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Effects of heat treatments and germination on trypsin inhibitor activity and polyphenols in jack bean (*Canavalia ensiformis* L. DC)

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Abstract. The application of dry heat to seeds and meal was not effective in inactivating the TI and reducing the polyphenol content. Soaking for 24 h followed by cooking for 20 min, was equally effective in destroying the TI activity. Germination of jack bean seeds for 40 h decreased the levels of TI and polyphenols by 31% and 35%, respectively.

Introduction

Food legumes are important sources of dietary protein in the developing countries. Among these, jack bean (*Canavalia ensiformis* L. DC) is considered to be a minor food legume. It has been commercially exploited for the isolation of concanavalin A (lectin), and urease [10]. Jack bean has been recently identifed as a potential food source for the tropics by the National Academy of Sciences of the United States of America [11]. It gives dependable yields under the dry farming and low soil fertility conditions where most other food legumes cannot be economically grown. It is a rich source of proteins, essential amino acids, thiamin, riboflavin and minerals [7]. However, the acceptability and utilization of jack bean seeds as food legume has been limited due to the presence of relatively high concentrations of certain antinutritional factors such as lectins, trypsin inhibitors and polyphenols. Simple processing like cooking, autoclaving and germination have been shown to reduce the levels of these antinutritional factors in several legumes [3, 9, 13]. Such information on jack bean is however lacking. The purpose of present investigation was to study the effects of heat treatments and germination on the trypsin inhibitor activity and level of polyphenols in jack bean seeds.

Materials and methods

Seeds of jack bean were obtained from Department of Horticulture, Mahatma Phule Agricultural University, Rahuri, India and stored at 4°C until experiments were conducted.

Dry heat treatment

The whole seeds and meal (60 mesh) were incubated at 50, 80 and 100 °C for 1 h in hot air oven. The heated seeds were powdered to 60 mesh.

Cooking in boiling water

The raw and soaked seeds (in water for 24 h; 1:3 w/v) were cooked in boiling distilled water for 5 to 30 min in open pan (1:3 w/v). The cooked seeds were dried at 60 °C to a constant weight and powdered to 60 mesh.

Autoclaving

The seeds and meal were autoclaved at 15 lb pressure for 5, 10, 15, 20 and 30 min and dried at 60 °C to a constant weight. The autoclaved seeds were milled to 60 mesh.

Germination

Ten-gram seeds, sterilized with 0.1% mercury chloride, were soaked in distilled water at 4 °C for 12 h, placed on double layer of filter paper in petridishes, and incubated at 30 °C. The filter papers were moistened at regular intervals of 12h. The germinated seeds were dried at 60 °C to a constant weight and powdered to 60 mesh.

Trypsin assay

The trypsin assay was carried out by the method of Erlanger et al. [5]. The reaction mixture contained: 0.15 ml enzyme (1 mg trypsin/ml in 10^{-3} M HCl), 0.15 ml 0.1 M acetate buffer, pH 4.9 and 2 ml BAPNA (21.8 mg Na-benzoyl-DL-arginine-p-nitroanilide dissolved in 1 ml of dimethyl sulfoxide and diluted to 100 ml with 0.05 M Tris-HCl buffer, pH 8.2). The reaction was carried out at 37 °C for 10 min and terminated by adding 1 ml of 20% acetic acid. The quantity of p-nitroanilide liberated by the enzyme was measured at 410 nm. One unit of trypsin activity was defined as 1 μmole of p-nitroanilide released/min by the enzyme.

Trypsin inhibitor assay

The inhibitor was prepared by extracting the meal in 5% NaCl for 60 min followed by centrifugation at 10,000 × *g* for 30 min. The trypsin inhibitor activity was measured in the appropriately diluted extract to obtain about 60% inhibition. The reaction mixture contained 0.15 ml inhibitor instead of acetate buffer. Remaining procedure was similar to assay of trypsin. One trypsin inhibitor unit (TIU) was the amount of inhibitor that inhibited one unit of trypsin.

Polyphenols

Polyphenol contents in the samples were estimated by using Folin-Denis reagent [2].

Results and discussion

Effects of heat treatments

When the dry seeds were heated at 100 °C for 60 min, the TI activity was reduced by 7%. Similar treatment to meal reduced TI activity by 24%. Thus, the inhibitor was found to be resistant to dry heat treatment. Similar observations have been recorded for the trypsin inhibitors of horse gram [6], moth bean [8] and winged bean [9]. The polyphenol content was reduced by 28% in seeds and 46% in meals when subjected to 100 °C for 60 min (Table 1).

The cooking of seeds in water for 60 min significantly decreased (50%) the

Table 1. Effects of dry heat on trypsin inhibitor activity and polyphenols in jack bean

Temperature (°C)	Trypsin inhibitor activity (TIU/g sample)		Polyphenols (%)	
	Meal	Seeds	Meal	Seeds
Control	12.4	12.4	1.31	1.31
50	12.0	12.4	1.11	1.18
	(3.2)	(0.0)	(15.3)	(9.9)
80	10.5	11.6	0.90	1.03
	(15.3)	(6.5)	(31.3)	(21.4)
100	9.4	11.5	0.71	0.94
	(24.2)	(7.3)	(45.8)	(28.2)

Values in parentheses indicate % of the control.

