Annals Of Agric. Sc., Moshtohor,
Vol. 35(1):357-374, (1997)

ISSN: 1110-0419

"BIOCHEMICAL STUDIES ON α-AMYLASE INHIBITORS IN SOME LEGUME SEEDS"

BY

El-Sayed, M.M. ; Ramadan, M. E.; Ali, H.M. and El-Morsi, E.A.
Biochem. Dept., Fac. of Agric., Minia Univ.

ABSTRACT

The crude inhibitors of α-amylases were extracted from the whole and dehulled seeds of three legume samples (two Kidney bean varieties, Giza 3 and Giza 6 and one cowpea variety Cream 7). Their inhibitory activities were examined against α-amylases from different origins, "human salivary α-amylase (HSA), porcine pancreatic α- amylase (PPA) and *Bacillus subtlus* α-amylase (BSA)".

The maximum action of inhibitors on α-amylases required pre-incubation of 20 min. The extent of inhibition was also dependent on pH during the interaction of the inhibitor with α-amylases. The obtained pH values were 6.0-6.5, 6.5-7.0 and 5.5-6.0 for inhibition of HSA, PPA and BSA, respectively. Inhibitory activities in whole seeds indicated that cowpea contained the highest levels (116 and 107 units/g seed) followed by Kidney bean var. Giza 6 (102 and 95 units/g), while kidney bean var. Giza 3 contained the lowest ones (81 and 78 units) against HSA and PPA, respectively. Removal of seed coat decrease the inhibitory activities against the three α-amylases by varying degrees. Kinetic studies using the crude inhibitor extract of kidney bean var. Giza 6 indicated that the inhibition of non-competitive type when examined against PPA.

Germination and other processing treatments decreased amylase inhibitor activities in the seeds of three legume samples with varying degrees and non of processing treatments resulted in complete removal of α-amylase inhibitors. Germination plus cooking or soaking plus cooking was more effective in reducing amylase inhibitors from the legume seeds than applying only one of these treatments. The seeds of the three legumes contained different levels of polyphenols (tannins) which are strong inhibitor of α- amylases.

However, with the exception of germination, all other processing treatments decreased tannins, and cooking the germinated seeds was the most effective processing in reduction of tannins and α-amylase inhibitor contents. This germination plus cooking are the recommended treatment for the three legumes to prepare a good quality meals for human utilization.

INTRODUCTION

Despite the nutritional potential of kidney beans and cowpeas as economic sources of significant amounts of proteins, calories and some B-vitamins, the utilization of these two legumes has not paralled to production (Junek *et al.*, 1980), due to the presence of certain antinutritional factors. Of The antinutritional factors reported: (1) trypsin inhibitor activity, (2) tannins (polyphenols), and (3) α- amylase inhibitors.

Although proteins which inhibit the proteolytic enzymes, trypsin and chymotrypsin, have been studied in details, the α- amylase inhibitors have received far less attention. These proteinaceous substances complex with α - amylase forming inactive amylase-inhibitors complexes (Wilcox and Whitaker, 1984).

The presence of amylase inhibitors in kedney beans was reported by Bowman (1945) and rediscovered by Jaffe and Lette (1968). They started to receive much attention because: 1) The α-amylase inhibitor content in *Phaseolus vulgaris* beans is usually high. 2) It is interest that some *P. vulgaris* species produce to kinds of α- amylase inhibitors,a heat-stable one and a heat-labile one (Fresh and Rupnow, 1985). 3) *P. vulgaris* beans are important in the human diet. The α-amylase inhibitors of white (Marshall and Lauda, 1975) red (Powers and Whitaker, 1977) and black (Tanizaki and Lajolo, 1985) combine with porcine pancreatic α-amylase to form a tight 1:1 complex at both pH 5.5 and 6.9. During screening of 150 bean (*Phaseolus vulgaris*) varieties for α- amylase inhibitors, Lajolo *et al.*, (1991), detected many different degrees of inhibitory activity (0.19-0.29 amylese inhibitor unit/mg of protein) with different degree of thermal stability at 80°C, as measured in crude extracts and no correlation between inhibitory activity and seed coat color was observed.

Grant *et al.*, (1995) determined the levels of the α- amylase inhibitor (α AI) in 18 seed samples. Kidney beans, haricat bean, pinto beans and runner beans had high contents of α AI (2-4 g equivalent kg^{-1} seed meal). Butter beans, blackeyed peas, field beans and sweet lupin seeds contained 0.1-0.2 g inhibitor equivalent kg^{-1} seed meal. No α-AI activity was detected in samples of adzuki bean, lentils, mung beans, peas, soybean, sunflower seeds or winged beans.

Hara and Honda (1990) reported that the inhibition of human salivary α - amylase by polyphenols of tea and its specificity was investigated *In Vitro* Carmona, *et al.*, (1991) found that biological utilization of legume seeds is limited by the presence of polyphenols, among other antinutritional factors. Tannin components of the complex polyphenols family, interact with diatary proteins and digestive enzymes, decreasing the nutritional value of ingested foods. Removal the undesirable components from the dry legume seeds is essential for improving their nutritional qualities. To achieve this, several

