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Lectins, Trypsin Inhibitors, BOAA and Tannins in Legumes and Cereals and the Effects of Processing

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ABSTRACT

Most of the legumes and cereals were found to contain lectins. Hemagglutinating activity differed when assayed at different temperatures (4, 18 and 37°C). Considerable variation was observed in hemagglutinating activity of different cultivars of a species. Lectin and β-oxalyl-amino-alanine (BOAA) content of different cultivars of Lathyrus sativus *showed significant variation whereas tannin, trypsin inhibitor and total protein content did not vary much. Cooked foods were found to have lower levels of antinutrients as compared to raw foods. Dry heating did not inactivate the antinutrients. Soaking the seeds in water and processing effectively removed the antinutrients.*

INTRODUCTION

Plant foods constitute a major component of diet in the developing countries. Commonly consumed cereals and pulses have been shown to contain a significant amount of lectins (Huprikar & Sohonie, 1961; Nachbar & Oppenheim, 1980; Nachbar *et al.*, 1980; Grant *et al.*, 1983). Some of the lectins which are resistant to *in vivo* digestion have been shown to reach the intestine in a biologically active form where they can exert deleterious effects (Nakata & Kimura, 1985). Several outbreaks of food poisoning due to consumption of foods containing lectins have been recorded (Noah *et al.*, 1980). While the majority of plant lectins are heat-labile (Boufassa *et al.*, 1986), some lectins are resistant to heat (Auricchio *et al.*, 1984; Freed, 1985)

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and in some cases the hemagglutinating activity may increase even up to seven-fold on heating at 80°C for 10 min (Bender & Reaidi, 1982). Thus, it appears likely that in some foods, processed at the low temperature employed in ordinary cooking, hemagglutinating activity may be higher than in raw foods (Coffey *et al.*, 1975). Pulses are also known to contain several other antinutritional and toxic factors like trypsin inhibitors, tannins, and in some cases, β-oxalyl-amino-alanine (BOAA) and other toxic factors (Roy, 1981).

There have so far been very few studies on lectin content of commonly consumed foods and their possible varietal differences. Varietal differences in the lectin content of pulses and cereals and the effects of traditional Indian household methods of processing of foods on their antinutrient content were therefore determined.

Lathyrus sativus, commonly known as khesari dhal, is a legume consumed as a staple food in some parts of Bangladesh, China, Ethiopia and India. It is frequently used to adulterate Bengalgram dhal. Chronic use of *L. sativus* as a staple has been shown to cause lathyrism (Roy *et al.*, 1986). This legume is known to contain trypsin inhibitors, tannins, lectins and BOAA (Liener, 1980). Fifteen cultivars of *L. sativus* and several other legumes and cereals were analysed for their lectin, tannin, trypsin inhibitor and BOAA content in order to assess the varietal difference. The influence of household processing on antinutrients was also studied in foods prepared from the whole seed powder or the pulse obtained from *Lathyrus sativus*.

MATERIALS AND METHODS

Fifteen authentic cultivars of *Lathyrus sativus* seeds were obtained from the Regional Agricultural Research Station, Raipur, India. Soyabean (*Glycine max.*), groundnut (*Arachis hypogea*), chick pea (*Cicer auretinum*) and pigeonpea (*Cajanus cajan*) were obtained from the International Crop Research Institute for Semi-Arid tropics, Hyderabad, India. Green gram (*Phaseolus aureus*), black gram (*Phaseolus mungo*), horse gram (*Dolichus biflorus*), maize (Zea mays), wheat (*Triticum vulgare*), rice (*Oryza sativa*) and sorghum (*Sorghum vulgare*) were obtained from Andhra Pradesh Agricultural University, Hyderabad, India. Remaining seeds were purchased from the local market.

Casein, vanillin and bovine serum albumin were obtained from Sigma Chemical Co., St Louis, USA. Trypsin was obtained from E. Merck, Federal Republic of Germany.

Human A, B and O erythrocytes were obtained from the Institute of Preventive Medicine, Hyderabad. Rat, rabbit and monkey erythrocytes were

obtained from the Laboratory Animals Information Service Centre, NIN, Hyderabad.

Food items were prepared using either dehusked *L. sativus* seeds or the powder of these seeds following the methods commonly used in Indian households (Swaran & Rebello, 1982). Processed foods containing moisture were air-dried. Defatted powder of the samples was used to extract the antinutritional factors.

