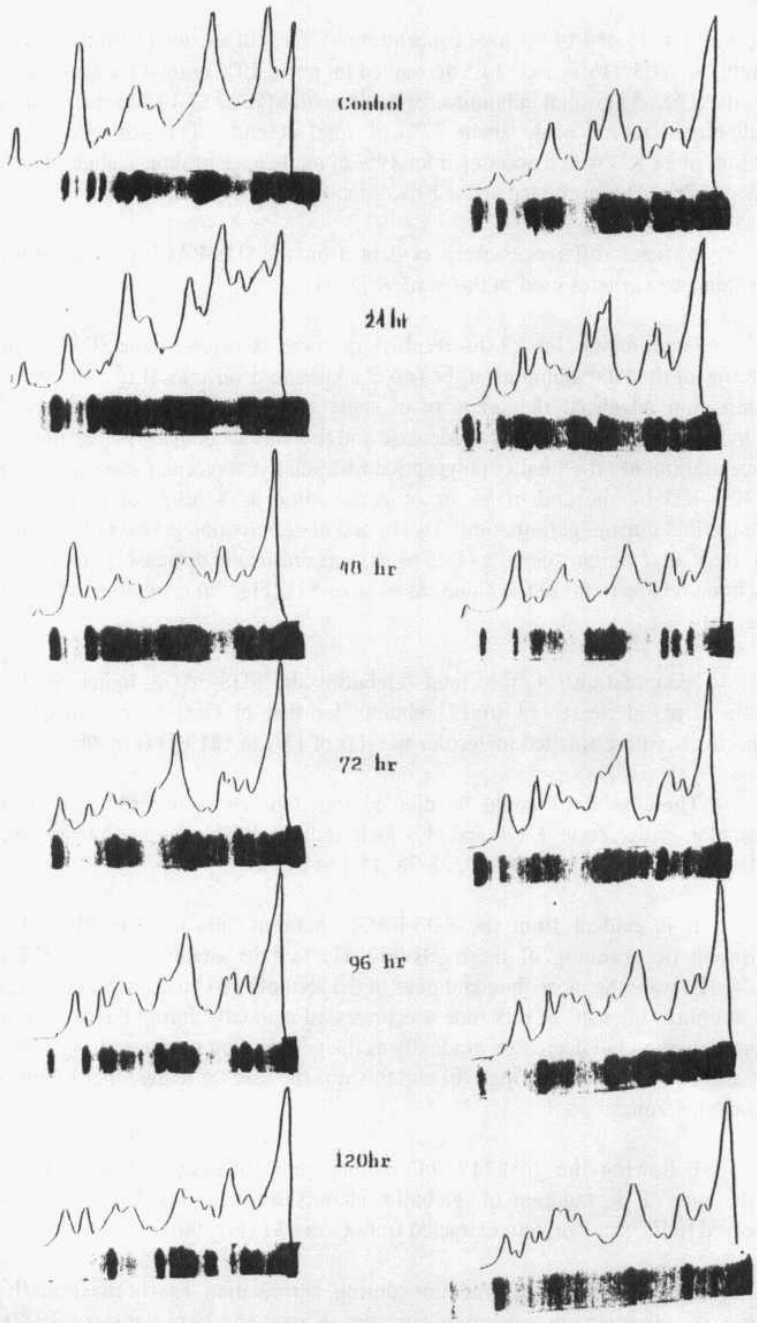

Fig.(10): SDS-PAGE patterns and densitometric scans of chickpea albumins

(account for 15.4% of the total concentration). Each of subunits with molecular weight of 64.5, 46.7 and 14.5 accounted for about 10% of total concentration. For the Giza-531, total albumins, subunit with MW of 17.4 KD was the most predominant which made about 27% of total albumins. This was followed by subunit of 38 KD which accounted for 19% of total concentration. Subunits with MW of 57.5 KD represented about 10% of total protein.

Varietal differences were evident from the SDS-PAGE patterns of the two chickpea varieties used in this work (Fig. 9a) .

Germination for 24 hr resulted in some changes in the SDS-PAGE patterns of the total albumins of the two chickpea seed varieties (Fig. 9a). As the germination advanced the intensity of some of the subunits with large and medium molecular size tend to decrease and this was accompaby increasing the concentration of the smaller polypeptide with relative molecular masses of 14.5 to 30.1 KD by the end of 96 hr of germination as a result of proteolytic degradation during germination. By the end of germination period (120 hr) the low molecular weight subunits (14.5 to 30) was drastically decrease and this was much more apparent in the albumins of Giza-531 (Fig. 9a) compared to Giza-1 (Fig. 9a).