convetional processing methods such as germination (Sathe *et al.*, 1983; Nielsen and Liener, 1988; Nnanna and Phillips, 1988; Chang *et al.*, 1989; El-Shakankery *et al.*, 1991; Soaking and cooking (Dhurandhar and Chang, 1990; Barampama and Simard, 1994; Vidal-Valverde *et al.*, (1994), have been used. In general, the above mentioned treatments reduced raffinose oligosaccharides and antinutritional factors but the effects varied with legume cultivars and treatments. Barampama and Simard (1994) studied the antinutritional factors in dry beans as affected by processing of these factors, tannin conent was decreased after soaking (28.75%) cooking (59.81%) and Soaking-cooking (84.64%). Tannin content of whole seeds of three different legumes was reported by Ismail *et al.*, (1995). It amounted of 5.3, 4.4 and 4.1 ml/g sample of kidney bean, field bean ansd cowpea, respectively. The same authores indicated reduction in tannin content to reach 52.5, 58.2 and 70.7% of its original levels after 72 hr. of germinating cowpea, field bean and cowpea, respectively. Grant *et. al.* (1995) reported that α- amylase inhibitor activity present in whole kidney beans relatively heat-resistant. However, it could be completely abollished by aqueous heat treatment of fully imbibed beans at 100°C for 5-10 min.

The aim of the present work was to determine the levels of α- amylase inhibitors and polyphenols (tannins) in the seeds of two legumes (kidney bean varieties Giza 3 and Giza 6, and cowpea variety Cream 7). Studying the effect of germination and other processing treatments on those two antinutritional (amylase inhibitors and tannins) factors was carried out and our altimate objective was evalue the most suitable processing method which produce meal that with low or no antinutritional factors of the final product of human consumption.

MATERIALS AND METHODS

The seeds of two kidney bean (*Phaseolus vulgaris*) varietties Giza 6 and Giza 3 and cowpea (*Vigna unguiculata*) seeds variety Cream 7 were obtained from the Department of Holticulture, Minia Faculty of Agriculture, Minia University.

Germination and preparation of sample :

The samples to be germinated were surface stirelized with 0.05 N Hg Cl_2 solution washed throughly with sterelized distilled water and soaked for 2 hr. Ten grams batches of the hydrated legume seeds were germinated in sterile beakers lined with wet filterpaper (Whatman No. 4) which placed in an incubator at 25°C. During germination, distilled water was sprinkled on seeds twice a day. Seeds germinated for 24, 48,72, 96 and 120 hr. were immediately frozen and freezedried. Unsoaked ungerminated seeds served as a control.

Dry seeds and freeze dried samples were ground in a coffee grinder and kept in refrigerator at 4°C until used. For some experiments the seed coat was

removed before grinding process. In some experimental work, the dehulled seeds were used instend of whole seeds.

Extraction of α- amylase inhibitors :

The powdered legume seed (3g) was homogenized in a mortor with a pestel with 15ml 0.1 N HCl. The suspension was stirred for 1 hr. at 4°C, filtered through muslin and then centrifugated at 15000 r.p.m. for 30 min by using a Beckman centrifuge model J21. The supernatant was ajusted to pH 7.0 by adding 1N NaOH. The precipitate formed during this step was removed by centrifugation at 10000 r.p.m. for 30 min. at 4°C. The clear supernatant crude amylase inhibitor was used for determining the α-amylase inhibitory activity and protein determination by the method of Lowry, *et al.*, (1951).

Preparation and assay of α- amylases :

Salivary of α-amylase was obtained from human salivia according to Bernfeld (1955). Procine pancreatic and bacterial (*Baullus subtillis*) α-amylase were obtained from Sigma Ltd. α- amylase activity was determined by method of Bernfeld, (1955), using 1.0 ml of 1% soluble starch in 0.02 M phosphate buffer pH 6.9. After equilibration at 37°C. 0.1 ml. of α- amylase was added and the reaction incubated for exactly 5.0 min. The reaction was stopped by the addition of 2.0 ml. of dinitrosalicylic acid reagent. The solution was heated for exactly 5.0 min. in a boiling water bath, cooled, diluted by adding 10 ml. of H_2O and measuring the absorbance at 540 nm. against a blank prepared in the same way without added α- amylase enzyme. The liberated reducing sugars were expressed as maltose. A calibration curve established with maltose was used to calculate the amylase activity. On unit of amylase activity is defined that amount of enzyme which liberates 1 mg. maltose under the given assay conditions (5 min., 37°C).

α- amylase inhibitor assay :

An inhibitor solution (50-100μl) was mixed with α- amylase dissolved in 0.02 M. phosphate buffer (pH 6.9) contain 0.02 M NaCl. The mixture was incubated at 37°C for 20 min and then the amylase reaction was initiated by adding 1% soluble starch (1ml). The reaction was allowed to proceed at 37°C for 5 min. and stopped by adding 2 ml of 1% 3.5 dinitrosalicylic acid reagent. The reaction mixture was heated for 5 min. in a boiling water bath, cooled and diluted with distilled H_2O. The inhibitor activity was difined as the amount of inhibitor (in mg) required to inhibit 1 unit of α- amylase activity by 50% (Lajola *et al.*, 1991)..

Effect of pH during preincubation of the crude inhibitor with α- amylase :

Suitable aliquots of crude inhibitor extracted from kidney bean (Giza6) was incubated with enzyme for 20 min. at 37°C in 0.3 ml. of 0.05 M buffers of different pH values, 0.01M HCl (pH 2); sodium acetate buffer (pH 3, 4, 5 and 6); sodium phosphate buffer (pH 6, 7 and 8) and glycin-NaOH buffer (pH 8, 9

and 10). The amylase assay was initial by the addition of 1.7 ml. of a 0.6% starch solution dissolved in 0.2 M phosphate buffer (pH 6.9), containing 0.02 M NaCl, controls without inhibitor was running simultaneously.

