Egypt. J. Food Sci. **35**, pp. 83-96 (2007)

Extraction and Characterization of Alpha-amylase Inhibitor from Some Cereals and Legumes

A.Y. Gebriel, A.A. Amin*, M.F. Madkour, H. A. El Banna* and K.F. Mahmoud*

*Dept. of Food Sci., Faculty of Agric., Ain Shams University, Shoubra El-Kheima and *Dept. of Food Sci. & Technol., National Research Centre,Cairo, Egypt.*

ALPHA-amylase inhibitor extracted from different sources, *i.e.*, wheat grains (Sohag 2 and Giza 164), legume seeds such as cow pea (Carim 7 and Giza 3), and kidney bean (Giza 6 and Giza 133) was purified and tested for activity using human salivary and pancreatic alpha-amylase.

Results showed that the alpha-amylase inhibitor activity from samples studied were 120 to 285 unit / mg protein.

The inhibitor was found to be stable at pH range from 2 to 4. It was also stable to digestion by proteolytic enzymes (pepsin and trypsin). The inhibition was faster at 37 °C than at 25 °C, The results showed that the degree of thermal stability of alpha-amylase inhibitor extracted from kidney bean (Giza 133) was at 35-37 °C, activity was decreased on 50 °C for 5 hr. Increased of pre-incubation time between inhibitor and both alpha-amylase enzymes at 37 °C increased the rate of inhibition of these enzymes and the complex formation between them from 60 – 80 % in 30 min, while addition of these enzymes to the mixture containing inhibitor and substrate (without pre-incubation) decrease the percent inhibition.

Therefore, an evidence of specific interference of the alpha-amylase inhibitor with starch availability was established. Such possibilities will have valuable interest in the field of special dietary food preparations for diabetes and over weight reduction purposes.

Keywords: Alpha-amylase inhibitor, Wheat, Legumes.

The naturally occurring substances present in some crops and food products are capable of inhibiting the action of some enzymes, and are the most widespread. Although the inhibition of trypsin and chymotrypsin by some proteins have been studied in detail. The physico-chemical properties, nutritional and physiological roles of alpha-amylase inhibitors have yet received far less attention. It was found that legume alpha-amylase inhibitors showed no inhibition towards plant or bacterial amylases but had similar inhibitory activity towards human pancreatic and salivary amylases.

Purified alpha-amylase inhibitors are glycoproteins and active against porcine pancreatic, human salivary and insect alpha-amylase. They are inactive against bacterial, mold and plant alpha-amylase (Frels & Rupnow, 1984 and Santimone *et al.*, 2004). Proteinaceous inhibitors of amylases have been detected in different organs of several vegetable species cereals and tubers (Shivara *et al.*, 1979) and fruits (Mattoo & Modi, 1970). The amount of inhibitor extracted from whole wheat bread or barley was from 60 to 69 % lower than the corresponding meal. Cooking of legume seeds at 100°C for 20 min markedly decreased the inhibitor activity (Mahmoud, 1987). Almost 15 % of the total inhibitor activity present in crude extract was lost after $(NH_4)_2SO_4$ fractionation and dialysis, (Daiber, 1975). Many inhibitors are destroyed by cooking, but some retain inhibitory activity even after baking (Richardson, 1991).

The stoichiometry interaction between the alpha-amylase and wheat inhibitor showed a binding complex of 1:1 enzyme : inhibitor. The purified alpha-amylase inhibitor from wheat or legumes presents great potential for use in *Phaseulus* genetic improvement programs (Octavio *et al.*, 2005).

Therefore the present work was aimed to study the extraction and purification of alpha-amylase inhibitor from some different sources. Moreover the inhibitors characteristics and enzyme inhibitor mechanism were also studied.

Material and Methods

Material

Wheat grains (Sohag 2 and Giza 164), cow pea seeds (Carim 7 and Giza 3), and kidney bean seeds (Giza 6 and Giza 133) (obtained from the Ministry of Agric. Cairo, Egypt). Human pancreatic and salivary alpha-amylase (obtained from Sigma Company, England) were used.

Methods

Extraction and purification of alpha-amylase inhibitor

Alpha-amylase inhibitor from some sources, *i.e.*, wheat grains; lupin and kidney seeds was extracted and purified according to Täufel *et al.* (1991). Grains and seeds were ground by laboratory mill (Brabender) and suspended in sodium phosphate buffer at pH 6.9 for 17 hr at 4 °C then centrifuged at 3000 xg for 60 min and the precipitate was purified sequentially as follow:

- Dialysis was made at 4°C overnight, precipitation and dialysis against water and then freeze-dried.
- The partially purified inhibitor was dissolved in acetate buffer (50 mM, pH 5.0) containing calcium chloride (5 Mm), and then fractionated on sephadex G-100 column (2.5 x 30 cm at 4 °C).
- The inhibitor solution was dialyzed against acetate buffer (10 mM, pH 4.0) and applied to CM-cellulose column equilibrated with the same buffer at room temperature (32°C ±2). Bound protein was recovered from the column by elution with sodium chloride (0 to 0.5 M) in acetate buffer (10 mM, pH 4.0), and dialyzed, then used for studying the characterization of the inhibitor's properties.