Examination of the total globulins by SDS-PAGE indicated the presence of at least 14 to 17 subunits for that of Giza-1 and Giza-531 respectively with estimated molecular weights of 15.1 to 141 KD (Fig. 9b).

These subunits could be divided into four zones according to their molecular sizes. Zone 1,2,3 and 4 which indicated subunits with respective molecular weight of > 66 , 44-66, 28-38, 15.1 to 24 KD.

It is evident from the SDS-PAGE patterns shown in (Fig. 9) and the densitomteric scanning of these gels (Fig. 11), that the subunits of zone 3 (MW 28-38 KD) are the most abundant ones in the seed of two chickpea varieties and the staining intensity of this zone was increased markedly during the first 24 hr of germination but decreased gradually as the germination progressed especially in the Giza-531 globulins (Fig. 9b) and this was the case for many other subunits of the other zones.

Following the first 24 hr of germination of chickpea seed variety Giza-1, the rate of degradation of globulin subunits in zone 3 was slower than that observed in the same protein extracted from Giza-531 (Fig. 9b).

Reserve protein degradation during germination has been similarly shown in other legumes including chickpea (Kumar and Venkataraman, 1978), peas (Konopska, 1979), mungbean (Chrispeels and Boulter, 1975) and great northern beans (Sathe *et al.*, 1983). However, the biological control(s) which delay reserve protein degradation during germination remain to be elucidated.

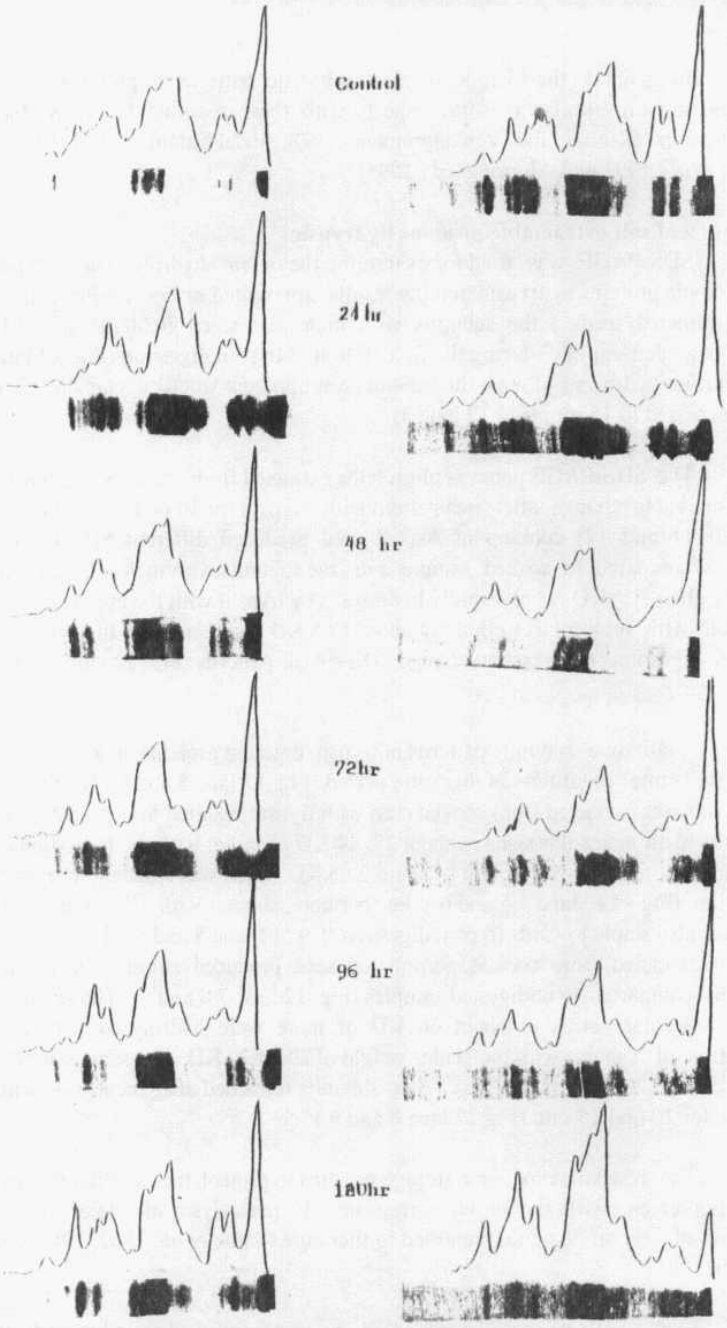

Fig.(11): SDS-PAGE patterns and densitometric scans of chickpea globulins

In general, the change in electrophoretic patterns of proteins during germination are similar in some aspects with those reported by many other investigators (Kumar and Venkataraman, 1978; Abdel-Salam *et al.*, 1991; El-Morsi *et al.*, 1992 and Ahmed *et al.*, 1995).

