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Toxic and Antinutritional Factors of New Varieties of Pea Seeds

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ABSTRACT

Legumes are the most common variety of food used by the humans largely by the low income group and vegetarians. In India and under developing countries legumes are recognized as one of the most important sources of edible vegetable proteins. In addition to being a relatively inexpensive source of dietary proteins, legumes possess desirable attributes such as abundance of complex carbohydrates, ability to lower serum cholesterol in humans, high fiber content, low fat content (excluding oilseeds), and high concentration of polyunsaturated fatty acids particularly the essential fatty acids which is highly essential for human health. Legumes may contain some toxic and anti-nutritional factors which may reduces the nutritional quality of the seeds which may cause more curse rather than boon to the poor and vegetarian people. In The present investigation commonly and cheaply available different new varieties of pea sample are selected and analysed for the presence of toxic and anti nutritional factors. Pisum sativum (Pea) seeds of different new varieties like Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6 were analysed for few toxic and anti nutritive properties like Cyanogenetic glycosides, Tannin content, Oxalate content, Trypsin inhibitor activity and Haemagglutinin activity by chemical and biochemical methods.

Key words: Toxic and anti-nutritional factors**,** Cyanogenetic glycosides, Tannin content, Oxlate content, Trypsin inhibitor activity and Haemagglutinin activity.

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INTRODUCTION

India is the world's largest producer and consumer of pulses. Legumes occupy a prominent place in our diets and the Indian agricultural economy, since they are major protein sources for the people. Legumes are usually grown for their edible seeds, and thus are also named grain legumes. In terms of production volume, the cereals are the most important as they furnish the carbohydrates that constitute the major portion of human and animal diet [1].

Protein-calorie malnutrition is a major nutritional syndrome affecting more than 170 million preschool children and nursing mothers in developing Afro-Asian countries. The present trend in population growth indicates that the Protein gap may continue to increase in the future unless well-plane measures are taken to tackle the situation. Provision of adequate proteins of animal origin is difficult and expensive. An alternative for improving nutritional status of the people is to supplement the diet with plant proteins. Attention, therefore, has to be directed to the nutritional evaluation of proteins from plant species. Legumes play an important role in human nutrition since they are rich sources of protein, calories, certain minerals and vitamins [2]. In Afro-Asian diets, legumes are the major contributors of protein and calories for economic and cultural reasons [3]. Pisum sativum belonging to this leguminacae family has a rich source of proteins, carbohydrates, fiber and minerals, freely and cheaply available food source and can be milled into flour, used to make bread, doughnuts, tortillas, chips, spreads, and extruded snacks or used in infant formulation.

Anti-nutritional factors are the chemical substances which, may although non-toxic but generate adverse physiological responses and interfere with the utilization and absorption of nutrients, even a single, toxin present in significant amount can turn a seed and its products unfit for human consumption

Although only a few legumes may contain all these anti nutritive factors, many contain a few of them. Hence study has been carried out for the determination and extent of toxic and anti nutritional factors in the commonly available cheap and edible legumes like Pisum sativum (Pea) having high levels of proteins carbohydrates source. The effects of toxic and anti nutritive factors are illustrated.

(A) **Cyanogens**:

Cyanide in trace amounts is fairly wide spread in the form of glycosides and hydrolysis of cyanogens yield glucose and either aldehyde or ketone compounds. Relatively high level can be found in grasses, pluses and root crops. The glycosides are non toxic in the intact tissue but when later are damaged or being to decay, a

hydrolytic enzyme is released liberating hydrogen cyanide. The hydrogen cyanide is rapidly detoxified in the liver by conversion to thiocyanate excess cyanide ion can quickly produce anoxia of the central nervous system through in activating the cytochrome oxidase system and death can result in few seconds[4].

Tannins:

Tannins are complex group of phenolic compounds which are astringent in taste that precipitate proteins. Tannins are widely distributed in plant kingdom. Tannins have major impact on animal nutrition because their ability to form complexes with molecules like carbohydrates, proteins, polysaccharides, enzymes involved in protein and carbohydrate digestion. Tannins are present in almost in all legumes predominantly [5]. Tannin causes growth depression in rats and in poultry [4, 6, 7]. This effect proves adverse due to reduced proteins and dry matter digestibility. The probable result from the tannin interference with digestive action of trypsin and alpha amylase either binding in the enzyme themselves or by combining with dietary proteins to form in digestible complex. In laying birds tannins will decrease the rate of lay adversely affect the efficiency of food utilization and increases the mortality [4].