Amylase activity determination

Alpha-amylase activity was determined according to the method described by Bernfeld (1955). The reaction mixture contained soluble starch, human serum albumin, calcium chloride and 2.6 µg of enzyme in sodium phosphate buffer (0.02M) and sodium chloride (0.05 M) at pH 6.9. This mixture was incubated at 37°C for 5 min. Blanks were run simultaneously. Reducing groups liberated from starch were measured by the reduction of 3,5-dinitrosalicylic acid colour reagent. Mix well and read at A_{540} versus blank by Uv/Visible spectrophotometer (Pharmacia LKB-CB44F9, UK). One unit of amylase activity is defined as the amount of enzyme that will liberate 1µ mol of maltose under the above assay conditions.

Alpha-amylase inhibitor assay

Assay of alpha-amylase inhibitor activity was based on the determination of the extent of inhibition of 100 µl of alpha-amylase, caused by an aliquot of inhibitor preparation. A known amount of the inhibitor solution was pre-incubated at 37°C for 20 min with α-amylase (1 unit), human serum albumin (2.0mg) and calcium chloride (2.0mg). After pre-incubation, soluble starch was added and the residual amylase activity was determined by the assay technique described above. Blank digest was run simultaneously.

One unit of inhibitor activity is defined as the amount which causes approximately 50 % inhibition of α-amylase activity under the assay conditions.

Protein determinations

Protein was determined in crude extract or purified inhibitor according to Lowry *et al.* (1951), and modification of the Hartree (1972) procedure.

Alpha-amylase inhibitor characteristics

Pre-incubation time and temperature : The inhibitor was pre-incubated at different periods and temperatures, *i.e.,* 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55min at either 25 °C or 37 °C according to Hoover & Sosulsk (1984). The inhibitor activity was then determined separately.

Inhibitor activity at pH values : The optimal pH for inhibitory activity was determined in digests (2.0ml) containing inhibitor (1.7 units), human serum albumin (2.0mg), calcium chloride (2.0mg) and sodium phosphate buffer 0.02-M containing sodium chloride 0.05-M of varying pH, *i.e.,* 5.0, 6.0, 7.0 and 8.0, sodium acetate buffer 0.02 M containing sodium chloride 0.05 M (pH 4.0 - 5.8), then added these mixtures to alpha amylase (1 unit), The above digests were preincubated at 37 °C for 20 min and the residual alpha amylase activity was determined at pH 6.9 using the standard assay conditions. (Johan & Carmen, 1975).

pH stability of inhibitor: The pH stability of the inhibitor was determined by incubating at various pH values, *i.e.,* 2, 4, 6, 7, 8, 9 and 10 for 4 hr at 37 °C. After 4 hr incubation the contents of amylase, inhibitor and starch were assayed

for inhibitory activity at pH 6.9 and 37 °C against α-amylase as described under the standard assay condition, described above.

Thermal stability of inhibitor: Digests (2.0ml) containing inhibitor (1.8μg protein), sodium acetate buffer (0.02 M), sodium chloride (0.05 M) at pH 5.7 were heat-treated for 20 min at temperatures ranging from 30, 40, 50, 60, 70, 80 and 90 °C. The solutions were then rapidly cooled and pre-incubated at (37 °C & pH 5.7) with α-amylase (1 unit), human serum albumin (2.0mg), calcium chloride (2.0mg) for 20 min. The residual α-amylase activity was determined at pH 6.9 using the standard assay conditions.

The interaction of alpha-amylase inhibitor and alpha-amylase at different salt ionic strength: Alpha-amylase inhibitor was incubated with alpha-amylase at various salt ionic strengths for 30 min at 30°C. The salt was varied, *i.e.*, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 mg [with the addition of various concentrations of NaCl and KCl to the sodium phosphate buffer 0.02 M, pH 6.9]. Controls were run without α-amylase inhibitor to determine the effect of each salt on none inhibited enzyme activity and the residual enzyme activity was also determined.