Digestion of salt extractable proteins by trypsin:

SDS-PAGE was used for examining the *in vitro* hydrolysis of chickpea salt soluble proteins by trypsin and the results arpresented in Fig. 12. For control (ungerminated seeds) the subunits with molecular sizes greater than 45 KD were degraded rapidly during the first 10 min of trypsin digestion (Fig. 12 lane 1,2) further hydrolysis of most the subunits was apparent when enzyme digestion was increased to 15 min (Fig 12 lane 3).

The SDS-PAGE patterns of proteins extracted from soaked seed showed no remarkable change after incubation with trypsin for 10 or 15 min (Fig. 12 lane 10,11 and 12) cooking of soaked seed produced different SDS-PAGE patterns compared to soaked sample and the subunits with molecular massed greater than 35 KD were rapidly hydrolysed by trypsin with the appearance of subunits with molecular weight of about 39.5 KD and increasing the period of trypsin digestion produced no change SDS-PAGE patterns (Fig. 12 lane 13, 14, 15).

Qualitative subunits patterns of trypsin digested proteins of cooked seed were the same regardless of digestion period (Fig. 12 lane 5 and 6). Incubation of the proteins extracted from cooked seed with trypsin resulted in degradation of subunits with molecular sizes of about 37, 44 KD or larger with the formation of subunits with molecular weight of about 29.5 KD which was resistant to trypsin digestion (Fig. 12 lane 4,5 and 6). Furthermore, subunit with MW of about 20 KD was also staple towards trypsin digestion (Fig. 12 lane 5 and 6). Treating the protein extracted from cooked germinated seed produced slight SDS-PAGE patterns compared to undigested samples (Fig. 12 lane 7,8 and 9). The subunits with molecular sizes of about 66 KD or more were hydrolyzed with the formation of subunit with molecular weight of about 23 KD. The same subunits of 29.5 and 20 KD as well as other subunits remained after incubation with trypsin for 10 and 15 min (Fig 12 lane 8 and 9).

The resistance of some storage proteins to proteolytic digestion *in vitro* is in agreement with the *in vitro* resistance to proteolysis of major storage proteins of several legumes reported in literature (Sathe *et al.*, 1982, 1983 and Nielsen 1991).

Heat treatment greatly improved the digestibility of most but perhaps not all, legume protein fractions. An *in vivo* study by Antunes and Sgurbieri (1980) and *in vitro* study of Sathe *et al.*, (1982) indicated that heat treatment improved the digestibility of both albumin and globulin fraction from *Phaseolus*