Oxalates:

Oxalic acid is found in both from free as well as combined state. Oxalates are in soluble complex which are formed by the action of oxalic acid and minerals. These complexes are extremely insoluble even at pH 3-4 and therefore are not readily absorbed from intestinal tract. Mineral ions which are generally affected are zinc, calcium, copper and manganese. The mode of oxalate in causing toxicity is obscure. Acute toxicity could be due to hypo calcemia while uremia from kidney damage may contribute to chronic toxicity. Oxalate inhibits a number of respiratory enzymes and also enzymes which are activated by calcium and magnesium may be inhibited, its anti nutritive effect may be due to complexing with calcium. There is evidence that in cattle and sheep rumen micro organisms can split off calcium as well as decompose oxalic acid. In pigs and poultry oxalates causes depression in growth and reduction in calcium retention although dissociation of some calcium oxalate occur in digestive tract [4, 8].

(B) **Trypsin inhibitor activity:**

Any substance that reduces the velocity of enzyme catalyzed reaction by whatever mechanism is an inhibitor. Trypsin inhibitor activity (TIA), which inhibit the

proteolytic activity of the digestive enzyme (trypsin) and can lead to reduced availability of amino acids hence inhibitor suppresses the release of amino acids and do not make the normal growth of animal. Enzyme inhibitors have practical importance in the fields of toxicology, pharmacology and food science [9].

(C) **Haemagglutinin activity:**

Haemagglutnin are proteins that have ability to cause agglutination to red blood cells (RBC). This effect which is highly specific for each protein results from binding to the erythrocyte plasma membrane and haemaggutinin are referred as lectins due to their specificity in binding [10].

Activities of haemagglutinin different according to origin of blood cells from different animals and source of material. It has been suggested that they have a high affinity to sugar molecules hence the microflora of alimentary tract may convert lectins to certain toxin and lectins attach to the cell lining the intestinal wall, causing a non specific interference with the digestion of nutrients and in particular protein digestibility [4]. Keeping the above in view toxic and anti nutritional constituents like cyanogenetic glycosides, tannins, oxalates, trypsin inhibitor activity and haemagglutinating activity in the selected new variety seed samples of Pisum sativum (Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6) were analysed and studied.

EXPERIMENTAL

In the present study, A new variety, healthy and matured legume seeds, Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15 and JM-6 of Pisum sativum, under consideration are collected from Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur cultivated under same climatic conditions and were studied for their toxic and antinutritional factors by chemical and biochemical methods

DETERMINATION OF CYANOGENETIC GLYCOSIDES

Cyanogenetic glycosides were determined in the seeds under study by the method of AOAC(Association of official analytical chemists, Washington DC)[11].

10-20 g of the sample were ground to pass through 20-mesh sieve and placed in Kjeldahl flask, with 200 ml-distilled water. It was kept for about 2-4 hour and then it was steam distilled. About 150-160 ml of distillate was collected in sodium hydroxide

solution (0.5g in 20 ml water). Then the distillate was diluted to 250 ml. To about 100 ml aliquot, 8 ml of ammonium hydroxide (6N), 2ml of potassium iodide (5 percent) were added and titrated against 0.02 N silver nitrate using, micro burette. The end point was faint but a permanent turbidity was easily recognized against black background.

1 ml of 0.02 N $AgNO_3$ ≡ 1.08 mg HCN.

DETERMINATION OF TANNINS

Tannins were determined in the seeds according to the method described in AOAC[11].

5g samples was boiled with 400ml of water for 30 min and diluted to 500 ml. To 10 ml of the aliquot of this extract, 25 ml of Indigo carmine solution(Solution containing 6g Indigo carmine and 50 ml concentrated sulphuric acid per liter.) and 750 ml of water were added. The potassium permanganate solution.(1.33g of potassium permanganate was dissolved in 1 litre of distilled water and its equivalent of 0.1 N oxalic acid was obtained.) was added from burette with stirring till the solution became light green and then bright yellow. The milliliter of potassium permanganate required was noted (Xml).

