J. Agric. Sci. Mansoura Univ., 25 (11): 7239 - 7247, 2000.

TRYPSIN INHIBITOR OF SOME FRESH AND DRY LEGUME SEEDS, ITS PARTIAL INACTIVATION BY GERMINATION OR BOILING.

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ABSTRACT

Trypsin inhibitor activity was determined in both fresh and dry seeds of four legumes namely; broad bean, cowpea, peas and kidney bean. The obtained results showed that the values of trypsin inhibitor activity (TIA) for all samples were higher in dry seeds than those in the corresponding fresh samples.

Dry kidney bean had the highest level of TIA (13.90 TUI/mg) followed by cowpea (12.6 TUI/mg) and pea (9.10 TUI/mg).

Germination of the legume seeds resulted in progressive decrease in TIA. After 72 hrs of germination, more than 60% of TIA were declined in all samples. Extending germination period to 96 hrs, slightly affected the retained activity of trypsin inhibitor in all samples.

Boiling dry faba bean seeds in water for 3 hrs, remarkably decreased TIA. The remained activity was 28% of the original TIA in the untreated sample. Boiling the other legume seeds for different periods resulted in a sharp decrease in TIA in all samples after boiling for 20 min. More than 70% of TI was inactivated, in all samples, after boiling for 60 min.

Neither germination nor boiling caused complete removal of TIA. Therefore, a combination of the two treatments might be more effective.

INTRODUCTION

legume seeds are considered as a rich source of dietary proteins, calories, some vitamins and minerals for a large section of the world's population particularly in the developing countries, since the majority of the people can not afford animal proteins as they are costly (Hughes, 1991; Morrow, 1991 and Attia *et al*., 1994).

A significant problem associated with the consumption of legume food resources is the presence of trypsin inhibitors. These inhibitors and other anti-nutritional factors were recently reviewed by El-Morsi (1996a). Trypsin inhibitors were shown to have an adverse effect on man and animal nutrition due to their inhibitory properties on major pancreatic proteinases (Sato and Herman, 1990; Arentoft *et al*., 1991 and Myers *et al*., 1991), as well as reduced animal weight gain (Kakade *et al*., 1973; Peace *et al*., 1991 and Herkelman *et al*., 1992).

It was reported by several workers that trypsin inhibitors could partially be eliminated or inactivated, to improve nutritive value of legume proteins, by various treatments like soaking, germination or heating, etc. (King and Puwastein, 1987; Bishnoi and Khetarpaul, 1994; Liener, 1994 and Barimalaa and Anoghalu, 1997).

The present investigation aims to estimate trypsin inhibitor activity in some legume seeds, produced in Egypt, in their fresh and dry forms. The

effect of germination and water boiling of the dry seeds on their contents of TIA were also studied in order to improve their nutritive values.

MATERIALS AND METHODS

Sampling:

Fresh and dry mature legume seeds of broad bean (*Vicia faba* var Giza 402), cowpea (*Vigna sincusis* var. Karim 7), peas (*Pisum sativum* var. Little marvine) and kidney bean (*Phaseolus vulgaris* var. Swiss blan) were obtained from Field Crop Institute, Agricultural Research Center, Giza, Egypt.

Germination

The dry mature seeds to be germinated were soaked for 2 hrs in water prior to germination in Petri dishes (lined with wet Whattman filter paper No. 4), which were placed in an incubator at 25°C during germination for 96 hrs. Samples of germinated seeds were collected at 24 hrs interval.

Boiling:

Dry seeds of broad bean were boiled in water for 3 hrs to get "medamis". Whereas, dry seeds of cowpea, peas and kidney bean were boiled in water separately for periods of 20, 30, 40, 50 and 60 min.

Defatting of samples:

Fresh, dry and treated (germinated and boiled) seed samples were decoated and ground separately in a coffee grinder, then fatty matters were extracted with n-hexane at ambient temperature for 24 hrs.

Extraction of trypsin inhibitor (TI):

Trypsin inhibitor was extracted from the defatted samples according to the method of Hamerstrand *et al*. (1981) using 0.01 N sodium hydroxide solution adjusted to pH 9.6 by 0.1 N HCl. The suspension was then centrifuged and filtered, respectively. The clear supernatant of each sample was used for the determination of trypsin inhibitor activity.