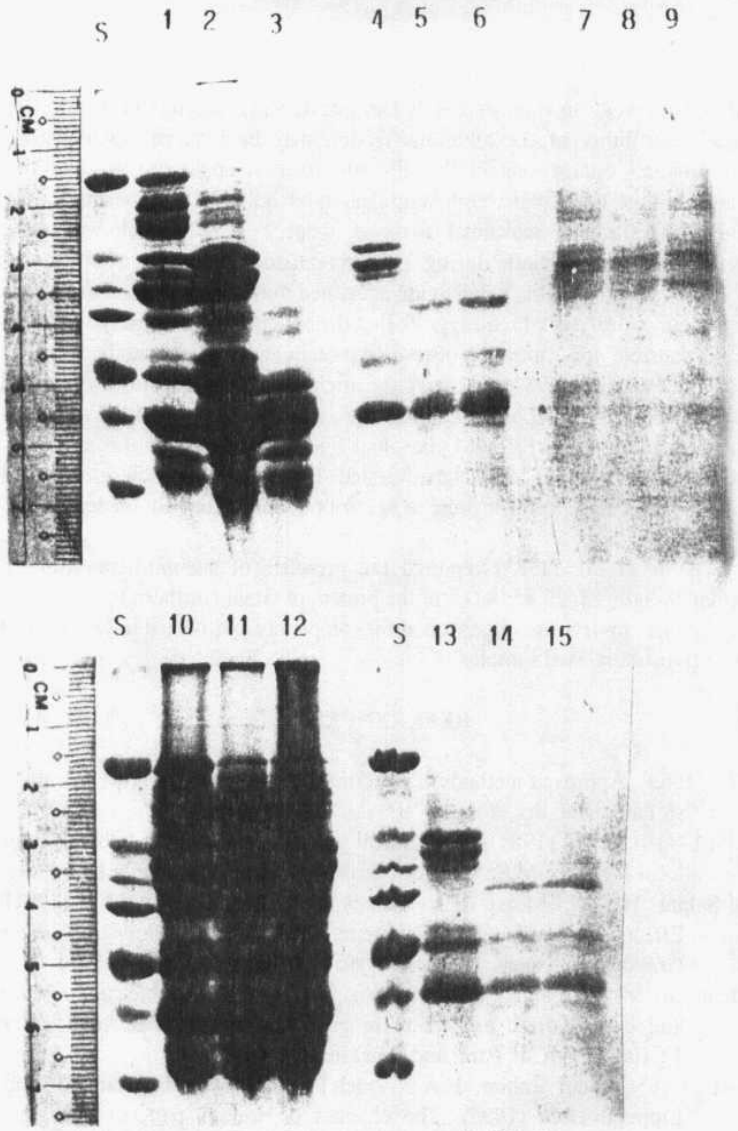

Fig.(12):SDS-PAGE patterns of treated globulins by trypsin and various processing of chickpea seed

1- Control
2- Control + trypsin (10 min)
3- Control + trypsin (15 min)
4- Cooking
5- Cooking + trypsin (10 min)
6- Cooking + trypsin (15 min)
7- Germination + Cooking
8- Germination + Cooking + trypsin (10 min)
9- Germination + Cooking + trypsin (15 min)
10- Soaking
11- Soaking + trypsin (10 min)
12- Soaking + trypsin (15 min)
13- Soaking + Cooking
14- Soaking + Cooking + trypsin (10 min)
15- Soaking + Cooking + trypsin (15 min)

vulgaris. However, *in vitro* studies by Deshpande and Nielsen (1987) suggested that the digestibility of the albumin fraction may be reduced upon heating. Protein-protein interactions in the albumin fraction apparently lead to the formation of high molecular weight aggregate not readily for enzyme attack. The subunit with estimated molecular mass of about 29.5 KD which was quite resistant to proteolysis both during germination and trypsin digestion (Fig. 12 lane 5, 6, 7, 8 and 9). This polypeptide generated during germination and the *in vitro* protein hydrolysis which suggests that the endopeptidase activity suggested to be responsible for initiation of reserve protein degradation and discussed by Mosse and Pernollet (1982) may have specificity similar to trypsin. Change and Satterlee (1982) have reported heating the major storage protein of the Great Northern bean at 90°C in 50 mM phosphate buffer (pH 7) had improved *in vitro* digestibility but was not completely digested. The reasons for such resistance of the major storage proteins of legume to *in vitro* proteolysis remain unclear.

Sathe *et al.*, (1983) reported the presence of subunit with estimated molecular weight of 29.85 KD, in the protein of Great Northern beans, which was resistance to trypsin digestion but it completely hydrolyzed in heat treated and 4 hr trypsin digested samples

REFERENCES

AACC, (1969): Approved methods of American Association of Cereal Chemists, st. Paul, MN, Sec. 20-30.

Abdel El-Naem, G.F. (1989): Biochemical studies on trypsin inhibitors in some local legumes. M.Sc. Thesis, Faculty of Agriculture, Minia University.

Abdel-Salam, H.S., El-Morsi, E.A., Ismail, H.A. and Hussein, M.Y. (1991): Effect of germination on proteins of fenugreek (*Trigonella foenum Graecum*, L.) seeds. Minia J. Agric. Res. & Dev. 13:1503-1521.

Abrahamsen, M. and Sudia, T.W. (1966): Studies on the soluble carbohydrate and carbohydrate precursors in germinating soybeans. Amer. J.Bot. 53:108-114. [C.F. Ring and Buwastein (1987]

Ahmed, F.A.; Abdel Rahim, E.A.; Abdel Fatah, O.M. ; Erdman, V. and Lippmann, C. (1995): The changes of protein patterns during one week of germination of some legume seeds and roots. Food Chemistry 52:433-437.

Amal Taha, A. S. (1996): Biochemical studies on peanut varieties cultivated in El-Minia Governorate. M.Sc. Faculty of Agric., Minia university, Egypt.

Antunes, P.L: and Sgarbieri, V.C. (1980): Effect of heat treatment on toxicity and nutritive value of dry bean (*Phaseolus vulgaris* var. Rosinha G2) proteins. J. Agric. Food chem. 28:935.

AOAC, (1975): Official methods of analysis .Association of official Analytical chemists 12th ed. Washington, C.O.