The 100 ml of aliquot of the extract was mixed with 50 ml gelatin solution (25g of gelatin was soaked in saturated sodium chloride for one hr. It was heated until gelatin dissolved, then cooled and diluted to one liter with saturated sodium chloride solution.) 100 ml of acid sodium chloride solution (975ml of saturated sodium chloride solution was acidified with 25ml of concentrated sulphuric acid.) and 10 g of kaolin. It was then shaken well for several minutes in a stoppered flask. The mixture was allowed to settle and filtered. To 25 ml of aliquot of the filtrate were added 25 ml of Indigo carmine and 750 ml of distilled water, then it was titrated with potassium permanganate solution, as above the milliliter of potassium permanganate solution required was noted (Y ml). A milliliter of potassium permanganate solution used was subtracted from that obtained above (X-Y). It gives the quantity of potassium permanganate required to oxidize tannin.

1ml of 0.1 N Oxalic acid ≡ 0.0042 g of Tannin.

DETERMINATION OF OXALATES

The total oxalates in the form of oxalic acid were determined by the method as given by Talpatra et al [12].

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The seeds, under investigation were dried and finely powdered. 2 to 5g sample was taken in a 600ml Pyrex beaker and 400ml of distilled water added, then it was kept on the sand bath, the top of beaker was covered with a suitable round bottom flask containing cold water to act as condenser, boiling was continued for half an hr and at the end of this period 10 ml of 20 percent solution of sodium carbonate was added and contents were stirred thoroughly and cooking was done for another half an hr with the occasional stirring.

When the cooking was over, the content was filtered hot by suction through a disc of cloth (65 threads to an inch) to a 7cm Buchner's funnel. The filtrate was collected and allowed to settle down when enough hydrochloric acid (1:1) was added drop by drop with constant stirring until the final acid concentration became 1 percent, at this stage the contents were transferred in to a 200 c.c. Volumetric flask and volume was made up to the mark .The precipitate was allowed to settle and supernatant liquid was filtered off through the filter paper.

An aliquot of the filtrate was taken in a 400ml beaker, diluted with water to 200 ml and made just ammonical and re-acidified with glacial acetic acid. In the medium was added 10ml of 10 percent solution of calcium chloride and mixture was well stirred to induce the precipitate of calcium oxalate to appear. The precipitate was allowed to settle overnight .The clear supernatant liquid was carefully decanted through a Whatmann 42 filter paper leaving as much as possible the precipitate undisturbed. The precipitate is then dissolved in hydrochloric acid (1:1) after allowing a few ml of acid to pass through filter paper and collecting it in the beaker containing precipitate.

Finally, the calcium oxalate precipitate was dissolved in dilute sulphuric acid and then titrated with 0.05N potassium permanganate solution and following relation was used for calculation.

1ml of 0.05 N $KMnO_4$ = 0.00225 g of anhydrous oxalic acid.

DETERMINATION OF TRYPSIN INHIBITOR ACTIVITY

Trypsin inhibitors (TIA), which inhibit the proteolytic activity of the digestive enzyme (trypsin) and can lead to reduced availability of amino acids and reduced growth. Trypsin inhibitor activity was determined according to the method described by Gupta and Deodhar[13].

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PREPERATION OF SAMPLE:

Selected seeds sample were finely ground in an electric grinder and extracted with 10ml of petroleum ether (60-80) °C at room temperature. The defatted material was passed through 40-mesh screen.

PREPARATION OF TRYPSIN INHIBITOR EXTRACT:

1g of sample was suspended in 200ml of 0.05 N hydrochloric acid and kept overnight at 4°C in the refrigerator. The insoluble matter was removed by centrifugation at 800 rpm. for 20 min. The supernatant was used as trypsin inhibitor extract and diluted 1:100 with distilled water before use.

A stock solution of casein was prepared by suspending 2g of casein in 100ml of 0.1 M sodium phosphate buffer (pH-7.6). The suspension was heated for 10 min in a boiling water bath to dissolve the casein completely. The solution was stored in refrigerator for further studies (almost 1 week). The solution of casein was incubated at 37°C for 5 min before use.