Proteolytic digestion of alpha-amylase inhibitor: Alpha-amylase inhibitor was incubated with 1.6 units of trypsin in sodium phosphate buffer (0.01 M, pH 7.5 at 37 °C). Trypsin activity was halted with the addition of 100 molar excess phenyl methyl sulfonyl fluoride (PMSF) in 10 % propanol at time intervals up to 6 hr. The pH was adjusted to 6.9 and α-amylase inhibitor was also digested with pepsin. The residual activity was also assayed at time intervals up to 6 hr.

Inhibition mechanism: The type of inhibition between the inhibitor and human pancreatic alpha amylase (HPA) was determined according to the procedures described by Lineweaver & Burk (1934). The rate of alpha-amylase action was determined at three steps using sodium phosphate buffer (0.02 M, pH 6.9) containing NaCl (0.05 M) at 30 °C with control sample for each steps after which the residual alpha-amylase was determined:

- Incubation of HPA with inhibitor at 35 °C for 30 min and addition of different starch concentrations from 1 to 8mg/ml.
- Incubation of inhibitor with starch at 35 °C for 30 min. and then addition of different concentration of HPA (0.5 to 2.0mg of enzyme).
- Incubation of human alpha-amylase with starch at 35 °C for 30 min. and then addition of different concentration of inhibitor (2.0 to 4.0 ml of a solution containing 1.76mg of protein).

Results and Discussion

Alpha-amylase inhibitor was extracted from wheat grains, lupin and kidney. The recovery of the inhibitor from these sources is given in Table 1. The inhibitor was purified from the crude extract with an overall recovery of approximately 68, 85, 78, 81, 67, and 71 % in kidney bean (Giza 6), kidney bean (Giza 133), lupin (Giza 3), lupin (Carim 7), wheat (Sohag 2) and wheat (Giza 164), respectively.

TABLE 1. Total inhibitor unit's from crude samples and steps of purification.

Sources	Steps	Total protein [mg/ml]	Total units inhibitor [UI/ml]	Specific activity [Units/mg]	Recovery [%]	Purification fold
Kidney bean Giza 6	Crude extract	383.718	1802.527	3.726	100	
	Ammonium sulfate	20.918	1661.605	79.430	92.182	21.317
	Sephadex G-100	11.463	1390.507	121.304	77.142	32.556
	CM-cellulose	9.918	1231.705	124.197	68.336	33.332
Kidney bean Giza 133	Crude extract	206.966	1018.419	4.920		
	Ammonium sulfate	11.051	1009.674	91.358	99.141	18.568
	Sephadex G-100	3.353	870.271	259.521	85.453	52.748
	CM-cellulose	3.034	867.006	285.756	85.133	58.080
Cow bean Giza 3	Crude extract	77.330	593.370	7.673		
	Ammonium sulfate	6.516	582.280	89.350	98.130	11.644
	Sephadex G-100	3.172	475.387	149.870	80.116	19.532
	CM-cellulose	2.948	461.164	156.433	77.719	20.387
Cow bean Carim 7	Crude extract	131.354	390.688	2.974		
	Ammonium sulfate	4.576	379.562	82.946	97.152	27.890
	Sephadex G-100	2.761	324.870	117.664	83.153	39.564
	CM-cellulose	2.642	319.050	120.761	81.663	40.605
Wheat Sohag 2	Crude extract	283.007	1052.927	3.720		
	Ammonium sulfate	11.712	901.138	76.937	85.584	20.681
	Sephadex G-100	6.230	732.224	117.532	69.541	31.594
	CM-cellulose	5.769	700.506	121.426	66.529	32.641
Wheat Giza 164	Crude extract	356.037	1183.257	3.323		
	Ammonium sulfate	12.129	1174.472	96.824	99.257	29.137
	Sephadex G-100	7.063	929.808	131.645	78.562	39.616
	CM-cellulose	6.316	841.550	133.241	71.121	40.096

The purified inhibitor was fractionated by using Sephadex G-100 column as shown in Fig. 1a and CM-cellulose (Fig. 1b).

Three peaks were obtained (from B which had higher inhibitory activity) and labeled BI, BII and BIII. These peaks were assayed against human salivary amylase (HSA) and only the BIII found to have inhibitory activity. The pool named BIII (fractions 200-225) was then concentrated and dialyzed against suitable different solutions for each of the subsequent assays. Peaks A, C, D, BI and BII did not show any activity towards alpha-amylase.

