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THE PHYTIC ACID CONTENT OF *LATHYRUS SATIVUS* CULTIVATED IN SOME STATES OF INDIA

Sujata Yerra[1]*, Eswar Kumar Kilari[2]

[1]Project Assistant, Advanced Analytical Laboratory, DST-PURSE PROGRAMME, Andhra University, Visakhapatanam, Andhra Pradesh, India

[2]Assistant Professor, Department of Pharmaceutical Sciences, A.U.College of Pharmaceutical Sciences, Andhra University, Visakhapatanam, Andhra Pradesh, India

*Corresponding author E-mail**: ys.gitam@gmail.com**

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ABSTRACT

Legumes form a basic part of human diet. *Lathyrus sativus*, popularly known as grass pea, is one such legume. All leguminous plants have ample anti-nutritional components which hinder the potentiality of the nutrients and their bioavailability. Some such anti-nutrients are phytates, tannins, saponins, trypsin inhibitors, glucosides and so on. Phytates, commonly known as phytic acid, binds minerals and hinders bioavailability causing mineral deficiencies and pellagra. Moreover, the phosphorus in phytate has been considered largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyze phytate in the small intestine. Phytate is storage form of phosphorus and abundant in foods having high fiber content. Hence, this work attempts to show the phytate content in various *Lathyrus sativus* samples collected from different states of India.

INTRODUCTION:

Legumes are the major sources of proteins in diet consumed in India, There are different kinds of legumes cultivated and consumed largely by Indian population as a chief source of protein. Legumes are the beans, peas, nuts and pulses. Although legumes constitute one of the most abundant and least expensive sources of protein in human/animal diets, their utilization is limited largely due to the presence of antinutritional/ anti-physiological compounds (Vijayakumari *et al*., 2007). Out of these, pulses are consumed more by Indians. Grass pea is one of the rich sources of protein obtained from the pulses. The ain limitation is the presence of various anti-nutritional factors and the neurotoxin ß-ODAP, which could greatly undermine the

potentials (Urga *et a*l., 2005), and is an important food crop in Asia and the Middle East where the whole seed is used in soups and ground to make unleavened bread. In India, Pakistan, Bangladesh and Nepal, the most common use of grass pea is a dhal (a soup-like dish). In India, the grains are sometimes boiled whole, but are most often processed through a dhal mill to obtain split dhal. Dhal is the most common method of retailing the crop in the Indian subcontinent (Tsegaye *et al*., 2007). In Canada, uses would be for high protein livestock feed and as a green manure crop or cover crop. Phytic acid binds trace and macro-elements such as zinc, calcium, magnesium, and iron, in the gastrointestinal tract and making dietary minerals unavailable for absorption and utilization by the body (Jansman *et al*., 1998). Moreover, the phosphorus in phytate has been considered largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyze phytate in the small intestine. Phytate is storage form of phosphorus and abundant in foods having high fiber content. Human body is unable to digest phytate like non-ruminant animals. It doesn't provide phosphorus but it chelates metal ions like iron, zinc, calcium and magnesium and vitamin niacin and makes them unavailable to the body and thus cause mineral deficiency and pellagra (Ali et al., 2010).

Anti-nutritional and toxic factors in grass pea:

Anti-nutrients have been defined as substances, which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Francis *et al*., 2001). In common with those of other grain legumes, grass pea seeds contain a variety of antinutritional factors. The most frequently occurring anti-nutritional substances in grass pea are protease and amylase inhibitors, lectins, tannins, saponins, alkaloids, phytates, and lathyrogens (Ramachandran and Ray, 2008). The main anti nutritional factors occurring in grass pea include protease inhibitors (trypsin inhibitors), phytic acid, tannins, and β-ODAP. It produces protein energy malnutrition and lathyrism appears frequently. One of the reasons is due to the presence of anti-nutritional factors, which inhibits the digestibility of food and the bioavailability of essential minerals and trace elements. The other more serious reason is that when the grass pea, if consumed by the people as staple food for 3-4 months it causes lathyrism (Malek et al., 1995). Overconsumption of grass pea for an extended period of time can cause spastic paraparesis of the legs in up to 6% of the population, affecting mainly the young males (Strickland GT, 1988). Since grass pea is deficient in cysteine and methionine, and consumption of cereals richer in these amino acids and condiments rich in antioxidants seem to be protective factors (Getahun, 2005), malnutrition and oxidative stress have to be considered as contributing factors in the etiology of neurolathyrism, together with the ingestion of the neurotoxin. The other more serious reason is that when the grass pea. If consumed by the people as staple food for 3-4 months it causes lathyrism (Malek et al., 1995). Overconsumption of grass pea for an extended period of time can cause spastic paraparesis of the legs in up to 6% of the population, affecting mainly the young males (Strickland GT, 1988). Moderate daily consumption of grass pea like other legumes has no deleterious effects, and some authors even mention beneficial effects for human health (Rao SLN, 2011). In common with all grain legumes there are a range of anti nutritional factors (ANFs) found in L. cicera and *L. sativus* grain. The ANFs commonly found in grain legumes include: tannins,