PROCEDURE:

The reaction mixture consisted of 1.0 ml trypsin (1g/ml of 0.001 N HCl), 0.8 ml of sodium phosphate buffer 0.1M, pH 7.6, 0.2ml of trypsin inhibitor extract and 2.0 ml of 2 percent casein in 0.1M sodium phosphate buffer, pH 7.6. The reaction was initiated by the addition of substrate (casein). After incubation for 20 min at 37°C in water bath, the reaction was stopped by the addition of 6.0 ml of 5 percent TCA. The deproteinised hydrolysate was used for the colorimetric determination of tyrosine with Folin phenol reagent after centrifugation at 800 r. p. m. for 20 min. The tyrosine released was determined by addition of 5.0 ml of sodium carbonate and 1.5 ml of phenol reagent to 2.5 ml of extract. The readings were taken after 5 min at 660 nm in exactly.

Spectronic 20 using red filter. The amount of tyrosine released and inhibited was read from standard curve.

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STANDARD CURVE FOR TYROSINE:

0.1g of tyrosine was transferred to 100 ml volumetric flask and volume was made up to mark. From this stock solution, 10 ml was pipette out into 100 ml volumetric flask and volume made 0.2, 0.4, 0.6, 0.8 and 1.0 ml were pipette into series of test tubes then diluted with 2.3, 2.1, 1.9, 1.7 and 1.4 ml distilled water respectively. To each tube, 5.0 ml of 2.8 N sodium carbonate and 1.5 ml diluted phenol reagent (Folin and Ciocalteus) (1:2) was added. The readings were taken exactly after 5 min at 660nm in Spectronic 20 using red filter.

Calculation: The extent of trypsin inhibition activity was calculated by the formula:

gm tyrosine / 30 min at 37°C/ 1 g seed $\frac{30}{0.2} \times \frac{Y_1 - Y_2}{1} \times \frac{10}{0.2} \times \frac{100}{1} \times 20$

Where,

Y1 = Amount of tyrosine released in control (without seed extract)
Y2 = Amount of tyrosine released in experiment (with seed extract)

DETERMINATION OF HAEMAGGLUTININS ACTIVITY:

The haemagglutinins activity of the seeds extract was tested by the serial dilution method of Liener and Hill[14] with modifications, according to the method of Liener[15].

PREPARATION OF R. B. C. SUSPENSION:

Different and healthy blood samples like chicken, goat, and human O +ve (3ml) were collected in a graduated centrifuge tube containing anti-coagulant and were centrifuged at 3000 rpm. for 20 min. The plasma was discarded and the residue (red blood cells) was washed with normal saline (0.85 percent) three times and centrifuged.

PREPARATION OF EXTRACT:

One gram of sample was suspended in normal saline (10 ml). It was kept in the cold for overnight and was then filtered through Whatman No.41 filter paper. It represents the agglutinin extract. Haemagglutinins plates (plexi-glass plates with wells, 8x10) were used for carrying out the experiment.

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PROCEDURE:

To each well, 0.5 ml saline was pipette. 0.5 ml of the agglutinin extract was pipette into the first well and was mixed thoroughly. 0.5 ml of this solution was pipette into the next well. This procedure was continued till the last well. A standard well with just 0.5 ml of normal saline was also included. To each of the wells, 0.5 ml of washed and diluted (0.5 percent) R.B.C. suspension was pipette out and kept in the cold for overnight. Agglutination was observed visually and recorded.

RESULT AND DISCUSSION

The seeds of different cultivated new varieties Pisum sativum like Arkel, Pusa pragati, IPF-99-25, JP-885, MM-15, JM-6 were analyzed for their cyanogenetic glucosides, tannins, oxalates, trypsin inhibitor activity and haemagglutinating activity. The results are given in Table I and II mentioned below.

All the samples were found with the presence of cyanides but having lesser quantities far below the fatal dose (50 mg/Kg)[16]. Pusa pragati variety reported least cyanide content with 2.63 mg/100 gm of sample. JM-6 reported little higher value than the remaining varieties with 3.35 mg/100 gm of sample. These results are in general agreement with the other samples of legumes reported earlier.

Tannin content of the all samples were within the range of 0.6225 to 0.0419 gm /100 gm. MM-15 reported with 0.6225 gm/100gm and least quantity was found in Pusa pragati variety with 0.0419 gm/100gm. The contents were found to be below the fatal dose (6000 mg / Kg)[16].