Effect of dialysis on α- amylase inhibitors :

The crude inhibitor extracts of the three legumes samples (10 ml each) were dialysis against 2 litre of distilled water for 48 hr. at 4°C. The dialysates were centrifuged at 10000 r.p.m. for 30 min. to remove the inert precipitate and the supernatent were examined for inhibitory activity against three different α-amylases.

Some processing treatments :

Sample of each legume was soaked at room temperature in tap water (1:3 w/v) for 12 hr. The soaked seeds were drained. Weighed and some ground and freez-dried. Some of seeds from the soaking process, germination process (96 hr.) in *Phaseolus vulgaris*, (72 hr.) in cow-pea, and untreated samples were separately cooked for 60 min in tap water (seed to water ratio 1:8 w/v). The cooked seeds were drained, weighed and some ground and freez dried.

Extraction and determination of Tannin :

The polyphenolic compounds were extracted from each defatted sample (500 mg) by refluxing with 50 ml of methanol containing 1% HCl for 4 hr. The extract was concentrated in a rotatory evaporator and brought to 25 ml with methanolic-HCl. The amount of phenols were estimated as tannic acid equivalent according to the Folin-Denis procedure (Swain and Hills, 1959).

Statistical analysis :

Means and standard deviation of means were calculated for all data (triplicate determination) according to the procedures of statistical analysis system (SAS, 1985).

RESULTS AND DISCUSSION

Extraction and assay conditions for α- amulase inhibitors :

The amylase inhibitors of the lested seeds were extracted using 0.1 M HCl. Extraction with acid would eliminate endogenous α-amylase activity according to Henson and Stone (1988). Crude inhibitor extract, prepared as described in materials and methods was used for assaying inhibitory activity against three α- amylases from different sources. The effect of several parameter (pre-incubation time, order of addition of reactants and pH) on the inhibtion of α - amylase has been investigated.

1. Effect of pre-incubation period :

The extent of inhibition of different amylases at different preincubation periods by the crude inhibitor of kidney bean (Var. Giza 6) was examined and the results are shown in Fig. (1) which indicated that the inhibition of the

enzymes was dependet on the time of interaction of the inhibitor and the enzymes. It is evident from the data presented in Fig. 1 that the maximal action of the inhibitor on the amylase required pre-incubation of the inhibitor with α-amylase for a minimum of 20 min. pre-incubation periods ranging from 15 to 30 min. were required to achieve maximum inhibition with α-amylase inhibitors from rye (Granum, 1978), colocasia tubers (Sharma and Pattabiraman, 1980). Pre-icubation of the inhibitor with strach for various periods did not alter the inhibitor activity significantly. This probably indicates a minimaldirect interaction involved between substrate and the inhibitor during the course of the reaction.

2. Effect of pH on the inhibitory activity :

It is evident from Fig. 2, that the extent of α- amylase inhibition was also dependent on pH during the interaction of the inhibitor with amylase and the pH optimal were 6-6.5, 6.5-7 and 5.5-6 for the inhibition of human salivary (HSA), porcine pancreatic (PPA) and *Bacillus subtillis* α- amylases (BSA), respectively. The inhibition of the three amylases was markedly decrease below pH 4.5 and above 7.5.

3. Effect of varying amounts of inhibitors on its activities :

A linear pattern of inhibition was observed towards PPA, BSA, and HSA up to 35, 50 and 65 % respectively. With all enzymes a large excess of inhibitor extract nearly complete inhibition was observed.

4. Effect of dialysis :

Dialysis of the crude inhibitors of the three legume samples against distilled water decreased their activities against PPA, and BSA by 35 and 52% respectively. However, their activities against BSA were completely lost after dialysis, even after prolonged incubation and adding NaCl to final concentration of 2m M restored the activity. Lajolo and Filho (1985) mentioned that chloride ion are known to function as activators of α- amylases by modulating its action through a suitable conformation change that resulted in a 340 times increase in calcium binding ability.

Levels and specificity of α- amylase inhibitors :

The crude inhibitors were extracted from the whole and dehulled seeds. Their inhibitory activities were examined against equivalent amounts of α-amylases from different origins and were active against HSA, PPA and BSA. The levels of these inhibitors are presented in Table (1). Cowpea seeds contain higher inhibitor than kidney bean seeds especially those active against HSA and PPA (116 and 107 units/g seeds) of the two kidney bean varieties Giza 6 contains higher levels of inhibitory activities against HSA (47 units/g), and PPA (95 units/g) than Giza 3 (81 and 61 unites/g), respectively. Irshad and Sharma (1981) found that peanut seeds α- amylase inhibitor did not inhibit the BSA and stated that this enzyme differ from those of PPA and HSA.

Table (1): Levels and specificity of amylase inhibitor activities of whole and dehulled legume seeds.