Hemagglutinating activity was determined as described by Allen *et al.* (1976), employing a 4% erythrocyte suspension prepared from freshly drawn blood. Agglutinability was tested at 4°C, 18°C and 37°C.

Trypsin inhibitor activity of the extract was determined by the method of Kakade *et al.* (1969) essentially as described by Roy (1972).

Protein content of the seed extracts was determined by the method of Lowry *et al.* (1951).

BOAA content of the samples was estimated as described by Roy and Bhat (1975).

Tannin content was determined as described by Price *et al.* (1978).

Total protein in the samples was determined by the Microkjeldahl method (Oser, 1965).

RESULTS AND DISCUSSION

Lectin extract was obtained from the powder of whole seeds. Of the 16 pulses and cereals tested, six (chick pea, pigeon pea, green gram, sorghum, rice and maize) did not show any hemagglutinating activity against the red blood corpuscles of any of the species tested (Table 1). Seed extracts from khesari dhal, white kidney bean, red kidney bean and *Vicia sativa* showed no species specificity and agglutinated erythrocytes from rat, rabbit, monkey and human A, B and O. Of the remaining six, groundnut, blackgram and wheat did not agglutinate human, monkey or rat RBC, but agglutinated rabbit erythrocytes. Goabean and horsegram agglutinated only human erythrocytes. Goabean extract agglutinated A, B and O erythrocytes of human, whereas horsegram showed agglutination only against human A erythrocytes. In the case of soybean, though it agglutinated both rabbit and human A and O type erythrocytes, the activity was much less against human erythrocytes as compared to its activity against rabbit RBC (Tables 2 and 3).

The influence of temperature on agglutination was assessed by carrying out the reaction at three different temperatures. Hemagglutinating activity was higher in some cases at low temperatures (Table 2 and 3) as observed earlier (Bird, 1953; Huprikar & Sohonie, 1961). Soyabean extract agglutinated the human erythrocytes only at low temperatures. But the

TABLE 1
Seeds Devoid of Hemagglutinating Activity

Variety	
Pigeon pea: 11 535	Green gram-3
Pigeon pea: 11 563	Green gram-4
Pigeon pea: 7035	Sorghum SPV-126
Pigeon pea: 7119	Sorghum SPV-346
Chick pea: ICCC 3	Sorghum CSY-421
Chick pea: ICCC 4	Sorghum SPY-245
Chick pea: ICCC 5	Rice—Masuri
Chick pea: ICCC 9	Wheat—1
Green gram-1	Wheat—2
Greem gram-2	Maize—Rohini

Hemagglutinating activity was checked against rat, rabbit, monkey, human A, B and O erythrocytes.

extracts of black gram showed agglutination against rabbit RBC only at higher temperature. These results indicate that the activity of some of the lectins is temperature-specific. Mitra *et al.* (1987) have shown that there are two lectins in hemolymph of *Achatina fulica* snail; one is active at 4°C whereas the other is active at 37°C. Hence, while screening any sample for the presence of lectins it may be advisable to carry out the agglutination test at different temperatures. Most of the samples studied here exhibited maximum agglutination activity at 4°C (Tables 2 and 3).

The relative hemagglutinating activity of different cultivars of each pulse or cereal tested, showed considerable variation (Tables 2 and 3). Out of the 11 samples which showed the presence of lectins, 9 agglutinated the rabbit erythrocytes. Besides that, the relative hemagglutinating activities of all these extracts were found to be higher against the rabbit RBC compared to their activity against erythrocytes from other species.

Lectins, trypsin inhibitor, tannins, BOAA and total protein content of the whole seeds of fifteen authentic cultivars of *Lathyrus sativus* are presented in Table 4. Whereas significant variation was observed in lectin (CV = 24·7) and BOAA (CV = 25·1%) contents, amongst the cultivars, the tannin, trypsin inhibitor and the total protein content did not vary much. In view of the wide variation in the lectin and BOAA content of these cultivars, identification of a cultivar with very low levels of these two antinutritional factors would be useful in the context of using khesari dhal as food. But none of the cultivars tested was found to contain very low levels of lectin or BOAA.

