

EFFECT OF VARIOUS PROCESSING METHODS ON THE MINERAL CONTENT OF *LATHYRUS SATIVUS* SEEDS CULTIVATED IN DIFFERENT STATES OF INDIA

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ABSTRACT

Pulses form a common part of human diet, grass pea is one among them. The grass pea (*Lathyrus sativus*) seeds cultivated in different States of India like Andhra Pradesh, Kerala, Odisha, West Bengal, Chattisgarh and Bihar were taken and the seeds were treated by using different processing methods of wet roasting, boiling and soaking+boiling and also further mineral analysis for the estimation of calcium, iron, copper, zinc, magnesium and phytic acid were determined using atomic absorption spectrophotometer method. Among all the States of India, *Lathyrus sativus* cultivated in Andhra Pradesh showed higher amounts of minerals compared to other States of India. In Andhra Pradesh, the soaked+boiled processed seeds showed a better amount of mineral values compared to raw, wet

roasted and boiled *Lathyrus sativus* seeds. The seeds have a higher concentration of magnesium followed by calcium. The trace minerals are also found to high and the levels of phytic acid was significantly altered.

KEYWORDS: Grass pea, *Lathyrus sativus*, processing methods, Minerals, Phytic acid

INTRODUCTION

Minerals are chemical constituents found in foods like pulses. They have important roles to play in many activities in the body.^[1,2] The macro-minerals found in pulses are calcium, phosphorus, sodium and chloride, while the micro-elements are iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulphur.^[2]

Grass pea is commonly used pulse in India and rich in minerals. The grass pea constitutes of major and minor elements. The calcium, magnesium, phosphorus and iron in the seeds were generally higher than the corresponding manganese, copper and zinc levels.^[3] The seeds of grass pea are also reported to contain many anti-nutritional compounds such as tannins, phytic acid, trypsin inhibitors, saponins and oligosaccharides.^[4] The presence of phytic acid is reported to interfere with mineral element absorption and utilization and also reacts with proteins to form complex products which have an inhibitory effect on peptic digestion.^[5]

β -ODAP is a neurotoxin compound abundantly found in the seeds of *Lathyrus sativus* and is the main cause for neurolathyrism.^[6,7,8,9] Neurolathyrism is an upper motor neuron disorder resulting from prolonged consumption of grass pea over a period of 3 to 6 months. The chemical composition of grass pea may vary according to varieties/ genotype, geographical region of their growing and maturity and environmental factors (soil fertility, nitrogen nutrition, temperature, and water stress and soil pH).^[10] The agroclimatic conditions like lack of water supply and lack of zinc and phosphorous content in fertilizers were found to be responsible for the elevation of ODAP.^[11]

Hence, the present study was aimed to evaluate the effect of different processing methods on the mineral composition of *Lathyrus sativus* seeds from different States of India.

MATERIALS AND METHODS

Materials

Chemicals: Reagents used for analysis were purchased from Sigma Aldrich Company. All chemicals and reagents used were analytical reagent grade except H₂O₂, which was laboratory reagent grade.

Sample Collection: *Lathyrus sativus*(LS) seed samples were collected from Andhra Pradesh (LS- AP), Odisha (LS- OD), Kerala (LS- KE), West Bengal (LS- WB), Chhattisgarh (LS- CH) and Bihar (LS- BI).

Sample Preparations: The seeds were cleaned manually to remove foreign matters, immature and damaged seeds. Different traditional processing methods are.

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.^[12]

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.^[12]

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 28⁰C (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.^[12]

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.^[12]

Mineral analysis

Preparation of sample: Sample (0.5 g) was digested by the wet digestion method. It was first digested with 10 ml HNO₃ at gentle temperature (60-70°C) for 20 min. Then the sample was digested with HClO₄, at high temperature (190°C) till the solution became clear. The digested sample was transferred to 250 ml volumetric flask and volume was made with distilled water and then filtered.^[13]

Estimation of mineral contents: The filtered sample solution was loaded to the atomic absorption spectrophotometer. Calcium, iron, copper, zinc, and magnesium were determined using atomic absorption spectrophotometer method of Osborne and Voogt (1978).^[14] The ash obtained after dry ashing at 550 ⁰C was treated with 5 ml of 6N HCl to wet it completely and carefully dried on a low temperature hot plate. 7 ml of 3N HCl was added and the dish was heated on the hot plate until the solution just boils. Then, it has been cooled and filtered through a filter paper in to a 50ml volumetric flask. Again 7 ml of 3N HCl was added to the dish and heated until the solution just boils. Finally, cooled and filtered into the volumetric

flask. For the determination of calcium, lanthanum chloride (1% w/v) was added to both standards and samples to suppress interference from phosphorus. The standard curve for each mineral was prepared by running samples of known strength. The mineral contents of the samples were estimated by using the respective standard curve prepared for each element.^[15] Using atomic absorption spectrophotometer, a calibration curve was prepared by plotting the absorption or emission values against the metal concentration in mg/100g. Reading was taken from the graph, which depicted the metal concentrations that correspond to the absorption or emission values of the samples and the blank. The metal contents were calculated by using the formula

$$\text{Metal content } \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{[(A - B) \cdot XV]}{10W}$$

