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Short Communication

The tannin content of *Lathyrus Sativus* cultivated in some states of India

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Abstract

Indigenous legumes like grass pea are an affordable important source of alternative protein for poor people that are predominantly consumed in tropical countries especially in Africa and Asia. Legumes are home to many valuable nutrients and anti-nutrients. The main anti-nutritional factors occurring in grass pea include protease inhibitors (trypsin inhibitors), phytic acid, tannins, and β-ODAP. Amongst them, tannins are polyphenolic compounds having intermediate to high molecular weight and have bitter and astringent taste which can be felt upon consuming its unripened fruit owing to their protein and alkaloid binding property turning the meal difficult to digest. The name 'Tannins' originated owing to their tanning quality and their ability in forming insoluble carbohydrates and proteins complexes, wherein the astringency in tannin-rich foods is due to the precipitation of salivary proteins. Additionally, they often form complexes with the vital minerals that reduce intestinal absorption and the subsequent utilisation at cellular level. This study depicts the tannin content in grass pea cultivated in various States of India namely, Andhra Pradesh, Odisha, Kerala, West Bengal, Bihar, Chhattisgarh showing their variability due to the effect of different food processing techniques applied on the samples. A notable change can be seen in the samples owing to their geographical as well as varied food processing methods applied to them.

Keywords: Legumes, grass pea, anti-nutrients, insoluble complexes, food processing.

Introduction

An increased interest regarding nutrition among a wider segment of society is vital and remarkable nowadays. The primary focus of the society is simply not only on having food for survival but also to be well acquainted of the components of food that we consume to achieve the best quality of life. Legumes are home to inumereable nutrients as well as anti-nutrients. Some of the anti-nutrients are phytates, tannins, saponins, protease inhibitors, haemaglutinins. In addition to phytates, tannins (phenolic compounds) are found in significant quantity causing astringency and bitterness of many legumes like grass pea and beverages[1]. Tannins are intermediate to high molecular weight compounds and the name had its origin owing to its tanning quality. These compounds aid in forming insoluble complexes with proteins, mineral elements and carbohydrates, too and the bitterness in tannin-rich foods is due to the precipitation of salivary proteins, also reduce intestinal absorption and the subsequent utilisation at cellular level[2]. We know that tannins are highly astringent due to their protein-binding abilities, also a decrease in the intake of feed may lower the productivity in animals. Many additional factors may also aid to the decreased feed efficiency ratios of tannin-rich diets. High consumption of tannin may lead to various bodily ailments like irritation of the bowel, damage of the liver, irritation in the kidney and stomach and acute pain in the stomach and intestines. These tannins chelate elements and hinder their bioavailability and their prolonged consumption may also lead to iron deficiency or anaemia. However, it is a well known fact that tannins decrease and hinder the bioavailability of iron especially non-haem iron from plant sources[3,4]. Tannins also reduce the absorption of vitamin B_{12}.

However, the term "tannin" is commonly used to refer polyphenolic compounds. Tannins are generally divided into hydrolysable (glucose polyesters of Gallic or hexahydroxydiphenic acids) and condensed tannins (proanthocyanidins)[5,6]. Some vital factors such as water stress, soil composition, atmospheric temperature and relative humidity affects the phenolic contents in plants[7]. The tannin content in plants differ during the developmental stages of the plant and are also responsive to the environmental alterations[8-10]. Indigenous legumes like grass pea are a vital and affordable dietary protein for the poor fraternity in tropical countries of Africa and Asia where they are predominantly consumed[11]. However, legumes contain a wide range of toxic components. The term antinutrients is defined as the substances, either by themselves or the metabolites in the system, hinder the utilization of food nutrients and also affecting the overall health and productivity in animals[12]. The contribution of legumes to the consumer nutrition is limited mainly owing to the occurence and action of various antinutritional factors[1]. The incidence of these antinutritional factors may eventually affect the bioavailability of the nutrients and produce erroneous outcomes[13]. Various toxicity manifestations from legume consumption with antinutritional factors range from severe decrease in the intake of food and nutritional bioavailability, to profound neurotoxicological effects and sometimes, death[14].