Legume	Inhibition (units/1 g seed)*								
	Human salivary amylase			Porcine pancreatic amylase			Bacterial amylase		
	Whole seed	Dehulled seed	% decrease on dehulling	Whole seed	Dehulled seed	% decrease on dehulling	Whole seed	Dehulled seed	% decrease on dehulling
Kidney bean									
Giza 6	102±1.8	74±1.1	27.5	95±1.6	55±0.8	42.1	56±0.7	44±0.6	21.4
Giza 3	81±1.1	61±0.9	24.7	78±1.2	37±0.6	52.6	86±1.1	76±0.9	11.6
Cowpea									
Cream 7	116±1.9	53±0.7	54.3	107±2.0	84±0.9	27	78±0.9	59±0.8	24.0

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

Removal of the seed coat decreased the activities of the inhibitors against the three amylases by varying degrees (Table 1). This decrease in inhibitory activities may be attributed to the removal of seed coat contain substantial amount tannins which is a strong inhibitor of α- amylases (Griffithus, 1981; Horigome, *et al.*, 1988). Furthermore, tannin from an insoluble protein-tannin complex was decreasing the extractability of inhibitors (Haslam, 1974). However, tannin in legumes are mostly located in the seed coat (Ma and Bliss, 1978) and thus its removal may expected to reduce tannins-similar reduction in tannin content on the removal of seed coat of finger millet (Ramachandra, *et al.*, 1977). Deshpande, *et al.*, (1982) reported increase in relative α-amylase inhibitors after dehuling of 10 bean cultivars.

Mode of inhibition :

Kinetic studies using the crude extract of kidney bean var. (Giza 6) indicated that the inhibitor is of non-competitive type when examined against PPA as indicated by Lineweaver Burk plot shown in Fig. (3). The inhibitor had no effect on the Michaelis constant (km), whereas the maximum velocity (Vmax) was appreciably decreased. These results are identical to those shown by others (Marshal and Lauda, 1975; Irshad and Sharma,1981) who observed a non-competitive inhibition of hog pancreatic amylase by phaseolamine and peanut seeds.

Effect of germination and other processing treatment on α- amylase inhibitor activity :

Change in α- amylase inhibitory activities during germination are presented in Fig. (4A, B and C). The inhibitory activities in kedney bean var. (Giza 3) were decreased to 37, 44 and 49% against PPA, HSA, respectively compared to control (Fig. 4A). Similar trend of decrease in α- amylase inhibitory activities were observed in kedney bean var. (Giza 6) and cowpea var. (cream 7) as shown in (Fig 4B and 4C), respectively. Our results are in accordance with that reported by Satha, *et. al.* (1983) who observed 67.1% decrease in α-amylase inhibitor in great northern beans (*Phaseolus vulgaris*) by the end of 5th day of germination. The decrease in α- amylase inhibitory activity in germinated seed may be attributed to the proteolytic degradation of the inhibitor during germination (Gupta and Wagle, 1980). The activity of proteolytic enzymes, which play a key role in biochemical mechanism of germination, have increased during the germination (Nnanna and Phillips, 1988; Hussein, 1991). Furthermore, decrease in inhibitors during germination as suggested by the action of proteolytic enzymes may gain support by the results of Frels and Rupnow (1985) who found that one of the two α-amylase inhibitor isolated from black bean (I-1) was more susceptible than (I-2) to the action of trypsin. After a 45 min. incubation, I-1 retained only 20% of its original activity while I-2 retained more than 50%.

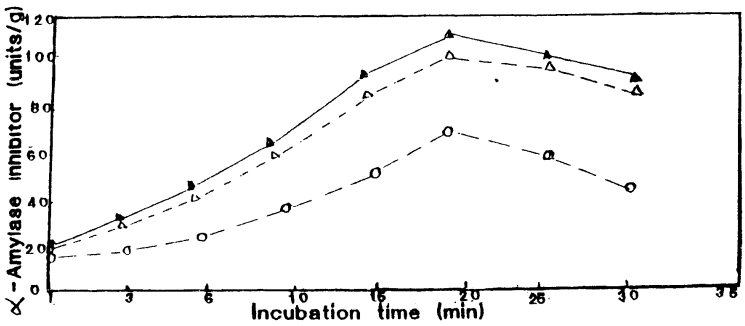

Fig. 1: Effect of preincubation time of the kidney bean inhibitor with α-amylase on its activity against human salivary α-amylase (Δ), porcine pancreatic α-amylase (Δ) and bacterial α-amylase (O).

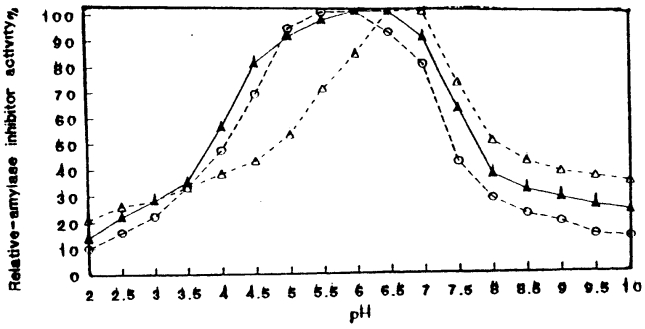

Fig. 2: Effect of pH on inhibitory activity of kidney bean inhibitor against human salivary α-amylase (Δ), porcine pancreatic α-amylase (Δ) and bacterial α-amylase (O).

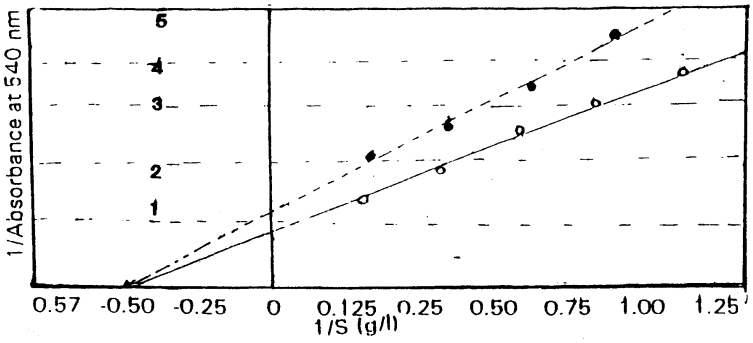

Fig. 3: Line Weaver-Burk plot of hydrolysis of starch by porcine pancreatic α-amylase in the absence (-•-) and presence (-•-) of inhibitor.