TABLE 2
Hemagglutinating Activity (units/mg Protein) in Food Grains

Variety	*Erythrocytes*								
	Rabbit			*Rat*			*Monkey*		
	4 ± 2°C	*18 ± 2°C*	*37 ± 2°C*	*4 ± 2°C*	*18 ± 2°C*	*37 ± 2°C*	*4 ± 2°C*	*18 ± 2°C*	*37 ± 2°C*
Soyabean ankur	363·0	363·0	363·0	nil	nil	nil	nil	nil	nil
Soyabean black	182·0	45·0	23·0	nil	nil	nil	nil	nil	nil
Soyabean Punjab	3·0	3·0	3·0	nil	nil	nil	nil	nil	nil
Soyabean brag	117·0	59·0	29·0	nil	nil	nil	nil	nil	nil
Groundnut-5175	11·0	3·0	nil	nil	nil	nil	nil	nil	nil
Groundnut-7348	9·0	2·0	nil	nil	nil	nil	nil	nil	nil
Groundnut-799	11·0	nil	nil	nil	nil	nil	nil	nil	nil
Groundnut-3039	4·0	nil	nil	nil	nil	nil	nil	nil	nil
Khesari dhal IARI P.24	31·0	64·0	31·0	8·0	4·0	4·0	16·0	16·0	16·0
Khesari dhal LSD-3	38·0	77·0	77·0	2·0	5·0	5·0	19·0	19·0	19·0
Khesari dhal LSD-3	80·0	80·0	80·0	5·0	34·0	10·0	20·0	20·0	10·0
Khesari dhal	33·0	33·0	67·0	17·0	4·0	4·0	8·0	8·0	4·0
Blackgram-1	nil	nil	9·0	nil	nil	nil	nil	nil	nil
Blackgram-2	nil	nil	9·0	nil	nil	nil	nil	nil	nil
Blackgram-3	nil	nil	5·0	nil	nil	nil	nil	nil	nil
Blackgram-4	nil	nil	9·0	nil	nil	nil	nil	nil	nil
White kidneybean	98·0	98·0	49·0	12·0	24·0	12·0	195·0	195·0	195·0
Red kidneybean	54·0	54·0	27·0	14·0	27·0	14·0	216·0	216·0	216·0
Vicia sativa	13·0	13·0	13·0	0·2	0·2	nil	0·2	0·2	0·2
Wheat-1	8·0	3·0	6·0	nil	nil	nil	nil	nil	nil
Wheat-2	3·0	3·0	3·0	nil	nil	nil	nil	nil	nil

TABLE 3
Hemagglutinating Activity (units/mg protein) in Food Grains

Variety	*Human erythrocytes*								
	Blood group A			*Blood group B*			*Blood group O*		
	4 ± 2°C	*18 ± 2°C*	*37 ± 2°C*	*4 ± 2°C*	*18 ± 2°C*	*37 ± 2°C*	*4 ± 2°C*	*18 ± 2°C*	*37 ± 2°C*
Soyabean ankur	11	nil	nil	nil	nil	nil	nil	nil	nil
Khesari dhal LSD-3	10	10	4	19	10	10	19	10	2
Khesari dhal IARI-P.24	16	16	4	31	16	16	16	8	4
White kidneybean	24	24	49	24	24	49	24	24	49
Red kidneybean	27	54	54	54	6	54	54	54	54
Vicia sativa	2	13	2	13	2	1	1	2	1
Goabean	36	36	9	36	36	36	36	36	36
Horsegram-1	12	12	2	nil	nil	nil	nil	nil	nil
Horsegram-2	8	4	1	nil	nil	nil	nil	nil	nil

TABLE 4
Antinutrients in Different Varieties of *Lathyrus sativus*

Sample number	*Cultures*	*Lectin (unit/mg protein)*	*Trypsin inhibitor (TUI/mg protein)*	*Tannins (g%)*	*BOAA (g%)*	*Total protein (g%)*
1	CVT-RL-311	85	139·0	0·23	0·94	26·54
2	LVT-RL-301	93	143·90	0·24	0·94	27·26
3	CVT-Rewa-21	64	118·30	0·19	1·24	20·12
4	LVT-RPLK-26	64	114·20	0·20	0·98	17·93
5	CVT-RL-211	71	103·10	0·14	0·85	24·50
6	CVT-LSD-6	71	107·10	0·16	1·32	27·65
7	CVT-RPL-29	64	105·80	0·28	0·72	24·25
8	CVT-RL-18	43	110·00	0·20	1·05	26·46
9	Khesari Kh-25	37	115·80	0·16	0·49	29·09
10	LVT-RPL-31	46	100·30	0·20	0·55	28·43
11	LVT-RL-211	61	9·10	0·16	0·60	28·40
12	CVT-RPLK-26	85	101·00	0·16	0·72	22·31
13	CVT-LSD-1	51	146·70	0·20	1·10	29·96
14	LSD-1	71	119·80	0·20	0·96	24·50
15	LSD-3	64	133·80	0·14	0·92	25·37
	$x \pm$ SD	64·70 ± 16·00	117·19 ± 16·28	0·19 ± 0·003 9	0·89 ± 0·241 0	25·51 ± 3·375 0
	CV	(24·73)	(13·89)	(20·45)	(27·04)	(13·22)