Table 2. Effects of cooking on trypsin inhibitor activity and polyphenols in jack bean

Time (min)	Cooking		Soaking and cooking	
	TIU/g sample	Polyphenols (%)	TIU/g sample	Polyphenols (%)
0	12.4	1.31	10.9	0.80
5	–	–	9.8	0.60
			(10.1)	(25.0)
10	11.6	1.11	3.7	0.53
	(6.5)	(15.3)	(66.1)	(33.8)
15	–	–	1.1	0.46
			(90.0)	(42.5)
20	10.9	0.88	ND	0.34
	(12.1)	(32.8)		(57.5)
25	–	–	ND	0.15
				(81.3)
30	8.6	0.65	ND	ND
	(30.7)	(50.3)		
60	6.4	0.53	ND	ND
	(48.4)	(59.5)		

– Assays not carried out.
Values in parentheses indicate % of the control.
ND = Not detected.

level of TI activity. However, when the seeds were soaked for 24 h prior to cooking, almost all the TI activity was destroyed within 15 to 20 min. When the dry seeds were cooked for 60 min, there was 60% reduction in polyphenol content (Table 2). However, when the seeds were soaked for 24 h and cooked, almost all the polyphenols were lost within 25 to 30 min.

Polyphenols are mostly located in the seed coat. Soaking as well as cooking results in loss of polyphenols due to leaching. In addition it has been suggested that due to heat treatment, either solubility or the chemical reactivity of polyphenols is changed which results in apparent decrease in assayable polyphenols [4]. The observed decrease in polypehnol content in jack bean seeds may be due to binding of polyphenols with other substances or due to alterations in their chemical structure that rendered them incapable of giving the colour reaction.

The moist heat, 120 °C for 20 min, destroyed all the inhibitor activity within 20 min in meal and within 30 min in seeds of jack bean. Similar results have been reported for moth bean and horse gram [6, 7]. The jack bean trypsin inhibitor exhibits similar thermal inactivation properties to that of horse gram, moth bean or winged bean. The moist heat treatment reduced the polyphenols by about 91% in meal and by about 70% in seeds of jack bean (Table 3). The reduction in polypehnols after autoclaving can be

Table 3. Effects of autoclaving (moist heat) on trypsin inhibitor activity and polyphenol contents in jack bean

Autoclaving (min)	Trypsin inhibitor activity (TIU/g sample)		Polyphenols (%)	
	Meal	Seeds	Meal	Seeds
Control	12.4	12.4	1.31	1.31
5	8.2	10.1	1.18	1.25
	(33.9)	(18.5)	(9.9)	(4.6)
10	3.3	4.9	1.03	1.11
	(73.4)	(60.5)	(21.4)	(15.3)
15	0.7	1.5	0.65	0.92
	(94.4)	(87.9)	(50.4)	(29.8)
20	ND	0.7	0.37	0.62
		(94.4)	(71.8)	(52.7)
30	ND	ND	0.12	0.40
			(90.8)	(69.5)

Values in parentheses indicate % of the control.
ND = Not detected.

attributed to the interaction of polyphenols with other components of seeds such as protein to form insoluble tannin-protein complexes.

Effects of germination

After 48 h germination, the trypsin inhibitor activity was reduced only by 26%. A drastic reduction in TI activity after germination has been reported in chickpea, broad bean, lentil and mung bean [1]. Similarly, Kadam et al [8] observed complete disappearance of TI activity in 48 h germinated moth bean seeds. However, similar treatment to horse gram did not decrease the

Table 4. Effects of germination on trypsin inhibitor activity and polyphenols in jack bean seeds

Germination period (h)	Trypsin inhibitor activity (TIU/g sample)	Polyphenols (%)
0	11.6	1.03
12	10.9	0.94
	(6.0)	(8.7)
24	10.2	0.85
	(12.1)	(17.5)
36	9.8	0.80
	(15.5)	(22.3)
48	8.6	0.65
	(25.9)	(36.9)

Values in parentheses indicate % of the control.

TI activity. The inhibitor in jack bean exhibited similar behaviour to that of horse gram inhibitor.

Germination decreased polypehnol content only by 37% in jack bean seeds (Table 4). The decrease in polyphenols due to germination can be attributed to the enzymatic hydrolysis [12] and to the leaching losses. Similar decrease in polyphenol content due to germination has been observed in horse gram [14]. The results revealed that sprouting of jack bean seeds reduces the polyphenol content and TI activity markedly. However, the treatments like moist heat and soaking followed by cooking are very effective in eliminating these antinutrients in jack bean seeds.

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