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Some anti-nutritional factors of mature sword beans *(Canavalia gladiata)*

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Abstract

The anti-nutritional factor evaluation of *Canavalia gladiata* seed flour indicated a high amount of phytic acid, which decreased with processing. The raw cotyledon fraction had the highest content of phytate (8.5 mgg^{-1}) whereas the dry-autoclaved cotyledon flour had only 4.3 mgg^{-1}. The seed coat contained an α-amylase inhibitor and a trypsin inhibitor. The presence of an α-amylase inhibitor in the seed coat was established for the first time. The trypsin inhibitor activity of whole seed flour and boiled whole seed flour was 4866 $TIUg^{-1}$ and 2745 TIU_g^{-1} respectively. The raw cotyledon was devoid of any inhibitor activity. The molecular weight of the trypsin inhibitor was found to be around 90 kD. The raw whole seed extract indicated a decrease in the α-amylase activity by 924 U/L indicating the presence of an inhibitor. There was complete inhibition of α-amylase activity by the raw seed coat extract. In the boiled seed coat extract the α-amylase activity increased (284 U/L). The different enzyme inhibitor activities were due to separate components present in the seed.

Key words: Anti-nutritional factors, *Canavalia* seeds

1. Introduction

The legume commonly known as sword beans (*awara*-Sinh., *Canavalia gladiata*), considered as an "old world tropic" belóngs to genus *Canavalia* of the family Leguminosae (Smartt, 1976). The protein content in the mature seed is comparatively higher than in most legumes and the amino acid profile compares well with that of the reference protein (casein). The content of starch, fat and the mineral profile, also indicate *C. gladiata* to be a good supplement to cereal based diets (Ekanayake, 1999). However, the results of animal feeding experiments with raw and processed samples of mature seed flour indicated the seeds to have a low net protein utilisation (NPU) (Ekanayake *et al.,* 2000).

The limited widespread utilization of *Canavalia* could be due to the effects of deleterious factors such as enzyme inhibitors, phytic acid (myo-inositol 1,2,3,4,5,6- hexadihydrogen phosphate, a naturally occurring organic compound found in plant seeds and/or grains, roots, tubers), saponins and polyphenolic compounds (Wickramanayake, 1996; Singh, 1984; Tan *et al.,* 1984; Knuckles *et al.,* 1985) which lower the NPU.

Phytate is a chelating agent for cations, and a form for storage of cations as well as phosphorous in many seeds (Oberleas, 1973). By binding (chelating) nutritionally important minerals, such as calcium, magnesium, copper, iron (Fe^{2+} and Fe^{3+}) and others, some of which are necessary as co-factors, phytic acid interferes with several essential metabolic processes as well as Causing reduced solubility of the metals (Oberleas and Prasad, 1969; Oberleas, 1973). *Canavalia* contains many essential minerals and their availability is important in its use as a food (Ekanayake, 1999).

Substances that have the ability to inhibit proteolytic activity of certain enzymes, are found particularly in legumes. The presence of trypsin inhibitors in the sword bean has been reported by Nagaraja and Pattabiraman (1991), Rajaram and Janardhanan (1992), Laurena *et al.*, (1994) .

The presence of α-amylase inhibitor in legume species has been reported as far back as 1945 (Bowman, 1945). The presence of such an inhibitor could interfere with starch digestion and thereby decrease the available energy content which could also limit the use as a food. This inhibitor is also known for its therapeutic value, by decreasing carbohydrate absorption in diabetic patients and in the obese (Garcia-Olmedo *et al.*, 1987; Menezes and Laljo, 1987).

The intention of the present work was to determine the presence of phytic acid, trypsin inhibitor and α-amylase inhibitor in the raw whole seed, cotyledon and some processed flour samples.

2. Materials and methods

Mature sword beans (*Canavalia gladiata; awara in Sinhala*) originally obtained from Galle, Sri Lanka, (where the red seeded, white flowered variety known as *rathu awara* is common) was cultivated in an experimental plot in Kandy and were utilized for the evaluation of anti nutritional properties.