Assay of trypsin inhibitor activity (TIA):

Determination of TIA was carried out as described by Hamerstrand *et al*. (1981), modified with respect to the initiation of TIA assay, i.e. trypsin was the last component added to the inhibitor-substrate mixture (Stauffer, 1993). Benzoyl-DL-arginine-P-nitroanilide hydrochloride (BAPA), Sigma chemical Co., St. Louis, USA, was used as a synthetic substrate for trypsin.

Trypsin inhibitor units (TIU):

One trypsin unit (TU) is arbitrarily defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of the reaction mixture at 37°C for 10 min. Trypsin inhibitor activity was expressed in terms of trypsin units inhibited under the same conditions.

RESULTS AND DISCUSSION

Trypsin inhibitor activities (TIA) were determined in both fresh and dry legume seed samples. The obtained results in Table (1) revealed that the values of TIA for all samples were higher in dry seeds than those in the corresponding fresh samples. This may due to that plants; during maturity; may synthesize trypsin inhibitor and translocate it to the seeds, where the inhibitor might has an important role in the dormant state (Elias *et al*., 1975 and Nielsen and Liener, 1988).

Table (1): Trypsin inhibitor activities (TIA) (*TUI/mg dry sample) in fresh and dry faba bean, cowpea, peas and kidney bean seed samples.

Legume sample	Fresh	Dry
Faba bean	3.30	7.50
Cowpea	10.60	12.60
Peas	4.20	9.10
Kidney bean	2.80	13.90

*** Trypsin units inhibited.**

Among legume seed samples under study, dry kidney bean had the highest level of TIA (13.90 TUI/mg) followed by cowpea (12.60 TUI/mg) and pea (9.10 TUI/mg). Similar observations were reported by Aboul-Fetouh *et al*. (1998).

TIA in dry faba bean (Giza 402) was 7.50 TUI/mg as shown in Table (1). This value agreed, in large extent, with those obtained by Wilson *et al*. (1972) and Valdebouze (1977), who showed that TIA in *Vicia faba* ranged between 5.70 and 7.30 TUI/mg. On the other hand, the obtained result for dry faba bean was lower than that recorded for Vietnami bean (11.9 TUI/mg) and higher than that for Czechoslovakian bean (1.1 TUI/mg) (Vinh and Dworschak, 1986 and Blahovec and Ivanko, 1990). It means that trypsin inhibitor could be found in legume seeds in different levels, which suggested that the inhibitor activities were under genetic control as reported by Meshram *et al*. (1980).

Effect of germination on TIA:

Changes in trypsin inhibitor activities (TIA) as affected by germination of all dry legume seeds under study are shown in Table (2) and Fig. (1). TIA decreased progressively in all samples as germination advanced, and after 72 hrs of germination, more than 60% of TIA were disappeared in all samples. Extending germination period to 96 hrs slightly affected trypsin inhibitor activities. In other words, the retained activities after 96 hrs of germination were 32.0, 27.0, 18.7 and 28.0 percent for faba bean, cowpea, peas and kidney bean, respectively.

Several workers reported a TIA decrease during germination. For instance, Rahma *et al*. (1987) found that trypsin inhibitor activity (TIA) in faba bean considerably decreased as a result of germination. Also, TIA were

decreased by germination of cowpea (El-Shakankery *et al*., 1991; Ismail *et al*., 1995; Aboul-Fetouh *et al*., 1998); peas (Abdel-Galil, 1998) and kidney bean (Ismail *et al*., 1995; Aboul-Fetouh *et al*., 1998). Furthermore, several investigators reported a considerable decrease in TIA after germination of other legume seeds, e.g. chick pea, mung bean and lentils (Savage and Thompson, 1993; Vidal-Valverde *et al*., 1994; Urbano *et al*., 1995 and Aboul-Fetouh *et al*., 1998). On the other hand, other workers observed little or no change in TIA after germination of mung bean and lentils (Noor *et al*., 1980 and Weder and Link, 1993).

On the contrary, Chang and Harrold (1988) indicated that germination of navy beans increased TIA by 46.2% and 39.2 after 3 and 6 days of germination, respectively.

Table (2): Effect of germination on trypsin inhibitor activity, TIA (*TUI/mg sample) in dry legume seed samples.