As many of the foods were found to contain antinutrients, it may be desirable to inactivate them through processing. Lectin, trypsin inhibitors, tannins and BOAA contents of the processed foods are given in Table 5. The majority of the cooked foods were found to have lower levels of those antinutrients as compared to the levels in the raw flour. Trypsin inhibitors and lectins were found to be inactivated to a considerable extent during cooking. But dry heating did not reduce the hemagglutinating activity and the trypsin inhibitor activity was also found to remain high, even after dry heating. Trypsin inhibitor activity was found to be destroyed only in certain cooked foods while, in many others, considerable amounts of trypsin inhibitors were still found (Table 5). Dry heating or deep frying for less than 10 min was found to inactivate the trypsin inhibitors only to an extent of 40 to 50%. But samples subjected to heat-treatment at temperatures above 100°C for longer periods in the presence of water did not contain any

TABLE 5
Antinutrients in Cooked Foods

Sample number	*Food item*	*TI (TUI/g)*	*Lectin (HU/g)*	*BOAA (g%)*
1	Raw powder	2440	1632	0·34
2	Popped seeds (popped at 250°C)	118 (95·17)	30 (98·17)	0·22 (36·85)
3	Papad (drying in sun and deep fat frying)	1306 (46·48)	60 (96·24)	0·28 (18·42)
4	Papad roasted (drying in sun and roasting)	1246 (49·00)	120 (92·65)	0·34 (nil)
5	Chapathi (Heating on a pan)	1248 (49·00)	1632 (nil)	0·26 (23·68)
6	Muruku (Deep fat frying of wet dough)	1394 (42·87)	Nil	0·29 (14·94)
7	Dosa (soaking fermentation and heating)	1028 (57·87)	Nil	0·017 (95·32)
8	Dokla (pressure cooking fermentation and steaming)	Nil	Nil	0·017 (95·32)
9	Kichidi (Presoaking and autoclaving)	Nil	Nil	0·087 (74·56)
10	Dhal (Boiling)	Nil	90 (94·48)	0·20 (42·10)

Values given in parentheses are percentage destroyed.

residual trypsin inhibitor activity. As the seeds used to prepare the food items were dehusked, tannin contents were found to be very low.

The BOAA content did not decrease much in prepared foods (Table 5). Dry heating, roasting, deep frying, or boiling did not alter the BOAA levels. In preparations where the seeds were presoaked in water and the soaked water was discarded, the BOAA content was found to be reduced considerably. In fact, the proposed process for the removal of BOAA from lathyrus seeds is based on this principle (Padmanaban, 1980).

Presoaking and autoclaving was found to remove all the antinutrients tested to a significant extent. Khesari dhal is mainly consumed in the form of chapathis where the flour is subjected to dry heating. This preparation was found to contain all the three antinutrients, lectin, trypsin inhibitors and BOAA. Apart from this, other preparations were also found to contain these antinutrients. This is in agreement with the observation of Lowgren and Liener (1986). Regular consumption of these foods may result in intake of these antinutrients, resulting in some deleterious effects.

REFERENCES

Allen, A. K., Desai, N. N. & Neuberger, A. (1976). The purification of the glycoprotein lectin from the broad bean (*Vicia faba*) and a comparison of its properties with lectins of similar specificity. *Biochem. J.*, **155**, 127–35.

Auricchio, S., Ritis, G. D., Vincenzi, M. D., Mancini, E., Minetti, M., Sapora, O. & Silano, V. (1984). Agglutinating activity of gliadin derived peptides from bread wheat. Implications for coeliac disease pathogenesis. *Biochem. Biophys. Res. Commun.*, **121**, 428–33.

Bender, A. E. & Reaidi, G. B. (1982). Toxicity of kidney beans (*Phaseolus vulgaris*) with particular reference to lectins. *J. Plant Foods*, **4**, 15–22.

Bird, G. W. G. (1953). Cold Agglutinin. *Curr. Sci.*, **22**, 273.