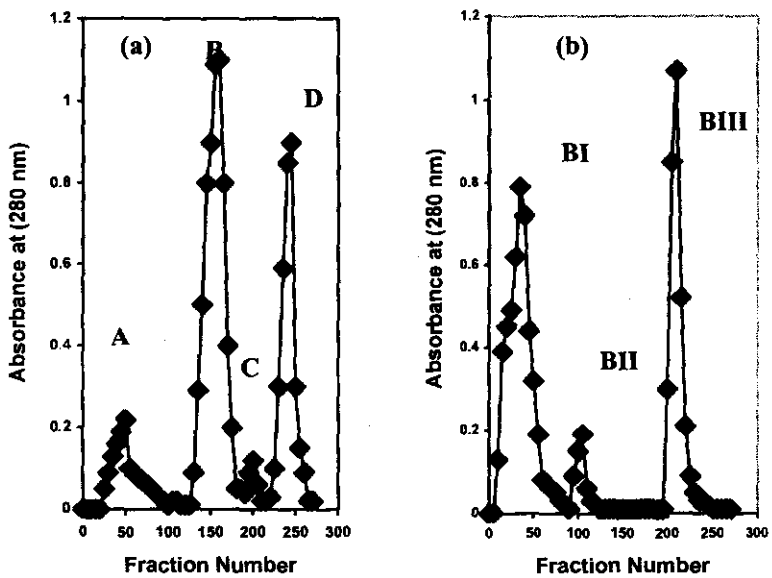

Fig. 1. Chromatography profiles obtained (a) during the Sephadex G-100 and (b) for the CM-cellulose chromatography.

Characteristics of alpha-amylase inhibitor

Heat stability of alpha-amylase inhibitor

Heat stability of alpha-amylase inhibitor was assayed separately at 35, 37, 40, 45 and 50 °C for 0, 1, 2, 3, 4, 5, 6 and 7 hr. The residual inhibitor activity was determined by using salivary and pancreatic alpha-amylase (Fig. 2).

Results showed that at 35 & 37 °C the inhibitory activity was slightly reduced, while such reduction increased at 40 & 45 °C to 10 – 30 % completely lost at 50 °C after 2 – 4 hr of incubation which is in agreement with Iulek *et al.* (2000).

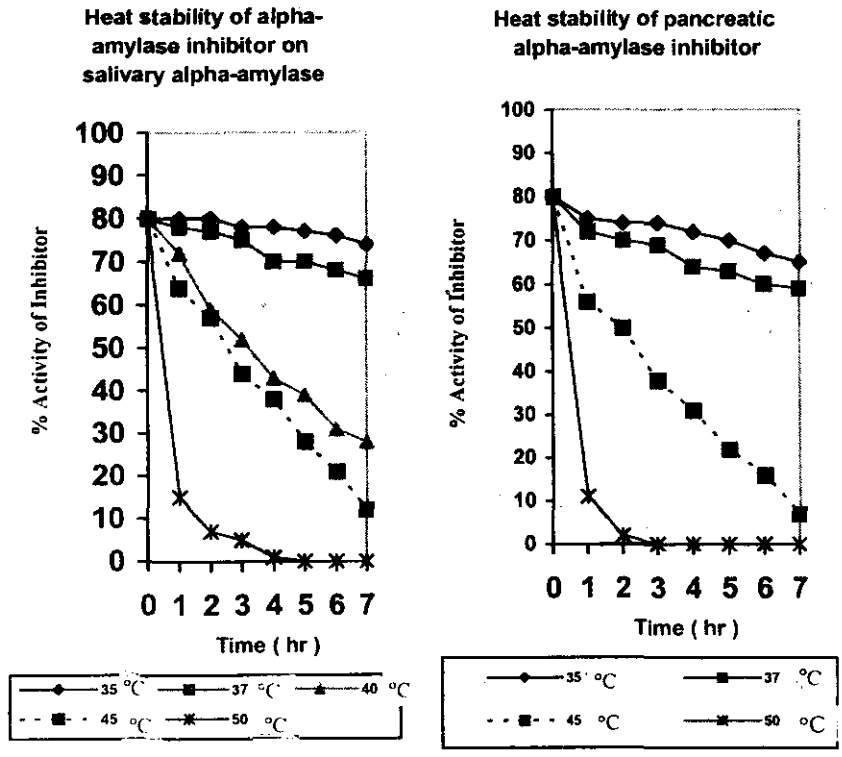

Fig. 2. Heat stability of alpha-amylase inhibitor.

Pre-incubation requirements

The rate of inhibition of either salivary or pancreatic alpha-amylase by inhibitor increased with increasing pre-incubation time up to 30 min and the complex formation between kidney beans alpha-amylase inhibitor and salivary alpha-amylase occurred more rapidly at 37 °C as shown in Fig. 3. Results also showed that the inhibition increased from 60 to 80 % as the time of pre-incubation increased from 0 to 30 min. While addition of enzyme to the mixture containing inhibitor and substrate (without pre-incubation) decreased the percent inhibition. In order to study the effect of pre-incubation on the amylase inhibitor, different pre-incubation periods were tested. A 30 min pre-incubation period was found the maximum.

