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Short Communication

Effect of sprouting on trypsin inhibitor of cowpea (*Vigna unguiculata*)

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With the increase role that legume protein plays in the world food supply, the nutritive quality related to trypsin inhibitor level is of major concern. The presence of trypsin inhibitors has been demonstrated in a wide variety of cereals and legumes. Rackis (1972) reported that an increase in nutritive value of soy flour paralleled the destruction of trypsin inhibitor activity. Liener (1974) suggested that trypsin inhibitors, along with other growth inhibitors that exist in addition to that resulting from phytohaemaglutinins in soybean seeds, accounted for growth inhibition noticed when these seeds are fed to rats. This study was carried to find out the extent of sprouting effect on the anti-nutritional factor' trypsin' in cowpea and it was discovered that there was a progressive reduction in the trypsin inhibitor as the germination period increases. The reduction was four fold of the original content.

Key words: Phytohaemaglutinins, aminomethane, benzoyl-DL-arginine-P-nitroanilide (BAPA), horsegram, dimethyl sulphoxide (BDH), *Phaseolus mungo*, immunoelectrophoresis, *Phaseolus aureus*, *Phaseolus mungoreous*, trypsin inhibitor.

INTRODUCTION

Germination has been investigated as a means of reducing the anti-nutritional effects of protease inhibitors. Desikachar and De (1950) found that trypsin inhibitor activity was not lost during germination of soybean. Similar results were also obtained by Charropadhyay and Banerjee (1953), for some germinated common Indian pulses. However, Kakade and Evans (1966) reported a slight decrease in the trypsin inhibitor activity with sprouts from navy beans. A similar trend was also reported for soybean sprouts by Bates et al. (1977). The slight loss in soybean trypsin inhibitor activity was attributed to leaching during the daily washing of the sprouts (Collins and Saunders, 1976). Freed and Ryan (1978) demonstrated only minor changes in soybean trypsin inhibitor activity using specific immunoelectrophoresis assay techniques. Pusztai (1972) concluded that the individual inhibitor components are in flux. Although, the trypsin inhibitor activity declined slowly after the 10^{th} day of sprouting, it was still present in sufficient quantity to be of potential concern.

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A high trypsin inhibitor activity of horsegram and moth bean was reported by Subbulakshmi et al. (1976). Although a high level of trypsin inhibitor was recorded for horsegram (950 x 10^3 TIU/g seed) in the ungerminated form, as compared to that of moth bean (4 x 10^3 TIU/g seed), the trypsin inhibitor activity in 72 h germinated samples was reduced by about 16% in the case of horsegram and 40% in the case of moth bean. Cooking was found to inactivate the trypsin inhibitor activity to a considerable extent, although reduction was more pronounced in horrsegram than in moth bean. Gupta and Wagle (1980) discovered that, during a 9 h sprouting of *Phaseolus mungoreous* (a cross between *Phaseolus mungo* and *Phaseolus aureus*), the trypsin inhibitor activity decreased from 101.6 to 51.6 TIU/g seed. A similar trend was observed in the parents *P. mungo* and *P. aureus*. They discovered that with the onset of germination, trypsin inhibitor activity increased slowly up to 295 TIU/g seed in 72 h in the cross, and then declined to 46.6 TIU/g seed after a period of 9 days. *P. mungo* and *P. aureus* were also observed to behave in a similar manner. They attributed the slight increase in trypsin inhibitor activity at the onset of germination to the transformation of the dormant state of the seed to the vigorous metabolic state. This effect was found to be

Table 1. Effect of sprouting on trypsin Inhibitor activity of cowpea expressed as (TUI/g) based on 4 separate determination.

Germination period (days)	TUI/g				
	1	2	3	4	Mean
0	1885	1855	1900	1860	1875
2	785	760	815	770	782
3	704	727	735	715	720
4	414	445	446	435	435
Standard error of difference (SED)					13.37
Variance ratio (F)					4479.8

Correlation coefficient (r = - 0.92)

similar to that reported by Puztai (1972). Gupta and Wagle (1980) also found that, the combination of germination and heating caused destruction of trypsin inhibitor. Trypsin inhibitor activity loss of about 71.8% was reported in *P. mungoreus* in a temperature range of 50 to 80°C after 24 and 36 h germination.