Arentoft, A.M.; Frokiaer, H., Sorensen, H. Sorensen, S. (1993): Determination of pea proteinase inhibitors using ELISA based on monoclonal antibodies. Acta Agric. Scand See b.

Arentoft, A. M.; Mortensen, K. S. Orensen, H. (1991): Pea proteinase inhibitor activity. *Aspects Appl. Biol.* 17: 211-215

Barampama, Z. and Simard, R.E. (1994): Oligosacchardies, antinutritional factors and protein digestibility of dry beans as affected by processing. J. of Food Sci., 59: 833-838.

Bhatty, R.S. (1982): Albumin protein of eight edible grain legumes species: Electrophoretic patterns and amino acid composition. J. Agric. Food Chem. 30:620-622.

Chang, K.C.; Harrold, R.L. (1988): Changes in selected biochemical components *in vitro* protein digestibility and amino acids in two bean cultivars during germi. J. Food Sci., 53: 783-787.

Change, K.C. and Satterlee, L.D. (1982): Isolation and characterization of the major protein from Great Northern beans (*Phaseolus vulgaris*) J. Food Sci. 46:1386.

Chrispeels, M. and Boulter, D. (1975): Control of storage protein metabolism in the cotyledons of germinating mung beans: Role of endopeptidase. Plant physiol. 55:1031.

Deshpande, S.S. and Nielsen, S.S. (1987): *In vitro* digestibility of dry bean (*Phaseolus vulgaris* L.) proteins. The role of heat-stable protease inhibitors. J.Food Sci. 52: 1330.

Dubois, M., Gilles, K.A.;Hamilton J.K., Rebers, P.A. and Smith, F. (1956): Colorimetric method for determination of sugar and related substances. Anal. Chem., 28: 350-356.

Dygert, S., Li, L.H.,; Florida, D and Thoma, J.A. (1965): Determination of reducing sugars with improved precision. Anal. Biochem. 13: 367-370.

El-Morsi, E.A, Lasztity, R., Abd El-Samei, M.B., Zaki, A.M. (1984): Extraction , Fractionation and characterization of cowpea (*Vigna unguiculata*) globulins. Periodica polytechnica 29: 130-137.

El-Morsi, E.A. (1982): Solubility and gel filtration chromatography of water extractable field bean proteins.Annals of Agric. Sci. (Ain Shams Univ.), 27: 23-36.

El-Morsi, E.A. (1996): Review: Studies on antinutritional factors in some Egyptian legume seeds. in "the proceeding of the fourth Arabic conference for horticultural crops, 25-28" March, Faculty of Agric., Minia university.

El-Morsi, E.A.; Abdel-Salam, H.S.; Ismail, H.A.; Aboul-Fetouh, S. A. and Fawzy, A.M. (1992): Effect of Gamma-Irradiation of trypsin inhibitor and subunit structure of proteins in field bean (*Vicia faba* L.) seeds during germination. Minia J. Agric. Res. & Dev., 14: 849-871.

El-Morsi, E.A.; Aboul-Fetouh, S.A.; Abdel-Salam, H.S. and Ismail, H.A. (1993): Studies on globulins and albumins of seeds of ten cowpea (*Vigna unguiculata*) varieties. Minia J. Agric. Res. & Dev., 15: 1-22.

El-Shakankery, M.M.; El-Morsi, E.A.; Metry, S.W. and Aboul-Fetouh, S.A. (1991): Changes in raffinose family sugars and trypsin inhibitor activity in cowpea (*Vigna ungiculata*) seeds during maturation and germination. Minia J. Agric. Res.& Dev., 13: 885-904.

Gornall, A.G.; Bardawell, C.J. and David, M.M. (1949): Determination of serum protein by means of the biuret reaction. J.Biol. Chem. 177: 751-766.

Gupta, K. and Wagle, D.S. (1980): Chain antinutritional factors during germination in *Phaseolus mungoreous* a cross between *Phaseolus mungo* (M 1-1) and *Phaseolus aureus* (T1): Food Sci., 45: 394-397.

Hafez, Y. and Mohamed, A. (1983): Presence of non-protein trypsin inhibitor in soy and winged beans. J. Food Sci., 48: 75-76.

Hamerstrand, G.E.; Black, L.T. and Glover , J.D. (1981): Trypsin inhibitors in soy products, modification of the standard analysis procedure. Cereal chem. 58: 42-45.

Hamza, M.A. Shehata, A.M.E-T, Stegemann, H. (1986): Studies on α-amylase and trypsin inhibitors in legume seeds using agar diffusion and isoelectric focusing techniques. *Qual. Plant Plant Foods Hum. Nutr.* 36: 139-146.