Generally, all grain legumes contain various anti nutritional factors as in *Lathyrus cicera* and *Lathyrus sativus* grain. The antinutritional factors predominantly seen in them are: tannins, phytic acid, oligosaccharides, protease inhibitors (trypsin and chymotrypsin inhibitors), amylase inhibitors and lectins[15], saponins, alkaloids, and lathyrogens especially, β-ODAP[16-18]. As an antinutritional factor, ODAP is specifically unique to the Lathyrus genus. There are only a few published studies on the contents and activities of the antinutritional factors, apart from ODAP, in *Lathyrus sativus*[19-24]. The levels of condensed tannins in grass pea lines occur from zero to 4.38g/kg[25] which include the tannin/protein complex formation making the protein unavailable, digestive enzymes inhibition, inadequate enzyme digestion leading to increased synthesis of digestive enzymes, and the loss of endogenous proteins is increased such as the gastrointestinal mucoproteins[26].

Contrarily, the hydrolysable tannins are destroyed easily in the biological system and form simpler and small compounds easily entering the blood stream which with time, increase the toxicity of the organs, namely, kidney and liver. Tannins are also highly interactive with other anti-nutrients like, the interactory activity of tannins and lectins hinder the amylase from being inhibited by the tannins, and the interactory activity of tannins and cyanogenic glycosides decreased the endangering aspects of the latter[12]. The tannin concentration was generally high and similar in all the samples, even for grass pea germ plasma[27]. The intensity of pigmentation is a vital factor the level of tannins in the particular food legume. The seed coats which are dark usually depicted elevated contents of tannins as similar as the research of Deshpande and Campbell, 1992. It was even noticed that the flower color in *Lathyrus sativus* is also closely related with the color of the seed, wherein, the flowers which were blue-pink or red-colored, generally produced seeds which were speckled and colored, whereas the flowers which were white, are related with the seeds which were white to creamy-yellow. The grass pea types with flowers which are blue, are concentrated in South-West Asia and Ethiopia whereas the grasspea varieties having either white or cream-colored seeds, are rarely found in Ethiopian or the Indian subcontinent accessions. The former grasspea types are even noted to show the best tolerance to abiotic factors such as heavy or poor soils, drought or water-logging, high and low pH, and other biotic factors[28]. For this reason, they undergo several primary processes of hulling (husking), puffing, grinding, splitting, etc.- before being incorporated in various food preparation techniques. This is done to improvise the nutritional quality and bioavailability of a given legume and to hinder the harmful effects of antinutritional factors and other toxic components. During any processing attempt of grass pea, it is important that toxic components be reduced to levels that pose no threat to health due the presence of inherent paralytic neurotoxin. The processing condition of grass pea should also take into consideration the degree of ripeness of grass pea. At different stage of maturity, grass pea contains different concentration of neurotoxin and other antinutritional factors. It is reported that unripe grass pea is the most toxic and should be avoided by all means for human consumption. In addition to this, processing parameters are also major determinant factors to what level any given processing method can improve the nutritional quality and reduce natural toxins and antinutritonal factors found in grass pea. This review of the literature examines how different processing methods affect the nutritional quality and level of antinutritional factors in grass pea. The report from different authors can be used as an input to propose the optimum food processing condition to achieve the intended nutritional quality bioavailability and also the elimination of the inherent neurotoxins found in grass pea. The daily consumption of grass pea like other legumes on a moderate scale has no deleterious effects, while some researchers even applaud the beneficial results for the upliftment of human health conditions[29].