Legume sample	Germination period (hrs)				
	0	24	48	72	96
Faba bean	7.5**(100)	5.8 (77.3)	3.6 (48.0)	2.8 (37.3)	2.4 (32.0)
Cowpea	12.6 (100)	8.1 (64.3)	6.6 (52.4)	3.9 (31.0)	3.4 (27.0)
Peas	9.1 (100)	6.3 (69.2)	4.6 (50.5)	2.0 (21.9)	1.7 (18.7)
Kidney bean	13.9 (100)	11.5 (82.7)	6.6 (47.5)	4.4 (31.6)	3.9 (28.0)

*** Trypsin units inhibited.**

**** Values in parenthesis are percent residual activities.**

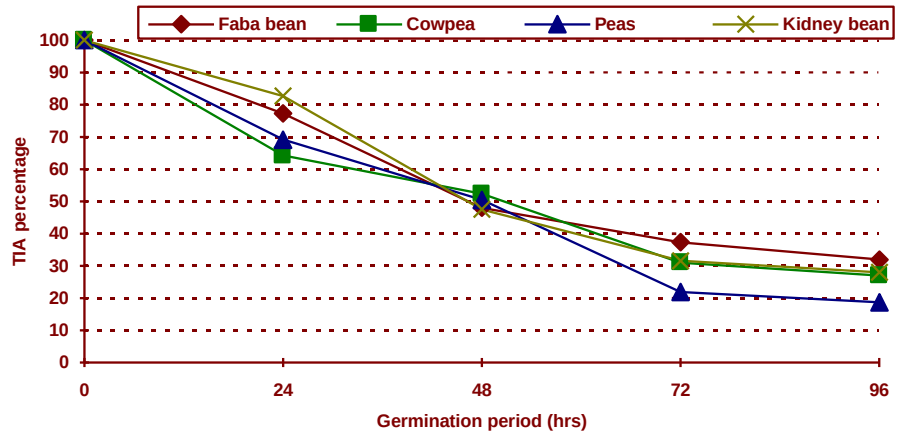

Fig. (1): Effect of germination on trypsin inhibitor activity (TIA) in dry legume seeds.

The reduction in TIA may be attributed to enzymatic degradation of proteins including trypsin inhibitor of legume seeds during germination as reported by Subbulakshmi *et al*. (1976) and Gupta and Wagel (1980). Abe *et al*. (1980) and Barros and Larkins (1990) showed that proteases, which play a key role in biochemical mechanism of germination have increased during germination of corn grains.

Effect of boiling on TIA:

Dry faba bean seeds were boiled in water for 3 hrs to get "medamis". At the end of heating time, the remained activity of TI was 2.1 (TUI/mg), representing 28% of the original activity in the untreated sample. This may due to heat disintegration of high molecular weight proteins to lower ones or polypeptides as indicated by Hamza *et al*. (1986).

The other legume seed samples were boiled separately in water for 60 min. Samples were taken after 20, 30, 40, 50 and at the end of boiling time (60 min.). The results shown in Table (3) and Fig. (2) revealed that a sharp decrease in TIA was achieved in all samples after boiling for 20 min., i.e., more than 40% of TIA was declined. Extending boiling period to 40 min., a gradual decrease of TIA was observed. At the end of boiling period (60 min.), less than 30% of TIA was remained in all samples, and no complete inactivation of trypsin inhibitors was reached by water boiling. This may be attributed to the presence of trypsin isoinhibitors, which more heat stable than trypsin inhibitor in addition to the relative heat stable nature of trypsin inhibitor itself as suggested by El-Morsi *et al*. (1996b).

Table (3): Effect of water boiling on trypsin inhibitor activity, TIA (*TUI/mg sample) in dry legume seed samples.

Legume Sample	Water boiling period (min.)					
	0	20	30	40	50	60
Cowpea	12.6**(100)	6.9 (54.8)	5.1 (40.5)	4.2 (33.3)	3.8 (30.1)	3.6 (28.6)
Peas	9.1 (100)	5.2 (57.1)	4.1 (45.0)	3.2 (35.2)	2.9 (31.9)	2.7 (29.7)
Kidney bean	13.9 (100)	6.2 (44.6)	4.4 (31.6)	3.2 (23.0)	3.0 (21.5)	3.0 (21.5)