Heba, M. Aly (1996): Biochemical studies on amylase inhibitors in some local legumes seeds. M.Sc. Thesis, Faculty of Agriculture, Minia University.

Herkelman, K.L. Cromwell, G.L. Stahly, T.S. Pfeiffer, T.W. Knape, D.A. (1992): A pparent digestability of amino acids in raw and heated conventational and low-trypsin-inhibitor soybean for pigs. *J. Anim. Sci.* 70:818-826.

Hsu, D. L.: Leung, H. K. Morad, M. M. , Finney, P. L. , and Leung, C.T. (1982): Effect of germination on electrophoretic, functional and bread-baking properties of yellow pea, lentil and faba bean protein isolates. Cereal chem. 59:344-350.

Ismail, H.A. (1995): Biochemical studies on the albumins and globulins of promising ten genotypes of mungbean (*Vigna radiata*) seed. Minia J. Agric. Res. & Dev., 17: 139-158.

Ismail, H.A.; Zaki, A.M.; Aboul-Fetouh, S.A. and El-Morsi, E.A. (1995): Effect of processing on raffinose oligosaccharides and some antinutritional factors in three of commonly consumed legumes in Egypt. J. Agric. Sci., Mansoura Univ., 20: 3903-3921.

Issa, M.A., Abdel-Salam, H.S., Hassan, M.S. and El-Malt, E.A. (1994): The effect of germination on carbohydrate content, trypsin inhibitors and protein digestibility (*in vitro*) of some local varieties of cowpea (*Vigna unguiculata*) seeds. Annals of Agric. Sci, Moshtohor, 32 :1545-1560.

Kadam, S.S. and Salunkhe, D.K. (1989): Production, distribution and consumption. In: CRC Handbook of world food legumes Nutritional chemistry, Processing Technology and utilization, Vol. ed Salunkhe, D.K., Kadam, S.S., CRC press Inc, Boca. Raton, Fl, USA, pp5-21.

Khan, M.A.; Ingeborg, J.; and Bjorno, E. (1979): Nutritive value of some improved varieties of legumes. J.Sci. Food Agric. 30: 395-400.

King, R.D. and Puwastein, P (1987): Effect of germination on the proximate composition and nutritional quality of winged bean (*Psophocarpus tetragonolobus*) seeds. J. Food Sci., 52:106-108.

Konopska, L. (1979): Changes in protein composition of aleurone grains of *Pisum sativum* L. during germination. Biochem. Physiol. Pflanzen. 174:275-

Kumar, K.G. and Venkataraman, L.V. (1978): Chickpeas seed proteins: Modification during germination. *Phytochem.* 17:605-

Laemmli, U.K. (1970): Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature 227: 680-685.

Lasztity, R., Abd El-Samei, M.B., El-Morsi, E.A, Zaki, A.M. (1985): Extraction, Fractionation and characterization of cowpea (*Vigna unguiculata*) albumins . Periodica polytechnica 29: 119-127.

Macrae,R. and Zand-Moghdlam, A. (1978): The determination of the component oligosaccharides of lupin seeds by high pressure liquid chromatography. J. Sci. Food Agric. 29: 1083-1086.

Metry, S.W., Hatour, F.S. ; Ghazy, M.A. and Abdel-Kader, S.M. (1988): Effect of germination on chemical composition , amino acid content and changes in albumin, globulin of chickpea (*Cicer arietinum* L.) seeds. Minia J. Agric. Res. and Dev. 10:127-143.

Mosse, J. and Pernollet , J.C. (1982): Storage progeins of legume seeds. in chemistry and Biochemistry of legumes. (ED) S.K. Aroa, p.III. oxford and IBH publishing Co., New Delhi.

Murray, D. F. (1979): A strong role for albumins in pea cotyledons. Plant, Cell Environ. 2:221- 226.

Myers, B.A., Hathcock, J., Shickh, N., Roebuck, B.D. (1991): Effects of dietary soybean trypsin inhibitor concentrate on inhibition and growth of putative prencolastic lesions in the pancreas of the rat. Food Chem. Toxic. 29: 437-443.

Nielsen, S.S.(1991): Digestibility of legume proteins. Food Technology. Septemper, 112:118-

Nnanna, I.A.; Phillips, R.D.; Mcwatters, K.H. and Hung,Y.G. (1990): Effect of germination on the physical, chemical and sensory characteristics of cowpea products: flour, paste and akara. J.Sci. Food Agric. 38:812-816.

Noor, M.S.; Bressani, R. and Elias, L.G. (1980): Changes in chemical and selected biochemical components, protein quality and digestibility of mungbean (*Vigna radiata*) during germination and cooking. Qual. Plant. Foods Human Nutr., 30: 135.