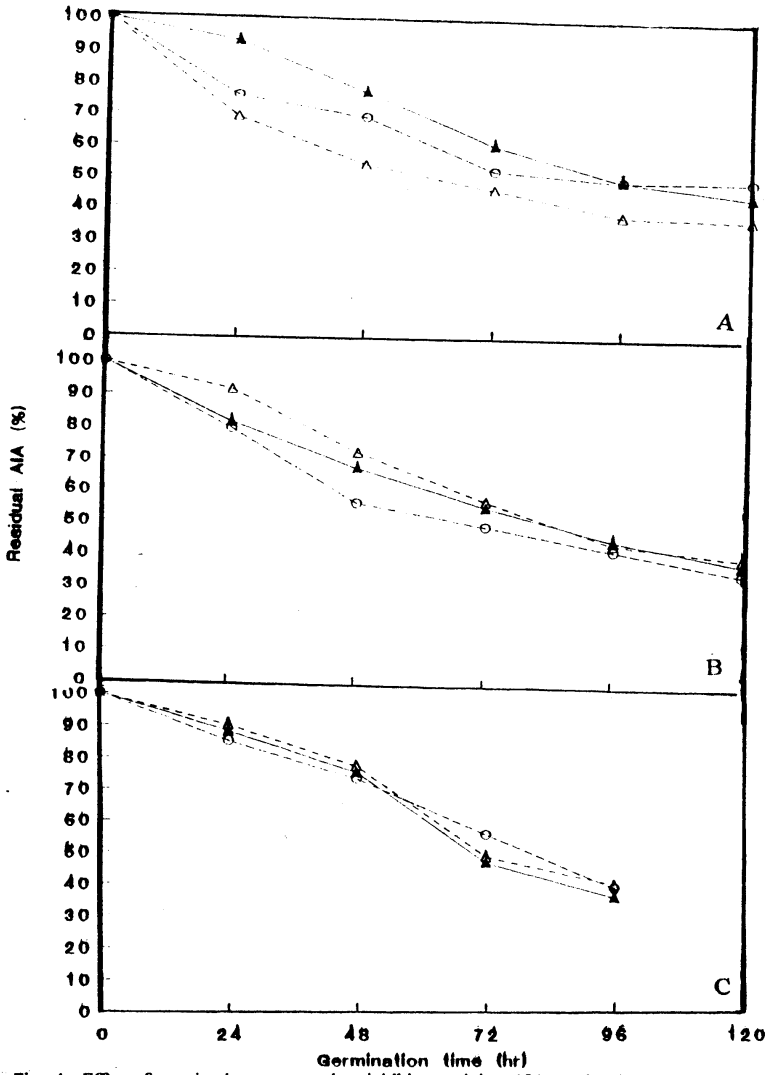

Fig. 4: Effect of germination on α-amylase inhibitor activity (AIA) against human salivary α-amylase (Δ), Porcine pancreatic α-amylase (Δ), and bacterial α-amylase (O).
(A) Kidney bean var. G.3, (B) Kidney bean var. G. 6, (C) cowpea.

The effect of different processing treatments on α- amylase inhibitor was studied and the results are presented in (Fig. 5B and C) for the inhibitors extracted from kidney bean variety Giza 3, Giza 6 and cowpea, respectively. It could be concluded from the data shown in Fig. 5 (A-C) that all the processing treatments decreased α- amylase inhibitor activity in the three legume samples with varying degree. Non of the processing treatments resulted in complete inactivation of α- amylase inhibitor but the highest decrease, compared to raw legume seed, was observed for soaked-cooked kidney bean variety Giza 6 (91, 89 and 87% of inhibition against PPA, HSA and BSA, respectively, Fig.(5B) and the same is true for cowpea inhibitor with decrease ranging from 82-90% for the three enzymes (Fig. 5C). The germinated-cooked kidney bean var. Giza 3 containt the lowest levels of inhibitor which is active against the three enzymes. However soaking or cooking in bothkidney bean varieties contained higher α-amylase inhibitors than the germination ones. In addition, the results revealed that germination plus cooking or soaking plus cooking was more effective in removing α- amylase inhibitors from the seeds of the three legumes than applying one these treatments (Fig. 5A, B, C). Soaking could be considered one of the processing to remove antinutritional factors, which can be eliminated with the discarded soaking solution, but some metabolic reactions can take place during soaking, affecting the content of some compounds (Vidal-Valverde *et al.*, 1992). Cooking generally inactivate heat-sensitive factors such as enzyme inhibitors. The cooking water may be discarded, but some other soluble compounds could be removed such as removal of 30-40% of the kidney bean polyphenols by cooking and discarding the cooking water solution.

Effect of germination and other processing treatments on tannins :

Tannins and polyphenolic content in three legume samples were determined and expressed as tannic acid. The seeds of kidney bean variety Giza 6 contained the highest level of tannic acid (5.3 mg/g seed) followed by Giza 3 sample (4.6 mg/g) wheres the cowpea seed contained the lowest amount (4.1 mg/g). These values are within the values reported by others (Ismaail *et al.*, 1995). After decorticating there was very little tannin detectable in cotyledons of the three legume studied, indicating that almost all the tannins of the seed are present in the seed-coat and this is in accordance with that reported by (Rao and Prabhavathi, 1982).