Secondary metabolites	Unit	Mean±SEM
Tannins, condensed (eq. catechin)	g/kg DM	2.1±1.6

Materials and methods

Samples: Samples of *Lathyrus sativus* seeds-LS: i. LS-Andhra Pradesh (LS-AP), ii. LS-Odisha (LS-OD), iii. LS- Kerala (LS-KE), iv. LS-West Bengal (LS-WB), v. LS- Bihar (LS-BI), vi. LS-*Chhattisgarh (LS-CH).*

Chemicals: The necessary reagents and chemicals were purchased from Sigma Aldrich.

Sample Preparations: The preliminary cleaning procedures for the samples of *Lathyrus sativus* were carried out. Different traditional processing methods[30].

Raw: The cleansed seeds (1Kg) were washed under running tap and rinsed with distilled water. Soon after, they were kept for drying in the drying oven at 55°C for about 12 hours. Later, the sample was ground using a grinder suitable for a 0.425mm sieve. Then they were packed in an air tight bottle and kept in storage room at room temperature till sent for further analytical work.

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 60min. (until soft) and consequently kept for drying in drying oven at 55°C for about 10-12 hours. Then, the seeds were ground by using a food grinder suitable for a 0.425mm sieve. Later, the ground sample was properly packed in an air tight container and kept at room temperature.

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

Soaking + Boiling: 100g sample soaked overnight (8-9hrs) 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 the estimation of tannin content in the LS samples: The level of tannins in the said samples were measured by Folin-Denis method[31]. Taking a 250ml conical flask, the ground sample (0.5g) was added with 75ml distilled water which was kept boiling for about 30-40 minutes. This mixture was sent for centrifugation at a speed of 2000rpm for 15-20 minutes. Later, taking a 100 ml volumetric flask, the supernatant was made up to the mark. Taking a 100ml flask, 75ml water, 1ml sample extract, 5ml Folin-Denis reagent along with 10ml sodium carbonate solution were added and making up the volume. Then, shaking the mixture well and later measuring the absorbance at 700nm stayed till 30 min. Finally, the preparation of blank was done with water instead of the sample and using 0-100μg tannic acid, the standard graph was drawn.

Results and discussion

Table-1 shows the estimation of tannins (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. The food processing techniques employed on grass pea is highly essential mainly owing to its high levels of anti nutrients leading to their indigestibility and biounavailability.

The tannins levels (mg/100gm) in *L. sativus* in the State of Andhra Pradesh was 658.06±4.36 in raw seeds, 691.56±4.84 in wet roasted seeds, 726.85±5.55 in boiled seeds and 856.45±6.32 in soaked seeds. As in the State of Kerala, the tannin levels (mg/100gm) of *L. sativus* seeds showed 625.45±5.12, 642.12±5.63, 698.26±4.36 and 820.48±4.12 in raw, wet roasted, boiled and soaked seeds respectively. The tannin levels (mg/100gm) in *L. sativus* in the State of Odisha was 569.36±6.66 in raw seeds, 589.48±4.71 in wet roasted seeds, 630.15±8.12 in boiled seeds and 781.33±5.19 in soaked seeds. The tannin levels in *L. sativus* seeds from the State of West Bengal as depicted in raw, wet roasted, boiled and soaked seeds as 532.45±3.58, 566.39±3.67, 604.26±3.64 and 753.45±4.44 respectively. As in the State of Chattisgarh, the tannin levels (mg/100gm) of *L. sativus* seeds showed 489.92±4.71, 530.15±5.75, 592.44±5.41 and 690.69±3.64 in raw, wet roasted, boiled and soaked seeds respectively. And, the tannin levels (mg/100gm) in *L. sativus* in the State of Bihar was 436.18±3.94 in raw seeds, 466.42±6.66 in wet roasted seeds, 512.36±7.70 in boiled seeds and 632.47±6.32 in soaked seeds.