Peace, R.W. , Sarwar, G., Touchburn, S.P. , Botting, H.G. (1991): Effects of soybean trypsin inhibitors and DL-ethionine on growth and serum parameters in young rats. Nutr. Res. 11: 1197- 1208.

Price, M.L., Hagerman, A.E. and Butler, L.G. (1980): Tannin contents of cowpeas, chickpeas, pigeonpeas and mungbeans. J. Agric. Food Chem. 28:459-

Pusztai, A., Watt, W.B. Stewart, J. C. (1991): A comprehensive scheme for the isolation of trypsin inhibitors and the egglutinin from soybean seeds . J. Agric. Food Chem. 39: 862-866.

Rahma, E.M.; El-Bedawey, A.A.; El-Adawy, T.A.; Goma, M.A. (1987): Changes in chemical and nutritional factors and functional properties of faba beans during germination. Lebensum. Wiss. Technol., 20: 271-276.

Reddy, N.R.; Pierson, M.D.; Sathe, S.K.;Salunkhe, D.K. (1985): Dry bean tannins: a review of nutritional implications. J. Amer. Oil. Chem. Soc., 62: 541-549.

Saini, H.S., Weder, J.K.P. and Knights, E.J. (1992): Inhibitor activities of chickpeas (*Cicer arietinum* L.) against bovine, porcine and human trypsin and chymotrypsin. J. Sci. Food Agric. 60: 28-295.

Salunkhe, D.K. and Kadam, S.S. (1989): CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization, 1:, CRC Press, Boca Raton, FL.

Sathe, S.K, Iyer, V., and Salunkhe, D.K. (1982): Functional properties of the Great Northern bean (*Phaseolus vulgaris* L.) proteins. Amino acid composition, *in vitro* digestibility, and application to cookies. J. Food Sci. 47: 8-

Sathe, S.K.; Deshpande, S.S.; Reddy, N.R.; Goll, D.E. and Salunkhe, D.K. (1983): Effects of germination on proteins, raffinose oligosacchardies and antinutritional factors in the great northern beans (*Phaseolus vulgaris* L.): J. Food Sci., 48: 1796-1800.

Sato, T., Herman, L. (1990): Morphometry and elemental analysis of rat exocrine pancreas following edministration of trypsin inhibitor. Acta Anat. 137: 65-76.

Savage, G.P.; Thompson, D.R. (1993): Effect of processing on the trypsin inhibitor content and nutritive value of chickpeas (*Cicer aeietinum*): Recent advances of research in antinutritional factors in legume seeds. Wageningen Pers: Wageningen, The Netherlands, pp. 435-440.

Singh, U. and Jambunathan, R.J. (1981): Studies on Desi and Kabuli chickpea (*Cicer arietinum* L.) cultivars: Levels of proteinase inhibitors, levels of polyphenolic compounds and *in vitro* protein digestibility. J. Food Sci. 46:364-1367.

Singh, U., Subrahmanyam, N., and Kumar, J. (1991): Cooking quality and nutritional attributes of some newly developed cultivars of chickpea (*Cicer arietinum*) J. Sci. Food Agric. 55: 37-46.

Stauffer, C.E. (1993): Trypsin inhibitor measurement: Effect of order of reagent addition. Cereal Chem., 70: 111-112.

Swain, T. and Hills, W.F. (1959): The phenolic constituents of *Prumus domestica* L. The quantitative analysis of phenolic constituents. J. Sci. Food Agric., 10: 63-69.

Tella, A. and Ojehomon, O.O. (1980) .An extraction method for evaluating the seed proteins of cowpea (*Vigna unguiculata* L.) Walp) J.Sci. Food Agric. 31: 1268-1274.

Vidal-Valverde, C.; Erias, J.; Estrella, I.; Gorospe, M.J.; Ruiz, R. and Bacon, J. (1994): Effect of processing on some antinutritional factors of lentils. J. Agric. Food Chem., 42: 2291-2295.

Weder, J.K.P., Muller, R. (1989): Reaction of lentil proteins inhibitors with human and bovine trypsin and chymotrypsin. J. Food Biochem. 13:81-103.

Weder, J.K.P.; Link, I. (1993): Effect of treatments on legume inhibitor activity against human proteinases. Recent advof research in antinutritional factors in legume seeds. Wageningen Pers: Wageningen, The Netherlands, pp. 481-485.