phytic acid, oligosaccharides, protease inhibitors (trypsin and chymotrypsin inhibitors), amylase inhibitors and lectins (Liener, 1989). ODAP is also an ANF and is almost unique to the Lathyrus genus. There are only a small number of published studies of levels and activities of ANFs, other than ODAP, in *L. sativus* (Latif et al., 1975; Deshpande and Campbell, 1992; Aletor et al., 1994; Urga et al., 1995; Srivastava and Khokhar, 1996; Wang et al., 1998), and less on L. cicera (Aletor et al., 1994)

Phytic acid and phytates:

Phytate (hexaphosphates of myo-inositol) is common in plant seeds. They are storage form of phosphorus in a plant (Urbano *et al*., 2000). They are necessary for germination. Phytic acid is a chelating agent, they can chelate with mono, di- and trivalent mineral ions such as Na+, K+,Ca_2+, Mg_2+, Zn_2+, Cu_3+ and Fe_3+ resulting in these ions becoming unavailable for body utilization or causes poor mineral bioavailability (Urbano *et al*., 2000). Since non-ruminants cannot break down phytates, their occurrence in feed reduces the availability of phosphorus to these animals. Phytates also form sparingly digestible Phytate–protein complexes, thus reducing the availability of dietary protein (Francis *et al*., 2001). It forms complexes with Fe, Cu and Zn, and can bind enzymes too (e.g. pepsin) – blocks absorption of these important minerals and causes starch to be less digestible – worse nutrition - In every plant, but highest concentrations in cereals, legumes, nuts and spices. They are heat-stable. On the other hand, they protect against cancer, one of the breakdown products is inositol triphosphate, which enhances natural killer cells activity.

MATERIALS AND METHODS

Samples of *Lathyrus sativus* seeds- LS

- LS- Andhra Pradesh (LS-AP)
- LS-Odisha (LS-OD)
- LS- Kerala (LS-KE)
- LS- West Bengal (LS-WB)
- LS- Bihar (LS-BI)
- LS- Chhattisgarh (LS-CH)

Chemicals

Reagents used for analysis were purchased from Sigma Aldrich Company. All chemicals and reagents used were analytical reagent grade except H_2O_2, which was laboratory reagent grade.

Sample Preparations:

The seeds were cleaned manually to remove foreign matters, immature and damaged seeds. Different traditional processing methods (Teklehaimanot et al., 1993):

Raw:

The cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water and immediately dried in drying oven at 55 °C for 12 h, under air circulation, and then grind by grinder to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until analysis.

Wet roasting :

Whole cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water, soaked with distilled water (1:2 w/v seed to water) for 3 hr., decant the soaking water and washed with another distilled water, placed in 2L of distilled boiling water at 96 °C and cooked for 60 min. (until soft) and immediately dried in drying oven at 55

°C for under air circulation, and then grind by grinder to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until required for analysis.

Boiling :

Whole cleaned seeds (1Kg) were washed with tap water, rinsed with distilled water, soaked with distilled water(1:5 w/v seed to water) at 28 ^{0}C (using water bath) for 20 h and then roasted at 200 °C for 40 min in baking oven placed in a baking try and turning with a fork, and then grind by grinder to pass through a 0.425 mm sieve, packed in air tight bottle and stored at room temperature (in the shelf) until required for analysis.

Soaking + Boiling:

100 g sample soaked overnight (8-9 hrs.) in water under room temperature and then boiled in sufficient water until the pulse seed is easily pressed soft by hand/spoon/ladle.

Method for estimation of Phytate content

Phytate content in legume meals was determined by procedure elaborated by Haug and Lantzsch (1983)

- **Preparation of phytate reference solution**

Sodium phytate (0.15 g) was dissolved in 100 ml de-ionized water. Reference solution was prepared by diluting the stock solution in the range of 1.2-11.7 ml stock solution (1.2, 2.7, 4.2, 5.7, 7.2, 8.7, 10.2, 11.7 ml) in 100 ml volumetric flask and made the volume with 0.2N HCl.

- **Preparation of ferric solution**

Fe (NH4)2(SO4)2.6H2O (0.2 g) was dissolved in 100 ml 2N HCl and volume was made to 1000 ml with de-ionized water.