Although leguminous seeds form the most abundant and economic source of rich protein, their utilization is minimised mainly owing to the occurrence of anti nutritional/ anti-physiological components in them[32]. During the processing of grass pea, the factors inhibiting the digestibility and bioavailability of proteins as well as mineral elements owing to the presence of various anti-nutritional factors. This is mainly due to the chelation of the mono, di and trivalent metal ions which form insoluble proteins and minerals reducing their molecular forms releasing the protein and the mineral components for utilisation. The food processing techniques of decortications, soaking, cooking, germination and fermentation immensely improve the beneficial aspects of leguminous seeds. Amongst them, germination and cooking play a pivotal role in influencing the utilization and bioavailability of nutrient components by improving their values and enhancing the palatability[1] Therefore, data on the effect of traditional food processes on the nutritive values, mineral contents and anti-nutritional factors could be evaluated. To detoxify the seeds, soaking repeatedly in hot water or boiling them and later, discarding the water in which soaking or boiling was done. This practise eliminates nearly 90% of the anti-nutrients which can be readily leached as they are soluble in water and can be leached. Similarly, we can roast the seeds at 140^{o}C for about 15-20 minutes which destroy the toxins and anti-nutrients by 80-90%.

Another practise of fermenting the seeds is also helpful in reducing the toxins. Some moist-heat methods of food processing techniques of steaming and boiling denature the protein inhibitors by depleting the protective sulfur amino acid[33].

The higher contents of tannins inhibit the role of digestive enzymes, interfering with the utilisation of essential nutrients. They also have a deleterious effect on the digestive tract mucosal cells and thence are essentially undesirable for consumption[32]. The processed grass pea samples showed a significance difference of ($P<0.05$) in the tannin levels. In the present study, the tannin content decreased by 54.06%, 33.21%, 91.67% and 83.11% in roasted, boiled, unleavened bread and sauce respectively.

Depletion of tannins, the polyphenolic compounds which are soluble in water and essentially noticed in the seed coats, may be due to solubility in water thereby leaching the contents and its sensitivity to heat[32]. Similar results of reduction in the tannin levels were recorded during various food processing techniques of legumes[32,34,35].

Table-1: Estimation of Tannins (mg/100g) Levels in *L. sativus* seeds of various States of India.

Parameters (%)	Raw seeds	Wet roasted	Boiled	Soaked+Boiled
LS-AP	632.47±6.32	512.36±7.70 (18.98)	466.42±6.66 (26.26)	436.18±3.94 (31.01)
LS-KE	690.69±3.64	592.44±5.41 (14.20)	530.15±5.75 (23.18)	489.92±4.71 (29.13)
LS-OD	753.45±4.44	604.26±3.64 (19.78)	566.39±3.67 (24.83)	532.45±3.58 (29.34)
LS-WB	781.33±5.19	630.15±8.12 (19.33)	589.48±4.71 (24.58)	569.36±6.66 (27.14)
LS-CH	820.48±4.12	698.26±4.36 (14.87)	642.12±5.63 (21.70)	625.45±5.12 (23.78)
LS-BI	856.45±6.32	726.85±5.55 (15.18)	691.56±4.84 (19.27)	658.06±4.36 (23.13)

The levels of tannins were found to be decreased. The processing of grass peas samples collected from different States of India were found to reduce tannin content greatly. The tannin levels were decreased by 18.98%, 26.26% and 31.01% in wet roasted, soaked, soaked+boiled respectively. Since tannins are soluble in water, the reduction in their levels may be attributed to the leaching of the anti-nutrient extracts into the soaked water. In addition, the levels of tannins were significantly eliminated by the wise application of various food processing techniques. Regarding the tannin content, preparing unleavened bread is the most beneficial method, followed by sauce making, roasting and boiling.

Conclusion

Hence, such simple and economic household food processing techniques should be employed for better utilization of grass pea, especially in the developing countries as they not only save time, energy and fuel consumption. These practices also improve the nutritive aspects of grass pea by reducing the anti-nutrient composition and enhancing the bioavailability of all vital nutrients like proteins and 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 reduce the anti-nutritional factors at a significant level, and hence should be practised and encouraged.

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