التغيرات البيوكيميائيه لبعض المكونات الكيميائية فى بذور الحمص أثناء الإنبات والتصنيع

عادل محمد زكى ، صالح أبوالفتوح ، حنان مهنى ، المرسى أبوالفتوح
قسم الكيمياء الزراعيه كلية الزراعه جامعة المنيا.

لقد تم دراسة تأثير الإنبات على التركيب الكيماوى والمكونات البروتينية والسكريات الذائبة الكلية ونشاط مثبط إنزيم التربسين وكذلك المركبات الفينولية فى بذور صنفين من الحمص. وتناولت الدراسة أيضا تأثير بعض المعاملات المختلفة مثل النقع والطبخ والنقع ثم الطبخ وطبخ البذور المنبتة على نشاط مثبط إنزيم التربسين وشملت الدراسة أيضا هضم البروتينات بالتربسين لمدد مختلفة وذلك لإختيار أنسب المعاملات التى توفر وجبة آمنة ذات جودة عالية للإستهلاك الآدمى من بذور الحمص وتتلخص أهم النتائج فيما يلى:-

١- تميل الجلوبيولينات والألبيومينات إلىالإنخفاض بزيادة فترات الإنبات بينما الجلوتين والبرولامين أظهروا عكس ذلك فى كلا من بذور الصنفين.

٢- ذوبانية الألبيومينات المستخلصه من البذور المنبته إنخفضت إنخفاضا بسيطا بالمقارنه بمثيلاتها المستخلصه من البذور الجافه مشيرا إلى إنخفاض بسيط على pH الحد الادنى للذوبانية.

٣- أظهرت الدراسة أن مستويات مثبط أنزيم التربسين الكلية والفينولات أعلى فى صنف جيزة ١ عن صنف جيزة ٥٣١.

٤- إنخفض مستوى مثبط التربسين إنخفاضا واضحا أثناء الـ ٢٤ ساعه الأولى من الإنبات ليصل إلى ١٢ر٤٧ و٥٥ر٥٠٪ من كميته الأصلية فى البذور الغير منبته لكل من جيزة ١ وجيزه ٥٣١.

٥- إنخفض مستوى المركبات الفينولية خلال الـ٢٤ ساعه الأولى من الإنبات ليصل إلى ٣ر٦٨ و ٤ر٨٠ ٪ من كميته الأصليه فى البذور الغير منبته للصنفيين .

٦- النقع فى الماء لمدة ١٢ ساعه أدى إلى إنخفاض نشاط مثبط التربسين إلى ٢ر٦٩ و ٣ر٧٤٪ من مستواه فى البذور الغير منقوعه. كان الطبخ أكثر فعالية مقارن بـالنقع فى إزالة مضادات التغذية محل الدراسة. النقع ثـم الطبـخ أدى إلـى إنخفـاض نشـاط مثبط التربسين إلى ٠ر٣٥ و٧ر٣٦٪ من كميته فى البذور الغير معاملـة أمـا طبـخ البذور المنبته كان أكثر المعاملات فعالية فى تقليل المثبط.

٧- التفريد الكهربـى للالبيومينـات باستخدام SDS-PAGE أدى إلـى تحلـل البيومينـات إلى١٦-١٩ تحت وحدة على الاقل أوزانها الجزيئية تتراوح من ٥ر١٤ - ٨٩ كيلو دالتون. تحت الوحدات ذات الأوزان الجزيئية العالية والمتوسطة إنخفضت بزيـادة فترات الاثبات. التفريد الكهربى الجلوبيولينات باستخدام SDS-PAGE أظهر وجـود عدد من تحت الوحدات يتراوح من ١٤-١٧ تحت وحدة على الاقل أوزانهـا الجزئية من ١ر١٥-١٤١كليو دالتون. هذه التحت وحدات أمكن تقسيمها إلى أربعـه مناطق أوزانها الجزئيـة أكبر من ٦٦ ومن ٤٤ -٦٦ و ٢٨ -٣٨ و١ و١٥-٢٤ كيلو دالتون.

٨- لقد تم استخدام SDS-PAGE لمعرفه نتائج تحلل البروتينات عند معاملتها بالتربسـين تحت ظروف المعمـل . أدى الهضـم بالتربسين لمـدة ١٠ دقـائق إلـى تحلـل سـريع للتحت الوحدات ذات أوزان جزئيه أكبر من ٤٥ كيلو دالتون. لـم تظهـر بروتينـات البذور المنقوعه أى تغيير عند معاملتهـا بالتربسين لمدة ١٠ أو ١٥ دقيقـه. طبـخ البذور المنقوعـه نتجت عنـه اختلافـات فـى أشـكال SDS-PAGE مقارنـة بالعينـات المنقوعه.