During the first 24 hr. of germination, tannins were decreased by 41.5% and 43.6% of its original amounts in ungerminated seeds of cowpea and kidney bean, respectively. In subsequent stages of germination the tannins tend to increase as the germination advanced to account for 139, 146 and 246% of its levels in control of kidney bean var. Giza 3, Giza 6 and cowpea, respectively by the end of germination period (Fig. 6). However, tannins can be linked to proteins or carbohydrate forming complexes. Soaking and germination liberate these complexes, therefore, tannin levels increase and the fact that these compounds are thermo and photochemically labile makes them more easily accessible to the analysis. This coincides with the observed decrease of α-

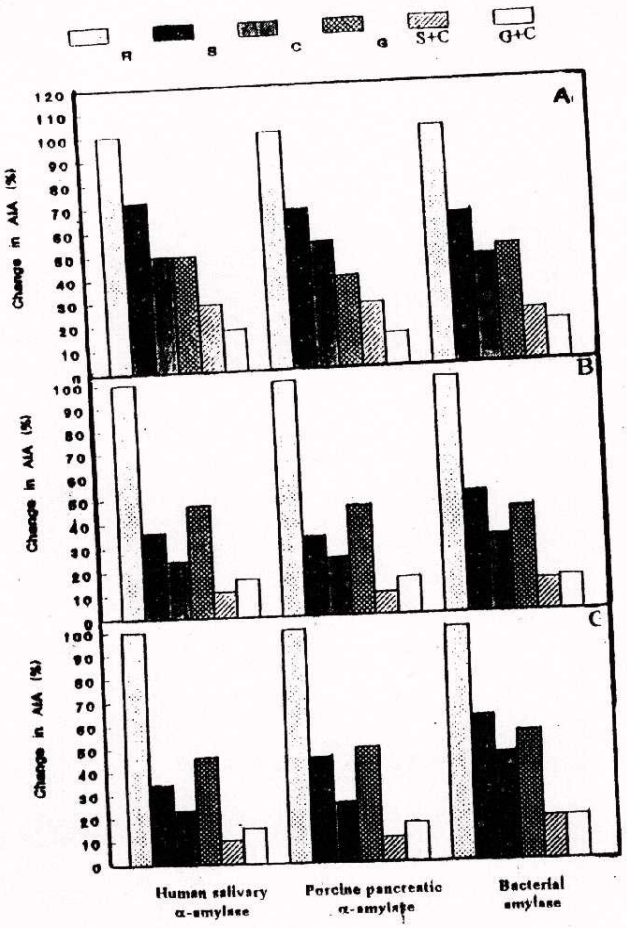

Fig. 5: Effect of processing treatments on α-amylase inhibitory activity (AIA) of crude inhibitor extracted from the seeds.

R: Raw seeds
C: Cooked seeds
S+C: Soaked-cooked seed
Kidney bean var. G.3, B)– var G.6 ;

S: Soaked seeds
G: 96 hr. germinated seed
G+C: 96 hr. germinated-cooked seed A)

galactosides (Vidal-Volverd, *et al.*, 1992) because these compounds are more soluble than tannins.

Other processing treatments were also investigated (Fig. 7) the results indicated that all other treatments decreased tannin. Non of the processing treatments in complete removal of tannin but the highest decrease compared to raw control legumes seeds was observed for germinated-cooked seeds (78, 77 and 69% for kidney bean Giza 3, Giza 6 and cowpea, respectively). One explanation of the decrease in tannins after cooking was probably due to the formation of insoluble tannin-protein complexes not extractable from the seeds (Barampama ans Simard, 1994). Ismail, *et al.*, (1995) observed that, cooking resulted in decrease of tannins and higher decreasing was noted in the soaked-cooked bean. Bressani and Elias (1980) noticed that about 30-40% of polyphenols can be removed from *Phaseolus vulgaris* by cooking and discarding the cooking water solution.

Generally, legume seeds contain antinutritional factors. Two of these factors, α- amylase inhibitor (heat labil factor) and tannins (heat-resistant factor) were studied here. It could be concluded that germinated-cooked or soaked-cooked processing decreased those two antinutritional factor as possible. Since niether amylase inhibitors nor tannins was completely removed by any processing treatments employed here, the hull contributes significantly to the total amylase inhibitory activity in the processed whole seeds.

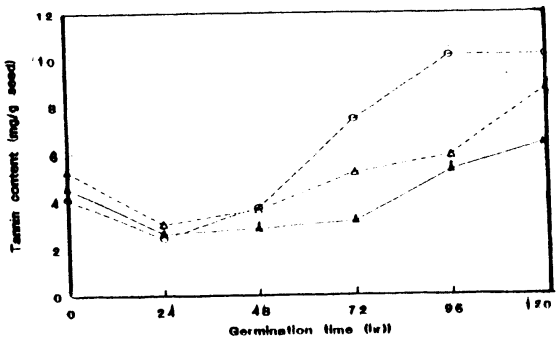

Fig. 6: Effcet of germination on tannin contents in the seeds of kidney bean variety Giza 3 (Δ), Giza 6 (Δ) and cowpea (O).