The seeds were removed from the mature pods, air dried and stored at 4°C pending analysis. Prior to analysis, seeds were washed with tap water, rinsed with distilled water and oven dried at 50°C overnight (12 hour).

The whole seeds and cotyledons were ground to a flour of particle size 40-60 mesh using a standard mill (Cyclotec 1093, Tecator, Sweden). Flour samples were stored in a desiccator until required for analyses. Some analyses were carried out with processed cotyledon flour as well. All reagents used were of analytical grade.

Processing

i) Autoclaving

A cotyledon flour sample was autoclaved at 121°C for 20 minutes wrapped in a muslin cloth. The sample was allowed to cool, homogenised and stored in the cold room (0°C).

ii) Roasting

A cotyledon flour sample was put in a nickel coated can and covered with an aluminum foil with pores to allow ventilation and roasted at 150°C for 50 min. attached to a rotating bar of the oven. Sample was cooled and stored in the cold room pending analysis.

iii) Preparation of extract for α-amylase inhibitor activity

Three extracts (i) cotyledon flour (0.5g) in phosphate buffer (3ml; 0.01M, pH7) kept shaking (Eyela, Japan, speed-75) for 15 min at room temperature (28°C). (ii) seed coat extract (0.5g) in phosphate buffer (6ml; 0.01M,pH7)kept shaking (Eyela, Japan, speed 75) for 15 min at room temperature (28°C). (iii) boiled seed coat (0.5g) in phosphate buffer (10 ml; 0.01M,pH7) held at 100°C in a water bath for 15 min were obtained.

All extracts were centrifuged at 2000rpm and supernatant taken for analysis.

iv) Preparation of extract for trypsin inhibitor activity

Whole seed flour or cotyledon flour (1g) were extracted with 0.1M NaOH (50ml) by shaking (Eyela, Japan, speed-75) for one hour.

Phytate

Phytate was determined as hexaphosphate equivalents, by the method developed by Harland and Oberleas (1986). The amount of phytate in the sample was calculated as hexaphosphate equivalent. Sodium phosphate solution was used as the standard to test the procedure.

α-Amylase inhibitor

The α-amylase inhibitor activity was investigated using Sigma kit for α-amylase detection in serum samples (Rauscher *et al.*,1986). The synthetic substrate, 4,6-diethylidene (G_7)-p-nitrophenyl (G_1)-α,D-maltoheptaside (ET-G_7PNP) is converted to p-nitrophenol and glucose by the action of α-amylase and α-glucosidase. Absorbance of pNP at 405nm is proportional to α-amylase activity and the absorbance decrease in the presence of an inhibitor.

Supernatants prepared as above (480μl) were used for inhibitor analysis. The samples were added to synthetic substrate (1ml) and α-amylase (20μl;sigma porcine α-amylase) and buffer (500μl) to make the final volume to 2ml. The alfa amylase was reconstituted in such a manner, so the control reaction reached a plateau in 7 to 10 min (0.1μl α-amylase diluted to 1ml with distilled water). The control had buffer in place of the sample solution.

The reaction was carried out in a cuvette. The substrate was added last in all samples. The absorbance was measured continuously from zero time until a constant absorbance was reached while the samples were incubating at room temperature against a reagent blank and a blank to compensate for the colour in the sample (extract 480µl+buffer 1.5ml+ α-amylase 20µl).

A crude extract was also eluted through a polyamide (polycaprolactam, polyamide cc 6; Conner *et al.*, 1990) column to determine the nature of the inhibitor. After eluting the crude extract, the column was washed with methanol (90%). The methanol solution used to wash the column was evaporated and reconstituted with phosphate buffer (pH7) and α-amylase activity tested.

Trypsin inhibitor activity

Tyrpsin inhibitor activity was determined by the enzymatic assay of Kakade *et al.*, (1974). *Canavalia gladiata* whole seed or cotyledon flour were extracted with NaOH (1g each in 50ml NaOH) and incubated with trypsin and a solution of benzoyl-D,L-arginine-*p*-nitroanilide hydrochloride (BAPNA; Sigma Chemicals Co., St. Louis, MO, USA) added. The reaction was terminated by adding 30% acetic acid. The absorbance measured at 410nm (ELICO, SL-150 UV/VIS spectrophotometer) against a reagent blank. One trypsin unit is expressed as an increase of 0.01 absorbance unit per 10ml reaction mixture at 410nm. Trypsin inhibitor activity is defined in terms of trypsin units inhibited per milligram of protein.