MATERIALS AND METHODS

Determination of trypsin inhibitor activity of cowpea

This Method is based on that described by Kakade et al. (1974). Tris-buffer (0.05 M, pH 8.2) containing 0.02 M $CaCl_2$: 6.05 g tris-(hydroxymethy) aminomethane (from Sigma Chemical Co.) and 2.94 g $CaCl_2.2H_20$ dissolved in 500 ml of distilled water. The pH was adjusted to 8.2 and the volume made up to 1L with distilled water.

Substrate solution

40 mg of benzoyl-DL-arginine-P-nitroanilide (BAPA) hydrochloride (Sigma Chemical Co) were dissolved in 1 ml of dimethyl sulphoxide (BDH) and diluted to 100 ml with tris-buffer prewarmed to 37°C. The BAPA solution was prepared daily and kept at 37°C while in use.

Trypsin solution

4 mg of accurately weighed trypsin (crystalline, salt free) (Sigma Chemical co) was dissolved in 200 ml 0.001 M HCl. The solution was stored in the refrigerator.

Cowpea samples

Raw cowpea (ungerminated), 2, 3, 5 day germinated cowpeas, and processed cowpeas were evaluated for their trypsin inhibitor activity.

Preparation of cowpea samples for assay

1 g of sample was extracted with 50 ml of 0.01 M NaOH. The extraction time was 1 h for the raw and germinated samples and 3 h for the processed samples. The pH of the suspension was usually 9.5 to 9.8. The raw and germinated sample extracts were diluted 1:50 while the processed sample extracts to 1:10 with distilled water.

Procedure

Portion (0, 0.6, 1.0, 1.4 and 1.8 ml) of the diluted cowpea suspensions were pipetted into duplicate sets of test tubes and adjusted to 2 ml with distilled water. After 2 ml of trypsin solution had been added to each test tube, the tubes were placed in a water bath at 37°C. To each tube, 5 ml of BAPA solution previously warmed at 37°C were added, and exactly 2 min later, the reaction was terminated by adding 1 ml of 30% acetic acid. After thorough mixing, the contents of each tube were filtered (Whatman No, 54`) and the absorbance of the filtrate was measured at 410 nm against a reagent blank. The reagent blank was prepared by adding 1 ml of 30% acetic acid to a test tube containing 2 ml each of trypsin solution and 2 ml distilled water, followed by the addition of 5 ml BAPA solution.

Calculation of trypsin inhibitor unit

In calculating the result, one trypsin unit (TIU) was arbitrarily defined as an increase of 0.01 absorbance units at 410 nm per 10 ml of reaction mixture under the condition described here. Trypsin inhibitor activity was defined as the number of trypsin units inhibitor (TIU)

RESULTS AND CONCLUSION

The effect of sprouting on trypsin inhibitor activity of cowpea expressed as TIU/g was investigated. The results in Table 1 are based on 4 separate determinations. The results clearly show that, there was reduction in the trypsin inhibitor activity of cowpea during sprouting. Reduction in trypsin inhibitor activity of cowpea and the period of germination have been found to be highly correlated (r = - 0.92).

Conclusion

The trypsin inhibitor activity of cowpea decreased with increasing germination period. A high correlation was found between reduction in trypsin inhibitor activity and the period of germination (r = -0.92). By the second day

of sprouting, 58% of the original trypsin inhibitor activity present in raw unsprouted cowpea was removed; fell to about 1/5th of the original trypsin inhibitor activity by the end of the fifth day. The values of the trypsin inhibitor shown in Table 1 is relatively small, compared to what has been reported present in other legumes. Soy bean for instance, was reported by Collins and Saunders (1976) to have a trypsin inhibitor activity as high as 226000 TUI/g.

Subbulakshmi et al. (1976) reported reduction in trypsin inhibitor activity on horsegram and moth bean during germination. A reduction of about 16% was reported for horsegram, and 40% in the case of moth bean. Similar report of reduction was also reported for soy bean during germination by Collins and Saunders (1976). Gupta and Wagle (1980), in their germination studies with *P. mungoreous*, reported a slight increase in trypsin inhibitor activity with the onset of germination, which later rapidly detailed with increasing germination period. The decrease in trypsin inhibitor activity during sprouting might be due to leaching during early stages of sprouting.

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