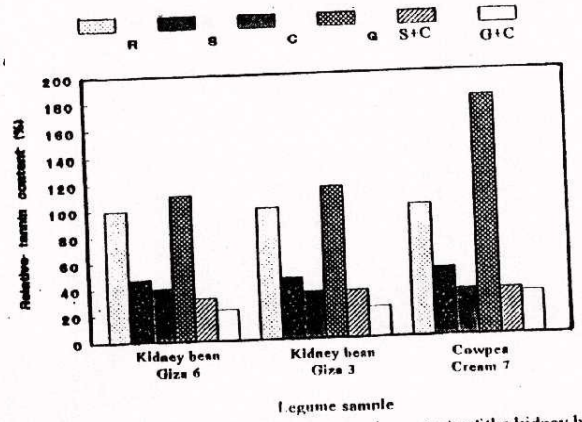

Fig. 7: Effect of processing treatments on tannin contents of the kidney bean and cowpea.

R: Raw seeds
C: Cooked seeds
S+C: Soaked-cooked seed
S: Soaked seeds
G: 96 hr. germinated seed
G+C: 96 hr. germinated-cooked seed

REFERENCES

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

Bernfeld, P. (1955): Amylases, α and β in Methods Enzymology. 1, 149-158.

Bowman, D.E. (1945): Amylase inhibitors of navy beans. Sci., 102, 358-359.

Bressani, R.; Elias, L.G. (1980): The nutritional role of polyphenols in beans. In polyphenols in cereals and legumes, ed Hulse L.H. International Development Research Center, Ottawa, pp. 61-68.

Carmona, A.; Seidl, D.S. and Jalfe, W.G. (1991): Comparison of eztraction methods and assay procedures for the determination of the apparent tannin content of common bean. J. Sci. Food Agric., 56, 291-301.

Chang, K.C.; Chang, D.C. and pha, L. (1989): Effect of germination on oligosaccharides and nonstrach polysacchardies in navy and pinto beans. J. Food Sci., 54, 1615-1619.

Deshpande, S.S.; Sathe, S.K.; Salunkhe, D.K. and cornforth, D.P. (1982): Effect of dehulling on phytic acid, polyphenols and enzyme inhibitors of dry beans (*Phaseolus vulgaris* L.) J. Food Sci., 47, 1846-1850.

Dhurandhar, N.V.; Chang, K.C. (1990): Effect of cooking on firmness, trypsin inhibitors, lectins and cystine/cysteine content of navy and red kidney beans (*Phaseolus vulgaris*). J. Food Sci., 55: 470-474.

El-Skakankery, M.M.; El-Morsi, E.A.; Metry, S.W. and Aoul-fetouh, S.A. (1991): Changes in reffinose family sugars and trypsin inhibitor activity in cowpea seeds during maturation and germination. Minia J. Agric. Res. & Dev., 13, 885-904.

Frels, J.M. and Rupnow, J.H. (1985):Purification and partial characterization of two α- amylase inhibitors from black beans (*Phaseolus vulgaris*). J. Food Biochem., 8, 281-301.

Crant, G.; Edwards, J. and Pusztai, A. (1995): α-amylase inhibitor levels in seeds generally available in Europe. J. Sci. Food Agric., 67, 235-238.

Granum, P.E. (1978): Purification and characterization of α-amylase inhibitor from rye (*Secale cereale*) flour. J. Food Biochemistry, 2, 103-120.

Griffiths, D.W. (1981): The polyphenolic content and enzyme inhibitory activity tests from bean (*Vicia faba*) and pea (*Pisum spp.*) varieties. J. Sci. Food Agric., 32, 797-804.

Gupta, K. and Wagle, D.S. (1980): Changes in antinutritional factors during germination in *Phaseolus vulgaris* a cross between *Phaseolus mungo* (M1-1) and *Phaseolus aureus* (T1). J. Food Sci., 45, 394-397.

Hara, Y. and Honda, M.C. (1990): The inhibition of α-amylase by tea polyphenols. Agric. Biol. Chem., 54(8): 1939-1945.

Haslam, E. (1974): Polyphenol-protein interactions. Biochem. J., 139, 285-288.

Henson, G.A. and Stone, I.M. (1988): Variation in α-amylase inhibitor activities in barley malts. J. Cereal Sci., 8, 39-46.

Horigome, T.; Kumar, R.; Okamoto, K. (1988): Effects of condensed tannins prepared from leaves of fooder plants on digestive enzymes in vitro and in the intestine of rats. British. J. Nut., 60, 275-285.

Hussein, M.Y. (1991): Biochemical studies on fenugreek seeds. M. Sc. Thesis, Fac. of Agric., Minia Univ.

Irshad, M.; Sharma, C.B. (1981): Purification and properties of an α-amylase protein-inhibitor from *Aracgis hypogaea* seeds. Biochemica et Biophysica Acta, 659, 326-333.

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

Jaffe', W.G.and Lette, C.L. (1968): Heat-labile growth inhibiting factors in beans (*Phaseolus vulgaris*). J. Nutr., 94, 203.

Junek, J.J.; Sistrunk, W.A. and Neely, M.B. (1980): Influence of processing methodology on quality attributes of canned dry beans. J. Food Sci., 45: 821.

Lajola, F.M.; Filho, F.F.and Menezes, E.W.(1991): Amy;ase inhibitors in *Phaseolus vulgaris* beans. Food Technology, September, 119-120.