Where, W = weight of samples (g)

V = volume of extract (ml)

A = concentration of sample solution (µg/ml)

B = concentration of blank solution (µg/ml)

Estimation of phytic acid: Phytic acid was estimated by the method of Davies and Reid 1979.^[16] One g of material was ground and extracted with HNO by continuous shaking, filtered and made up to suitable volume with water. To 1.4 ml of the filtrate, 1 ml of ferric ammonium sulphate solution (21.6 mg in 100 ml water) was added, mixed and placed in a boiling water bath for 20 min. The contents were cooled and 5 ml of isoamyl alcohol was added and mixed. To this, 0.1 ml ammonia solution was added, shaken thoroughly and centrifuged at 3000 rpm for 10 min. The alcoholic layer was separated and the colour intensity was read at 465 nm against amyl alcohol blank after 15 min. Sodium phytate standards were run along with the sample. The results were expressed as mg phytic acid/100 g dry wt.

RESULTS AND DISCUSSION

Table I: The mineral composition of *Lathyrus sativus* seeds from Andhra Pradesh in India (LS-AP)

Parameters (%)	Raw seeds	Wet roasted	Boiled	Soaked+boiled
Calcium	98.2±0.16	102±0.23	110±0.56	113±0.66
Iron	5.66±0.13	5.89±0.30	6.15±0.19	6.98±0.09
Copper	0.82±0.02	0.86±0.01	0.89±0.03	0.94±0.02
Zinc	4.31±0.02	4.44±0.02	4.52±0.01	4.68±0.03
Magnesium	126.56±1.26	132.15±1.95	151.23±1.33	170.23±2.13

Table II: The mineral composition of *Lathyrus sativus* seeds from Kerala in India (LS-KE)

Parameters (%)	Raw seeds	Wet roasted	Boiled	Soaked+boiled
Calcium	97.46±0.15	98.02±0.13	99.23±0.10	100±0.09
Iron	5.51±0.10	5.76±0.16	6.09±0.12	6.13±0.11
Copper	0.80±0.01	0.83±0.03	0.87±0.06	0.90±0.05
Zinc	4.26±0.02	4.40±0.01	4.47±0.04	4.51±0.05
Magnesium	120.56±1.26	127.15±1.41	150.23±1.32	161.23±0.06

Table III: The mineral composition of *Lathyrus sativus* seeds from Odisha in India (LS-OD)

Parameters (%)	Raw seeds	Wet roasted	Boiled	Soaked+boiled
Calcium	95.23±0.13	96.14±0.11	97.14±0.16	99.02±0.12
Iron	5.44±0.15	5.67±0.13	6.01±0.12	6.10±0.14
Copper	0.77±0.02	0.80±0.06	0.82±0.03	0.87±0.08
Zinc	4.30±0.03	4.38±0.06	4.42±0.05	4.48±0.06
Magnesium	119.56±1.31	132.15±1.26	149.23±1.41	158.62±1.33

Table IV: The mineral composition of *Lathyrus sativus* seeds from West Bengal in India (LS-WB)

Parameters (%)	Raw seeds	Wet roasted	Boiled	Soaked+boiled
Calcium	94.13±0.15	95.21±0.16	96.35±0.13	98.26±0.11
Iron	5.34±0.11	5.63±0.13	5.98±0.15	6.08±0.12
Copper	0.75±0.01	0.78±0.06	0.81±0.03	0.89±0.04
Zinc	4.35±0.04	4.36±0.01	4.41±0.06	4.46±0.05
Magnesium	117.23±1.46	138.15±1.38	144.23±1.58	158.23±1.69

Table V: The mineral composition of *Lathyrus sativus* seeds from Chattisgarh in India (LS-CH)

Parameters (%)	Raw seeds	Wet roasted	Boiled	Soaked+boiled
Calcium	93.26±0.13	95.01±0.16	97.10±0.13	97.13±0.11
Iron	5.31±0.14	5.49±0.15	5.46±0.12	5.82±0.13
Copper	0.70±0.06	0.72±0.05	0.79±0.03	0.85±0.04
Zinc	4.39±0.02	4.19±0.04	4.47±0.06	4.59±0.07
Magnesium	116.26±1.59	137.16±1.46	145.93±1.39	153.29±1.66