Inhibitor activity at pH values

The inhibitor was incubated at different pH values, *i.e.*, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 at 37 °C for 20 min, and the inhibitor activity was determined (Fig. 4).

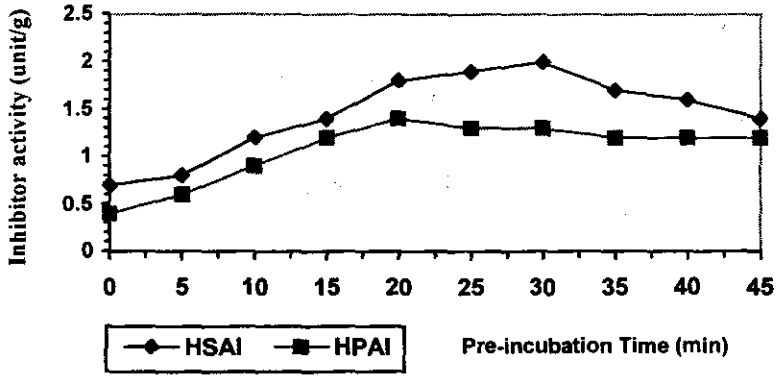

Fig. 3. Pre-incubation period on inhibitor activity.

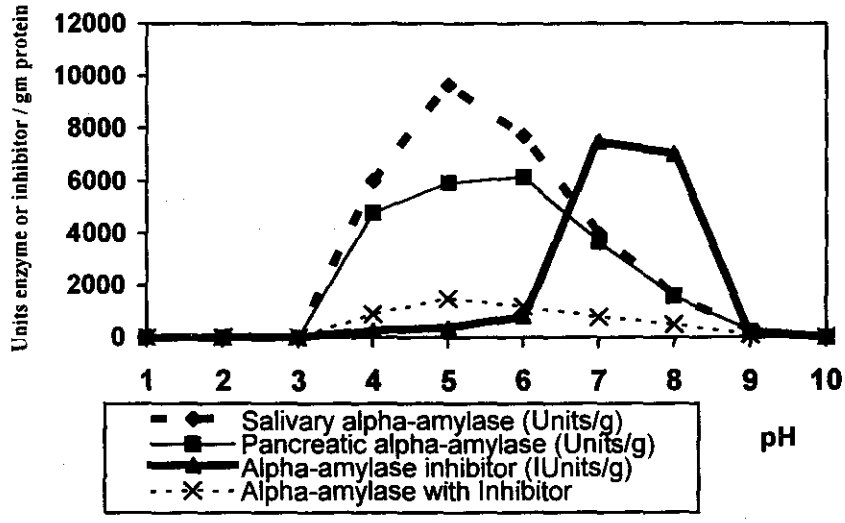

Fig. 4. Inhibitor activity at pH values.

Results show that the inhibition decreased salivary alpha-amylase activity with inhibitor, less than that without inhibitor. The pH optimum for salivary alpha-amylase, pancreatic alpha-amylase, and the inhibitor (from kidney bean) was 5, 6 and 6.9, respectively. These results are in agreement with that reported by O'Donnell & McGeeney (1976) and Täufel *et al.* (1991).

pH stability of the inhibitor

The pH stability for alpha-amylase inhibitor was from 2 to 4 and the activity was decreased as the pH increased up to 10 as shown in Fig. 5.

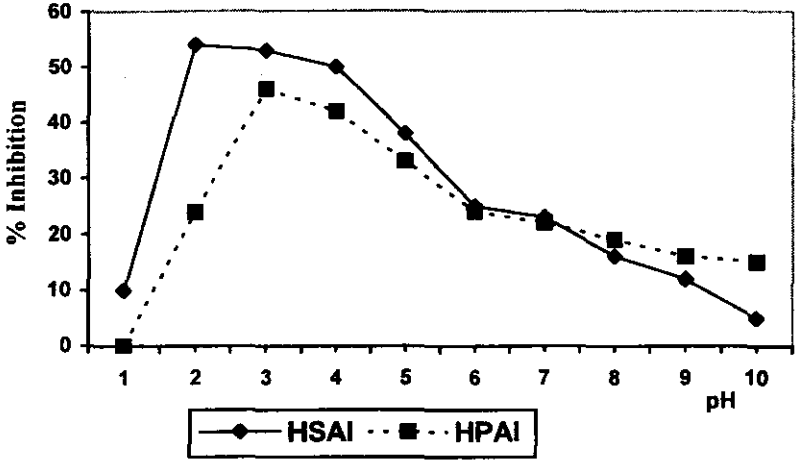

Fig. 5. Stability of alpha-amylase inhibitor at different pH values.