Appreciably Oxalate content were also found with lesser than the fatal dose (10gm/100gm)[16] and the contents were found equal in JP-885, MM-15 and JM-6 with 0.101 gm/100gm. Arkel reported less content of oxalates with 0.06 gm/100gm of sample.

Trypsin inhibitor activity was also found to be present in all the samples. MM-15, reported the highest quantity of trypsin Inhibitor activity with 15.23 TIU/mg protein. Lesser quantities were reported in JM-6 and Arkel with 7.63 and 8.89 TIU/mg protein, respectively. More over the samples were found to have lesser quantities of TIA compared with the other samples reported earlier[17,18].

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Table – I: Toxic And Anti-Nutritive Factors In New Variety Seeds Of Pisum Sativum (Pea).

Name of the sample varieties of Pisum sativum.	Cynide content as HCN.in mg/100g	Tannin content gm/100gm	Oxalate content as anhydrous oxalic acid gm/100gm	Trypsin inhibitor activity TIU/mg protein	Haemagglutnin activity with		
					Chicken blood erythrocytes. (Erythrocytes : Sample) in ml: gm)	Goat blood erythrocytes. (Erythrocytes : sample) in (ml: gm)	Human blood erythrocytes. (Erythrocytes :sample)in (ml:gm)
Arkel	2.83	0.1046	0.06	8.89	+ve (1:5)	+ve (1: 10)	+ve (1:0.625)
Pusa pragati	2.63	0.0419	0.068	12.42	+ve (1:5)	+ve (1: 10)	+ve (1:0.625)
IPF-99-25	2.81	0.2486	0.075	11.63	+ve(1: 5)	+ve (1: 10)	+ve (1:0.625)
JP-885	3.26	0.2291	0.101	10.00	+ve(1: 5)	+ve (1: 10)	+ve (1:0.625)
MM-15	3.20	0.6225	0.101	15.23	+ve(1: 5)	+ve (1: 10)	+ve(1:0.625)
JM-6	3.35	0.1046	0.11	7.63	+ve(1: 5)	+ve (1: 10)	+ve (1:0.625)

* The values given in the tables are the mean of the triplicate values obtained.

Table - II: The effect of haemagglutnin activity after various treatment process on the seed samples.

Treatmet process	Haemagglutinin activity with chicken, goat and human blood erythrocytes after the different treatment process of the seeds of different varieties of Pisum sativum.					
	ARKEL	PUSA PRAGATI	IPF-99-25	JP-885	MM-15	JM-6
Boiling with water for 15 Minutes	-ve	-ve	-ve	-ve	-ve	-ve
Soaking in water for 24 Hours	-ve	-ve	-ve	-ve	-ve	-ve
Roasting in Micro oven for 5 Minutes	-ve	-ve	-ve	-ve	-ve	-ve

Haemagglutnin activity of all the sample varities were found positive with chicken (avian), goat (caprine) and human blood erythrocytes. The intensity of haemagglutination was maximum in human blood rather than chicken and goat erythrocytes. The activity of human blood erythrocytes to all the samples were 1: 0.625(erythrocytes: sample) in ml: gm. Chicken blood erythrocytes to all the samples were 1: 5 (erythrocytes: sample) in ml: gm. And goat blood erythrocytes reported the least susceptibility with 1:10 (erythrocytes: sample) in ml: gm. The higher proportion of haemagglutinin activity in human might be due to presence of specific antigen for agglutination in human blood rather than in chicken and goat blood. Alarmingly all the samples reported no haemagglutinin activity with the same blood after subjecting the samples to various treatment process like boiling with water for 15 minutes, soaking in water for 24 hours and roasting in micro oven for 5 minutes.

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Soaking, heating, roasting, sprouting, cooking, boiling has reduce or eliminate most of the toxic factor of the selected peas [19-21].

Most of the anti nutritional factors are heat-labile and since only humans consume legumes after cooking, it would not constitute any major health hazard. In the other animal feed or compositions soaking and roasting for limited period would be beneficial to in increase the nutritional quality of the feed. And the extent of the ant nutritional may be reduced. Heat stable compounds such as polyphenols are, however, not easily removed by simple soaking and heating. These could may be reduced by germination or fermentation [22].

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