Table VI: The mineral composition of *Lathyrus sativus* seeds from Bihar in India (LS-BI)

Parameters (%)	Raw seeds	Wet roasted	Boiled	Soaked+boiled
Calcium	94.10±0.19	95.59±0.16	97.00±0.12	97.95±0.14
Iron	5.29±0.14	5.51±0.16	5.62±0.13	5.90±0.15
Copper	0.68±0.04	0.70±0.02	0.74±0.03	0.84±0.06
Zinc	4.29±0.03	4.30±0.01	4.32±0.03	4.41±0.02
Magnesium	111.11±1.60	133.00±1.46	142.23±1.51	156.62±1.57

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

Parameters (mg/100gm)	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	982.58±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)

^{***} P<0.001, significance followed 2 way ANOVA, Bonferonni's post test when compared with Raw seeds.

Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body.

Grass pea being an important source of protein and calories, it is also rich in minerals. The seeds have a higher concentration of zinc, magnesium and iron followed by calcium.^[3,10] But the level of neurotoxicity was found to be more by consumption of grass pea as a staple food because of presence of metal chelator like phytic acid. The presence of phytic acid is reported to interfere with mineral element absorption and utilization and also reacts with proteins to form complex products which have an inhibitory effect on peptic digestion.^[5] The pulses have an inherent drawback due to the presence of a metal chelating agent named as phytic acid (PA) limiting absorption of certain essential mineral nutrients such as Fe, Zn and Ca.^[17,18] Inhibitory effect of PA has been shown with respect to retention of Ca, and on the absorption of Zn, where the retention of Ca was at lower molar ratios than for Zn.^[19] High Ca levels in foods can promote the PA induced decrease in Zn bioavailability.^[10]

The initial connection between Zn and neurotoxicity stemmed from the incidence of the disease in soils low or depleted in plant available Zn.^[20] It has been suggested that Zinc deficiency in the soil leads to a greater expression of the neurotoxin in the seeds, thus increasing the toxic hazards from consuming this food.^[11] Loss in calcium content might be due to dehulling as minerals are more concentrated in the testa rather than in the cotyledon.^[21]

In the present investigation the processing methods showed beneficial effect on enhancing the bioavailability of metal micronutrients in grass pea. Firstly to determine the levels of phytic acid, as well as minerals like Ca, Fe, Cu, Zn and Mg. The phytic acid levels were found to be low in LS-AP compared to LS-CH. The increase in phytic acid levels is in the order of LS-AP < LS-KE ≤ LS-OD < LS-WB < LS-BI < LS-CH. The processing methods reduced the content of phytic acid to a great extent. The wet roasted, boiled and soaked+boiled were found to lower the phytic acid levels to the extent of 17.68%, 26.82% and 36.53% respectively. The decrease in phytic acid content may be due to leaching of phytate ions during soaking into the water by concentration gradient. Soaking+boiling were found to further reduce the content of phytates. This might be due to the hydroxylation of phytates as reported by earlier researchers Weaver and Kannan, 2002.^[5]

Traditional processing methods were found to be beneficial for lowering the phytate content and improving the bioavailability of dietary essential minerals in grass pea. There was only an approximate reduction of phytic acid by about 36% in soaked+boiled seeds of *Lathyrus sativus* collected from Andhra Pradesh as shown in table 7. This reduction has been sufficient to increase the bioavailability levels of metal nutrients content of *Lathyrus sativus* in different processing methods used in the present investigation.

Among all the States of India, the mineral analysis of the seeds of *Lathyrus sativus* showed higher amounts of minerals in Andhra Pradesh. In Andhra Pradesh, the soaked+boiled processed seeds showed a better amount of mineral values compared to raw, wet roasted and boiled processed *Lathyrus sativus* seeds. Whereas in Bihar, the values were the lowest as shown in table 1,2,3,4,5 and 6. The seeds have a higher concentration of magnesium followed by calcium. The trace minerals are also significantly high. And these levels were corresponding to the phytic acid levels.

CONCLUSION

The results revealed that the calcium, iron, copper, zinc and magnesium levels in samples from LS-AP were found to be slightly more and lower phytic acid levels than other States and LS-BI was found to have the least values. The processing method of soaking+boiling was found to slightly elevate the free mineral content and lowers the phytic acid levels in the samples. Education to the people on the dangers of consuming improperly processed foods especially legumes which are reported to contain very high concentrations of anti-nutritional

factors is very important. Soaking and other pre-processing methods are essential for better utilization of the legumes in human body.

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