- **Preparation of 2, 2- bipyridine solution**

10 g 2, 2- bipyridine and 10 ml thioglycolic acid were dissolved in de-ionized water and made the volume 1000 ml.

- **Procedure**

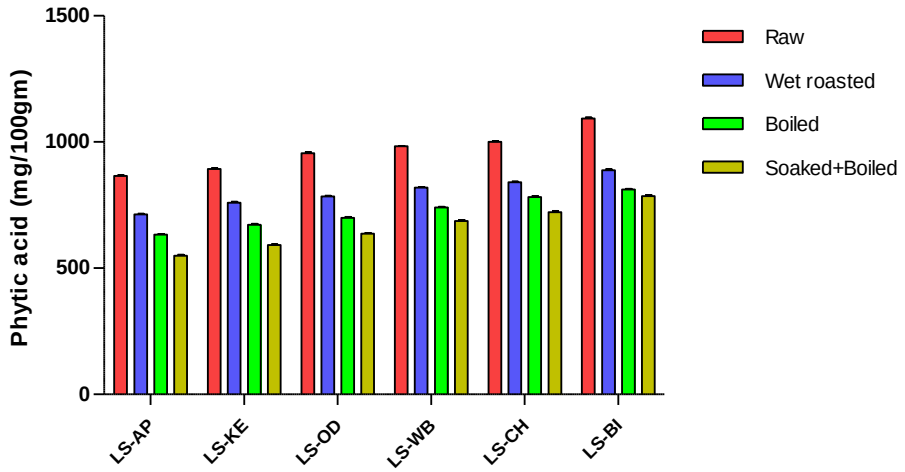
Sample (0.06) g was extracted in 10 ml 0.2N HCl solution in a test tube by shaking for half an hour. Then 1 ml extract was taken in a test tube. 2 ml ferric solution was added into the test tube and covered with a stopper. The test tube was fixed with clip and then heated in a water bath for 30 min. Then test tube was cooled in ice water for 15 min and allowed to adjust at room temperature. 2, 2- bipyridine solution (4 ml) was added into the test tube and the contents were mixed. The absorbance was checked at 519 nm by spectrophotometer against de-ionized water after 30-60 sec. The method was calibrated with reference solution as a substitute for the sample solution for each set of analysis.

RESULTS AND DISCUSSION:

Table: Estimation of Phytic Acid (mg/100g) Levels in *L. sativus* seeds of various States of India:

Parameters (%)	Raw seeds	Wet roasted	Boiled	Soaked+Boiled
LS-AP	865.23±12.30	712.56±8.79 (17.68)	633.06±12.31 (26.82)	549.12±11.30 (36.53)
LS-KE	893.15±11.65	759.13±9.32 (15.00)	672.46±8.79 (24.74)	591.54±9.58 (33.81)
LS-OD	956.06±9.15	783.89±10.24 (18.09)	699.28±10.35 (26.88)	637.23±12.13 (33.36)
LS-WB	982.589±10.23	819.46±11.52 (16.59)	741.16±12.22 (24.54)	687.14±8.32 (30.04)
LS-CH	1000.16±13.51	840.26±9.99 (16.00)	782.23±11.07 (21.8)	721.49±10.66 (27.90)
LS-BI	1093.18±8.46	888.49±12.35 (18.75)	811.46±9.35 (25.80)	785.33±9.99 (28.17)

Graph: Estimation of Phytic Acid (mg/100g) Levels in *L. sativus* seeds of various States of India:



PHYTIC ACID:

Table shows the estimation of phytic acid (mg/100gm) levels in *L. sativus* seeds of various States of India. The samples of *L. sativus* obtained from farmers of Andhra Pradesh (LS-AP), Kerala (LS-KE), Odisha (LS-OD), West Bengal (LS-WB), Chattisgarh (LS-CH) and Bihar (LS-BI) for the present study. The obtained samples of *L. sativus* were subjected to traditional processing methods basing on people consume them into raw, wet roasted, boiled and soaked + boiled as described in Processing methods for grass pea is very important primarily due to the high content of antinutrients and the difficulty in their digestion. The phytic acid levels (mg/100gm) in *L. sativus* in the State of Andhra Pradesh was 865.23±12.30 in raw seeds, 712.56±8.79 in wet roasted seeds, 633.06±12.31 in boiled seeds and 549.12±11.30 in soaked seeds. As in the State of Kerala, the phytic acid levels (mg/100gm) of *L. sativus* seeds showed 893.15±11.65, 759.13±9.32, 672.46±8.79 and 591.54±9.58 in raw, wet roasted, boiled and soaked seeds respectively. The phytic acid levels (mg/100gm) in *L. sativus* in the State of Odisha was 956.06±9.15 in raw seeds, 783.89±10.24 in wet roasted seeds, 699.28±10.35 in boiled seeds and 637.23±12.13 in soaked seeds. The phytic acid levels in *L. sativus* seeds from the State of West Bengal as depicted in raw, wet roasted, boiled and soaked seeds as 982.589±10.23,819.46±11.52, 741.16±12.22 and 687.14±8.32 respectively. As in the State of Chattisgarh, the phytic acid levels (mg/100gm) of *L. sativus* seeds showed 1000.16±13.51, 840.26±9.99, 782.23±11.07 and 721.49±10.66 in raw, wet roasted, boiled and soaked seeds respectively. And, the phytic acid levels (mg/100gm) in *L. sativus* in the State of Bihar was 1093.18±8.46 in raw seeds, 888.49±12.35 in wet roasted seeds, 811.46±9.35 in boiled seeds and 785.33±9.99 in soaked seeds. The samples of *L. sativus* obtained from farmers of Andhra Pradesh (LS-AP), Kerala (LS-KE), Odisha (LS-OD), West Bengal (LS-WB), Chattisgarh (LS-CH) and Bihar (LS-BI) for the present study. The obtained samples of *L. sativus* were subjected to traditional processing methods basing on people consume them into raw, wet roasted, boiled and soaked + boiled as described in Chapter III (materials and methods). Processing methods for grass pea is very important primarily due to the high content of antinutrients and the difficulty in their digestion. Boiling in water or repeated steeping in hot water and discarding the extracts can detoxify the seeds. Roasting of seeds, at 140^{o}C for 15 to 20 minutes, result in 80 to 90 % destruction of the neurotoxins. Some people soak the seeds overnight and decant the water before cooking. This eliminates about 90% of the toxin. Toxic amino acids are readily soluble in water and can be leached. Fermentation is useful to reduce ODAP content. Moist heat (boiling, steaming) denatures protein inhibitors, which otherwise adds to the toxic effect of raw grass pea through depletion of protective sulfur amino acid (Rao SLN, 2001). In addition, tannin and phytic acid were significantly reduced by the processing methods. Among the processing methods, preparing sauce was found to be the best method to reduce phytate, followed by unleavened bread, roasting and boiling grass peas seeds. When grass pea is processed, the protein inhibitor and other anti nutritional factors, which inhibit the protein digestibility and chelate the mono, di and trivalent metal ions and form insoluble complexes will be degraded to a smaller molecular form and release the protein and the essential elements. The food processing methods including soaking, germination, decortications, fermentation and cooking greatly influence the nutritive values of

legumes. Of these, cooking and germination plays an important role as it influences the bioavailability and utilization of nutrients and improves palatability, which incidentally may result in enhancing the digestibility and nutritive value (Ramakrishna *et al*., 2006). Therefore, data on the effect of traditional processes on the nutrient composition, mineral contents and ant nutritional factors could be evaluated. The loss in phytates in roasted, boiled and sauce samples of grass pea may be due to leaching of phytate ions during soaking into the soaking water under the influence of a concentration gradient (difference in chemical potential) which governs the rate of diffusion. In addition, it may be ascribed to the activation of the endogenous phytase during the long soaking treatment and possible enzyme action continued during drying step. Similar results for reduction in phytate in the soaked and cooked peas have been reported in common other legumes (*Bauhinia purpurea* L. and *Prosopis chilensis* seeds) by Vijayakumari *et al*. (1997; 2007) and in pigeon pea (*Cajanus cajan)* by Duhan et al. (2002). Phytic acid exerts its inhibitory effect on the absorption of zinc and iron by forming insoluble complexes in the gut under physiological condition. The formation of such chelates depends on the ratio of the content of zinc, iron or calcium relative to that of phytate in the food (Umeta et al., 2005). In a region where grass pea is used as staple food, protein energy malnutrition and lathyrism appear frequently. One of the reasons is due to the presence of anti nutritional factors, which inhibits the digestibility of food and the bioavailability of essential minerals and trace elements. The other more serious reason is that when the grass pea is consumed by the people as staple food for 3-4 months it causes lathyrism (Malek *et al*., 1995).

CONCLUSION

Hence, for proper utilization of grass pea, especially in developing countries, these simple and economic household processing and cooking methods should be followed, as they not only save time, energy and fuel consumption but also enhance the nutritional quality of the grass pea by lowering the content of anti-nutrients and increasing the bioavailability of minerals. Overall, it can be concluded that when we compare the processing effect on the nutrient composition, mineral content and anti-nutrients, boiling, preparing sauce and roasting respectively are the most appropriate methods to consume grass pea. Although, scale-up and applicability of these processing, to large- or commercial-scale should be overlooked. However, traditional household practices can decrease the antinutritional factors significantly, and thus need to be encouraged.

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