Lajola, F.M. and Finardi Filho, F. (1985): Partial characterization of the amylase inhibitor of black bean (*Phaseolus vulgaris*) var. Rico 23. J. Agric. Food chem., 33: 132-138.

Lowary, O.J.; Rosebrough, N.J.; Parr, A.L. and Ransall, R.J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem., 193, 265-275.

Ma, Y. and Bilss, F.A. (1978): Tannin content and inheritance in common bean. Crop Sci., 18: 201.

Marshall, J.J. and Lauda, C.M. (1975): Purification and properties of phaseolanin, an inhibitors of α-amylase, from the kidney bean, *Phaseolus vulgaris*. J. Biol. Chem., 250: 8030-8037.

Nielsen, S.S. and Liener, I.E. (1988): Effect of germination on trypsin inhibitor and hemagglutinating activities in *Phaseolus vulgaris*. J. Food Sci., 53: 298-301.

Nnanna, I.A. and Phillips, R.D. (1988): Changes in oligosaccarides content, enzyme activities and dry matter during controlled germination of cowpea (*Vigna unguiculata*). J. Food Sci., 53: 1782-1786.

Powers, J.R. and Whitaker, J.R. (1977): Effect of several experimental parameters on combination of red kidney bean (*P. vulgaris*) α-amylase inhibitor with porcine pancreatic α-amylase J. Food Biochem., 1: 239-260.

Ramachandra, G.; Virupaksha, T. and Shadaksharaswany, M. (1977): Relationship between tannins and invitro protein digestability. J. Agric. Food chem. 25: 1101-1104.

Rao, N.B.S. and Prabhavathi, T. (1982): Tannin content of foods commonly consumed in India and its influence on ionisable iron. J. Sci. Food Agric., 33: 39-96.

S.A.S. (1985): " SAS User's Cuide: Basics, Version, 5 Edition "SAS Institute, Inc., Cary, NC.

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

Sharma, K.K. and Pattabiraman, T.N. (1980): Natural plant enzyme inhibitors-isolation and characterization of two α-amylase inhibitors from *Colocasia antiquorium* tuber. J. Sci. Food Agric., 31: 981.

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.

Tanizaki, M.M. and Lajolo, F.M. (1985): Kinetics of the interaction of pancreatic α-amylase with a kidney bean (*Phaseolus vulgaris*) amylase inhibitor. J. Food Biochem., 9: 71-90.

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.

Vidal-Valverde, C.; Erias, J.; Valverde, S. (1992): Effect of processing on the soluble carbohydrate content of lentils. J. Food Prot. 55: 301-304.

Wilcox, E.R. and Whitaker, J.R. (1984): Some aspects of the mechanism of complexation of red kidney bean α-amylase inhibitor and α-amylase, Biochemistry, 23: 1783-1791.

"دراسات كيميائية حيوية لمثبط إنزيم الفا أميليز فى بذور بعض البقوليات"

محمد مصطفى السيد ، محمد السيد رمضان ، هبة محمد على ، المرسى أبو الفتوح المرسى
قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة المنيا

تم إستخلاص المثبط الخام لإنزيم الفا أميليز من بذور كاملة وبذور منزوعة القصرة لصنفان من الفاصوليا صنف جيزة ٣ ، جيزة ٦ وصنف واحد من اللوبيا صنف كريم ٧ . وقدر نشاط هذه المثبطات ضد أميليز اللعاب (HSP) ، أميليز البنكرياس (PPA) وأميليز البكتريا(BSA) .

ووجد أن أعلى معدل لنشاط مثبطات ألفا أميليز يلزمها فترة تحضين لاتقل عن ٢٠ دقيقة وأن أعلى تثبيط على درجات pH 6.0 - 6.5 ، ٦,٥ – ٧,٠ ، ٥,٥ – ٦,٠ ضد PPA, HSA, BSA على التوالى . هذا وعند قياس نشاط التثبيط فى البذرة ككل وجد أن أعلاها فى اللوبيا (١١٦ ، ١٠٧ وحدة/جم بذرة) يليها الفاصوليا صنف جيزة ٦ (١٠٢ ، ٩٥ وحدة/جم) وأقلها الفاصوليا صنف جيزة ٣ (٨١ – ٧٨ وحدة/جم) ضد HSA ، PPA على الترتيب . وعند إزالة القصرة من البذور نقصل نشاط المثبط للإنزيمات الثلاثة بدرجات متفاوتة . هذا وقد كانت نتائج دراسة kinetic للمثبط المستخلص من بذور الفاصوليا صنف جيزة أوضحت أنه من نوع المثبط غير التنافسى ضد PPA.

أوضحت النتائج أن الإنبات والمعاملات الأخرى كالنقع والطبخ أدت الى نقصا فى نشاط مثبط الفا أميليز بدرجات مختلفة وأن أى من عمليات الإنبات لم توقف النشاط تماما . عملية الإنبات ثم الطبخ أو النقع ثم الطبخ أدت الى فقد أكبر نسبة من نشاط تلك المثبطات . هذا بالإضافة الى أن العمليات السابقة قد أدت الى تناقص كمية التانينات والتى تعمل كمثبط قوى لإنزيمات الأميليز.

عموما عمليات النقع فالطبخ أو الإنبات فالطبخ أدت الى فقد معظم نشاط مثبط الأميليز وكذلك محتوى البذور من التانينات لذا يوصى بإستخدام تلك المعاملات لتقليل كفاءة تلك المثبطات وتحسين الصفات الغذائية لتلك البقوليات فى تغذية الإنسان.