Boufassa, C., Lafont, J., Rouanet, J. M. & Besancon, P. (1986). Thermal inactivation of lectins (PHA) isolated from *Phaseolus vulgaris*. *Food Chem.*, **20**, 295–304.

Coffey, D. G., Uebersa, M. A., Hosfield, G. L & Brunner, J. R. (1985). Evaluation of the hemagglutinating activity of low temperature cooked kidney beans. *J. Food Sci.*, **50**, 78–81.

Freed, D. L. J. (1985). Lectins. *Br. Med. J.*, **290**, 584–6.

Grant, G., More, L. J., McCenzie, N. H., Stewart, J. C. & Pusztal, A. (1983). A survey of the nutritional and haemagglutination properties of legume seeds generally available in the UK. *Br. J. Nutr.*, **50**, 207–14.

Huprikar, S. V. & Sohonie, K. (1961). Haemagglutinins in Indian pulses; Part-I-Detection of haemagglutinins effect of autoclaving and germination on haemagglutinating activity. *J. Sci. Indust. Res.*, **20C**, 82–4.

Kakade, M. L., Simons, N. & Liener, I. F. (1969). An evaluation of natural versus synthetic substrates for measuring the antitryptic activity of soyabean samples. *Cereal Chem.*, **46**, 518–26.

Liener, I. E. (1980). *Toxic Constituents of Plant Foods.* Academic Press, New York.

Lowgren, N. & Liener, I. E. (1986). The effect of slow cooking on the trypsin inhibitor and haemagglutinating activities and *in vitro* digestibility of brown beans (*Phaseolus vulgaris*, varistella) and kidney beans (*Phaseolus vulgaris* var. Montcalm). *Qual. Plant. Plant Foods. Hum. Nutr.*, **36**, 147–54.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with folin phenol reagent. *J. Biol. Chem.*, **193**, 265–75.

Mitra, D., Sarkar, M. & Allen, A. K. (1987). Further characterization of the cold agglutinin from the snail *Achatina fulica. Biochem. J.*, **242**, 331–8.

Nachbar, M. S. & Oppenheim, J. D. (1980). Lectins in the United States diet: A survey of lectins in commonly consumed foods and a review of literature. *Am. J. Clin. Nutr.*, **33**, 2338–45.

Nachbar, M. S., Oppenheim, J. D. & Thomas, J. O. (1980). Lectins in the US diet, Isolation and characterization of a lectin from the tomato (*Lycopersicom esculentum*). *J. Biol. Chem.*, **255**, 2056–61.

Nakata, S. & Kimura, T. (1985). Effect of ingested toxic bean lectins on the gastrointestinal tract in the rat. *J. Nutr.*, **115**, 1621–9.

Noah, N. O., Bender, A. E., Reaidi, G. B. & Gilbert, R. J. (1980). Food poisoning from raw kidney beans. *Br. Med. J.*, **281**, 236–7.

Oser, B. L. (Ed.) (1965). *Hawk's Physiological Chemistry* (14th edn). McGraw Hill Co., New York.

Padmanaban, G. (1980). Lathyrogens. In *Toxic Constituents of Plant Foods*, ed. I. E. Liener, Academic Press, New York, pp. 239–63.

Price, M. L., Van Scoyoc, S. & Butler, L. G. (1978). A critical evaluation of vanillin reaction as an assay for tannin in sorghum grain. *J. Agric. Food Chem.*, **26**, 1214–18.

Roy, D. N. (1972). Further purification, fractionation and properties of trypsin inhibitor isolated from *Lathyrus sativus. J. Agric. Food Chem.*, **20**, 778–80.

Roy, D. N. (1981). Toxic amino acids and proteins from *Lathyrus* plants and other leguminous species. A literature review. *Nutr. Abstr. Rev.*, **51**, 691–707.

Roy, D. N. & Bhat, R. V. (1975). Variation in neurotoxin, trypsin inhibitors and susceptibility to insect attacks in varieties of *Lathyrus sativus* seeds. *Environ. Physiol. Biochem.*, **5**, 172–7.

Roy, D. N., Spencer, P. S. & Nunn, P. B. (1986). Toxic components of *Lathyrus.* In '*Lathyrus and Lathyrism*'. *Proc. Internatl. Symp., 9–13 Sept. 1985,* ed. A. K. Kaul & D. Combes, Third World Med. Res. Foundation, New York. pp. 286–97.

Swaran, P. & Rebello, L. M. (1982). *Some Common Indian Recipes and Their Nutritive Value.* National Institute of Nutrition Publication, Hyderabad, India.