Results showed that the inhibitor was affected at all pH values. Such effect was reflected on alpha-amylase activity. The activity was decreased at all pH values. The inhibition was at maximum values, *i.e.*, 55 and 45 % for HSAI and HPAI and decreased beyond these values.

This indicates that the inhibitor activity was at the maximum (HSAI & HPAI) between 2 – 4 pH and decreased beyond these values.

Alpha-amylase inhibitor as affected by proteolytic enzymes

The action of either trypsin or pepsin on alpha-amylase inhibitor was shown in Fig. 6. Results showed that the inhibitor lost about 33 % of its activity when incubated at 37 °C for 60 min with 1.6 units of trypsin, while alpha-amylase without trypsin retained more than 98 % after 60 min incubation. The % loss in alpha-amylase inhibitor activity was 37 % after 4 hr digestion. This loss was slightly observed after 6 hr incubation at pH 2.0 and 37 °C more than a control sample. While alpha-amylase inhibitor with 1.22 units of pepsin retained only 96% of its original activity and decreased slowly when incubated at pH 2 for 60min and the % loss in alpha amylase inhibitor activity was 18 % after 4 hr digestion, which is in agreement with Jane & John (1985).

These results indicated that the inhibitor was slowly hydrolyzed by trypsin, but was resistant to pepsin.

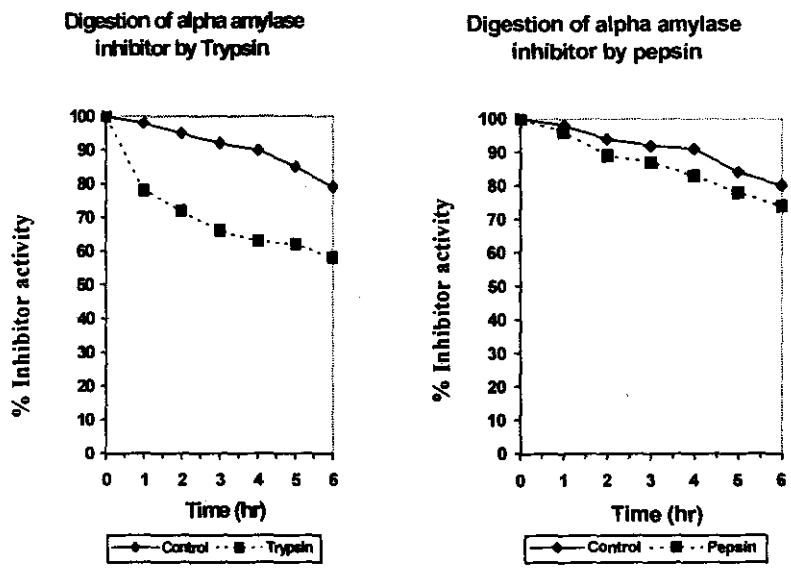

Fig. 6. Proteolytic digestion of alpha-amylase inhibitor.

Effect of salts on the inhibitor activity

The ionic strength of the pre-incubation salt had a profound effect on the inhibitory activities of alpha-amylase inhibitor (Fig. 7).

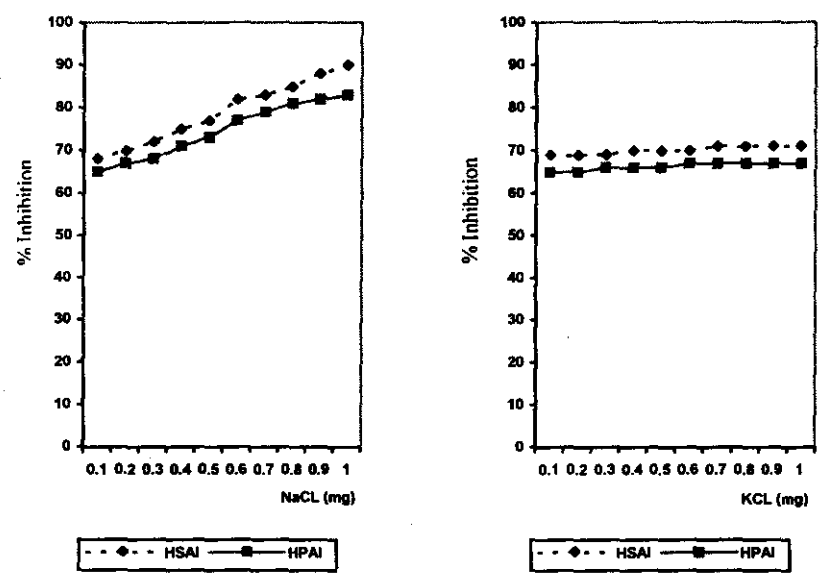

Fig. 7. Inhibitor activity at different salts ionic strength.