3. Results and discussion

Phytic acid

The phytic acid content of raw and processed *Canavalia gladiata* seed flour is given in Table 1. Phytic acid content decrease significantly ($p<0.05$) with heat processing and the decrease is higher in the autoclaved sample. This could be due to hydrolysis of phytic acid. Since heat in any form can at least partly decrease the phytic acid activity, processing with heat when preparing food will lower the deleterious effects due to phytic acid.

Table 1. Phytic acid content in raw and processed Canavalia gladiata seed flour (mgg^{-1})

Sample	Phytic acid mean±sd
raw whole seed	7.02±0.41
raw cotyledon	8.47±0.81
dry-autoclaved cotyledon	4.32±0.80
roasted cotyledon	6.05±0.20

sd=standard deviation; n=3

The high phytic acid content of raw *Canavalia gladiata* cotyledon (8.47 mgg^{-1}) could be due the accumulation site of phytic acid in mono-cotyledonous and dicotyledonous seeds being the aleurone particles or grains in the aleurone layer and globoids (Lott and Buttrose, 1978). The fact that phytate is also reported to accumulate in the seeds during the ripening period could be another reason for the high phytic acid content in the raw cotyledon fraction (Abernethy *et al.*, 1973; Nahapetian and Bassiri, 1975). The high phosphorous content from the mineral analysis also coincides with high phytate content in the seed cotyledons (Ekanayake, 1999; Mohan and Janardhanan, 1994)

α-Amylase inhibitor activity

The α-amylase inhibitor activity of raw ahole seed, raw cotyledon, seed coat and boiled seed coat was determined. The raw whole seed extract indicated a decrease in the α-amylase activity by 924 U/L (48% decrease) indicating the presence of an inhibitor or inhibitor activity. The cotyledon fraction was devoid of any inhibitor activity. The α-amylase activity increased by 1278 U/L in this fraction (total activity 3196 U/L) when compared with the control (1918 U/L). This confirms the high α-amylase activity of the cotyledon fraction (Ekanayake, 1999). There was complete inhibition of α-amylase activity by the raw seed coat extract. In the boiled seed coat extract the α-amylase activity increased (284 U/L) indicating partial destruction of inhibitor activity by heat.

This study established the presence of an α-amylase inhibitor in the seed coat for this species. However, the effect from amylase inhibitor on food preparations of *Canavalia* will be negligible since the seed coat will be removed at processing.

The inhibitor activity of a seed coat extract after eluting through a polyamide column was lost whereas the initial extract had inhibitor activity. The polyamide specifically bind polyphenolic compounds. The nature of the inhibitor was assumed to be polyphenolic since the activity was lost following elution through the polyamide column (Conner *et al.*, 1990). This could be due to a structural deterioration in the column or due to the inhibitor being irreversibly bound on the column.

Trypsin inhibitor activity

The trypsin inhibitor activity of the whole seed was 4866TIU/g. A cooked sample of whole seed flour also gave an inhibition of 2745TIU/g, but there was no inhibitor activity in the raw cotyledon. Boiling the seed extract had reduced the trypsin activity by 2121 TIU/g (56%). The extracts with trypsin inhibitor activity were run through a polyamide column to determine if the activity was due to the same component responsible for α-amylase inhibition. The initial activity (4866TIU/g) was retained in the eluent after runing through the polyamide column which showed that the inhibitor activity was not linked to the α-amylase inhibitor. Probably the activities were not of a double-headed enzyme.

The molecular weight of the trypsin inhibitor was found to be around 90kD using the low molecular weight calibration molecular weight markers on Sephadex G-100. This value is higher than the values reported so far (Nagaraja and Pattabiraman, 1991).

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