*** Trypsin units inhibited.**

**** Values in parenthesis are percent residual activities.**

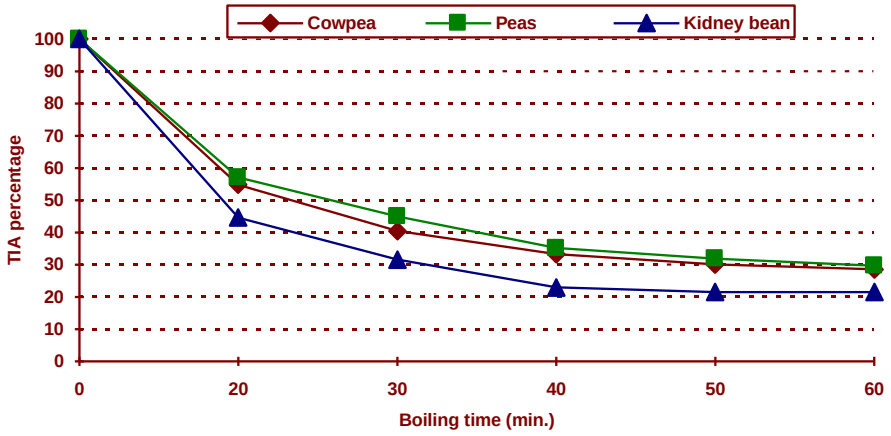

Fig. (2). Effect of water boiling on trypsin inhibitor activity (TIA) in dry legume seeds.

From the results mentioned before, it could be noticed that neither germination nor boiling caused complete removal of TIA. Therefore, a

combination of the two treatments might be more effective. In this respect, Zaki *et al*. (1999) could achieve a 100% inactivation of trypsin inhibitor by autoclaving guar seeds previously germinated for 96 hrs.

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J. Agric. Sci. Mansoura Univ., 25 (11), November, 2000.

مثبط إنزيم التربسين فى بذور بعض البقوليات الطازجة والجافة0 التثبيط الجزئى لنشاط مثبط الإنزيم بالإنبات أو الغليان0

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تم تقدير نشاط مثبط إنزيم التربسين فى كل من البذور الطازجة والجافة لأربعة أنواع من بذور البقوليات هى الفول البلدى والبسلة واللوبيا والفاصوليا0 أوضحت النتائج إرتفاع قيمة نشاط مثبط الإنزيم فى البذور الجافة عن مثيلتها فى البذور الخضراء فى جميع العينات تحت الدراسة 0 وجدت أعلى قيمة نشاط لمثبط الإنزيم فى بذور الفاصوليا الجافة (13.90 وحدة مثبط / ملليجرام عينه) يليها بذور اللوبيا (12.6 وحدة مثبط / ملليجرام عينه) ثم البسلة (9.10 وحدة مثبط / ملليجرام عينه)0

أدى إنبات بذور البقوليات الجافة تحت الدراسة إلى نقص متدرج فى نشاط مثبط الإنزيم حيث وصل النقص إلى أكثر من 60% من قيمة النشاط الأصلى فى البذور الجافة عند إنبات جميع العينات لمدة 72 ساعه0 أدت إطالة مدة الإنبات إلى 96 ساعة إلى خفض المتبقى من نشاط مثبط الإنزيم بدرجة طفيفة0

أدى غليان بذور الفول البلدى الجافة فى الماء لمدة 3 ساعات (للحصول على المدمس) إلى خفض نشاط مثبط الإنزيم بدرجة ملحوظة حيث وصل مقدار النقص إلى 72% من قيمة نشاط مثبط الإنزيم فى البذور الجافة0 أما غليان بذور البقوليات (البسلة واللوبيا والفاصوليا) لمدة 20 دقيقة أدى إلى نقص حاد فى نشاط مثبط الإنزيم0 وعند زيادة مدة الغليان إلى 60 دقيقة وصل مقدار النقص فى نشاط مثبط الإنزيم إلى أكثر من 70% من قيمة النشاط الأصلى فى البذور الغير معاملة0

من هذا يتضح أن كل من المعاملتين (الإنبات أو الغليان) أدت إلى نقص جزئى فى نشاط مثبط إنزيم التربسين ولم تنجح أى من الطريقتين بمفردها فى الإيقاف الكامل لنشاط مثبط الإنزيم0 لذلك قد يكون من المناسب دمج المعاملتين أى غليان البذور المنبته للحصول على نتائج أفضل0