The activity of inhibitor at ionic strength 0.9 was 25 % more than that at 0.1. These results are in agreement with the activities reported by Jane & John (1985).

These results suggested that an increase in ionic strength might facilitate inhibition by shielding charge groups on the enzyme or inhibitor, which could cause a conformational change or an alkaline shift at the optimum pH. Although O`Donnell & McGreeney (1976) reported that increasing phosphate buffer concentrations from (0.1 to 50 mM) did not have any effect on the degree of inhibition of salivary alpha-amylase by inhibitor isolated from wheat, while the addition of NaCL (1 mM) to phosphate buffer (0.1 mM), increased the inhibitor activity from 68 to 85 %.

The results showed that addition of KCL up to (1.0 M) have no significant effect on the inhibition of human salivary and pancreatic alpha-amylase by the inhibitor.

Mechanism of inhibition

The mechanism of inhibition of alpha-amylase inhibitor on HPA is presented in Fig. 8.

Results indicated that the inhibitor interact with human pancreatic alpha-amylase via a non competitive mechanism. These results are in agreement with Marshall & Lauda (1975)

The experimental data showed also that inhibition was observed when alpha-amylase was incubated with the inhibitor in the presence of starch.

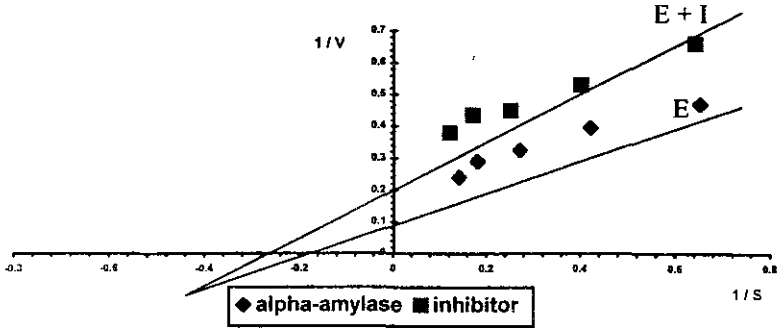

Fig. 8. Mechanism of inhibition of alpha-amylase.

where: V is velocity, S is substrate concentrate, I is inhibitor and E is enzyme.

Conclusion

It could be concluded that the yield of extracted and purified kidney bean alpha amylase inhibitor was 285 unit/mg protein higher than all other examined samples.

It could be concluded that the purified alpha-amylase inhibitor from kidney bean Giza 133 was the most economical effective one for both human alpha-amylase.

References

Bernfeld, P. (1955) Amylases, α and β. In: "*Methods in Enzymology*", Vol. 1, pp. 149-154.

Daiber, K.H. (1975) Enzyme inhibition by polyphenols of sorghum grain and malt. *J. Sci. Food Agric.* **26,** 1399.

Frels, J.M. and Rupnow, J.H. (1984) Purification and partial charactrization of two alpha-amylase inhibitors from black bean (*Phaseolus vulgaris*). *J. Food Biochem.* **8,** 281.

Hartree, E.F. (1972) Determination of protein: a modification of the Lowery method that gives a photometric response. *Anal. Biochem.* **48,** 422.

Hoover, R. and Sosulsk, F.S. (1984) Characteristics and concentrations of alpha-amylase inhibitor in *Phaseolus vulgaris* biotypes. *Starch/Stärke* **36** (7), 246.

Iulek, J., Franco, O.L., Silva, M., Slivinski, C.T., Bloch, C., Rigden, D.J. and Groosi-de-Sá, M.F. (2000) Purification, biochemical characterrization and partial primary structure of a new alpha-amylase inhibitor from secal cereal (rye). *Int. J. Biochem. Cell Biol.* **32,** 1195.

Jane, M.F. and John, H.R. (1985) Characterization of tow alpha-amylase inhibitors from black bean (*Phaseolus vulgaris*). *J. Food Sci.* **50** (1) 72.

Johan, J.M. and Carmen, M.L. (1975) Purification and properties of phaseolamin, an inhibitor of alpha-amylase, from the kidney bean, *Phaseolus vulgaris. J. Biol. Chem.*, **250** (20) 8030.

Lineweaver, H. and Burk, D. (1934) The determination of enzyme dis- sociation constants. *J. Am. Chem. Soc.* **56,** 658.

Lowry, O.H., Rosenbrough, N.J., Farr, A.L. and Randall, R.J.J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193,** 265.

Mahmoud, R.M., Gamal N.F. and Kerrolles, S.Y. (1987) Cereal and legume alpha-amylase inhibitors and their importance in special food preparations. *Annals of Agric. Sci., Moshtohor* **25** (1), 469.

Marshall, J.J. and Lauda, C.M. (1975) Purification and properties of phaseolamin an inhibitor of alpha-amylase, from kidney bean, *Phaseolus vulgaris. J. Biol. Chem.* **250** (20), 8030.

Mattoo, A.K. and Modi, V.V. (1970) Partial purification and properties of enzyme inhibitors from unripe mangoes. *Enzymologia* **39,** 237.

Octavio, L.F., Francislete, R.M. and Paulo, A.M. (2005) Characterization of tow acanthoscelides obtectus alpha-amylase and their in-activation by wheat inhibitors. *J. Agric. Food Chem.* **53**, 1585.

O'Donnell, M. D. and McGreeney, K. F. (1976) Purification and properties of an alpha-amylase inhibitor from wheat. *Biochem. Biophys. Acta* **422,** 159.

Richardson, M. (1991) *"Methods in Biochemistry"*, Vol. 5, pp. 259-305, Academic Press.

Santimone, M., Koukiekolo, R., Moreau, Y., Le-Berre, V., Rouge, P., Marchis-Mouren, G. and Desseaux, V. (2004) Porcine pancreatic alpha-amylase inhibition by the kidney bean inhibitor and structural changes in the alpha-amylase inhibitor complex. *Biochim. Biophys. Acta* **1696,** 181.

Shivara, J.B., Sharma, K. K. and Pattabiraman, T.N. (1979) Partial extraction and properties of alpha-amylase inhibitors from potato tubers. *India J. Biochem. Biophys.* 16, 52.

Täufel, A., Behnke, U., Emmer, I. and Gabor, R. (1991) Studies on the germination specific alpha-amylase and its inhibitor of rye. *Z Lebensm Unters Forsch.* **193,** 9.

(*Received* 8 / 5 / 2007;
accepted 27 / 12 / 2007)

فصل ودراسة خواص مثبط إنزيم الألفا أميليز من بعض الحبوب والبقوليات

أحمد يوسف جبريل ، عزة أنور أمين* ، محمد فهمي مدكور ، هناء عبد المنعم البنا* و خالد فهمي محمود*
قسم علوم الأغذية – كلية الزراعة – جامعة عين شمس – شبرا الخيمة و* قسم الصناعات الغذائية – المركز القومى للبحوث – القاهرة - مصر.

في هذا البحث تم فصل مثبط إنزيم الألفا أميليز من عدة مصادر طبيعية مثل حبوب القمح (سوهاج ٢ و جيزة ١٦٤) و البقوليات مثل اللوبيا (كريم ٧ و جيزة ٣) والفاصوليا (جيزة ٦ و جيزة ١٣٣) ، ثم تنقيته واختباره تجاه إنزيمات الألفا أميليز اللعابي والبنكرياسي من المصدر الإنساني ودراسة خواص هذا المثبط من حيث درجة الثبات الحراري وفترة التحضين ودرجة الأس الأيد روجيني (pH) المثلي ودرجة الثبات علي الـ (pH) ومدي تأثير الإنزيمات الهاضمة علي تحليل المثبط وكذلك تأثير إضافة الأملاح عليه.

أظهرت النتائج أن نشاط مثبط إنزيم الألفا أميليز في العينات المستخدمة في البحث كانت من ١٢٠ إلى ٢٨٥ وحدة / ملليجرام بروتين. ويعطي المثبط ثبات علي (pH) من ٢ إلى ٤ وكذلك يكون ثابت للتحلل بواسطة الإنزيمات الهاضمة للبروتينات مثل التربسين والببسين ، وعملية التثبيط تكون أسرع علي درجة حرارة ٣٧ °م عن ٢٥ °م.

وأن درجة الثبات الحراري لمثبط الألفا أميليز الذي تم فصله و تنقيته من ١٣٣ kidney bean Giza كانت عند درجة حرارة ٣٥ و ٣٧ °م ، ويحدث له فقد في النشاط علي ٥٠ °م لمدة ٥ ساعات.

ولقد أدت إطالة فترة التحضين بين المثبط وكل من إنزيمي الألفا أميليز علي درجة حرارة ٣٧ °م إلى زيادة معدل تثبيط هذه الانزيمات والمعقد المتكون من ٦٠ إلى ٨٠ ٪ بعد ٣٠ دقيقة. وتؤدي اضافة هذه الانزيمات إلي مخلوط المثبط ومادة التفاعل (بدون تحضين) إلي خفض النسبة المئوية للتثبيط.

وتشير النتائج أن هذا المثبط يؤثر علي نشاط إنزيمات الألفا أميليز اللعابي والبنكرياسي وبالتالي يقلل من هضم النشا وتحليلة وتمثيلة في الأغذية النشوية ويمكن استخدامه في الأغذية الخاصة لمرضي السكر وإنقاص الوزن.