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PROMIMATE COMPOSITION OF THE SEEDS OF LATHYRUS SATIVUS FROM SOME STATES OF INDIA

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ABSTRACT

Lathyrus Sativus L., commonly known as grass pea or as Kesari dal in India has immense potential as a food feed, fodder as well as green forage manure. It is an annual crop widely grown as a pulse and its dried seeds are harvested and consumed as human feed since ancient times. In India, several grass pea germplasm of *Lathyrus sativus L.* are present and, among them the edible seeds of plants grown in some States of Bihar, Chattisgarh, West Bengal, Odissa and Andhra Pradesh are most common for the local cuisine. Since there is no nutritional data available on the L-sativus grown in India, we have gone for investigation of the nutritional composition of these seeds. In the present study, *L. sativus L.*, seed samples collected from the traditional cultivation areas in the country were analysed for characteristics such as crude protein, crude fibre, moisture, fat, total ash, total carbohydrate content and calorific value. Various treatments were given to the seeds of *L. sativus* to know their effect on the proximate composition.

Keywords: *Lathyrus sativus*, grass pea, β-ODAP, neurolatyrism, composition, treatment, pulse protein

1. INTRODUCTION:

Food has played a significant role in the national and international diplomacy [1]. An ever increasing global demand for both food and feed resources and the need to diversify modern cropping systems [2], the legume genus *Lathyrus* in receiving overwhelming attention by academic as well as scientists. There are about 150 species in the genus *Lathyrus* that comprise 15 sections among which grass pea is one. *Lathyrus Sativus L.*, commonly known as grass pea or as Kesari Dal in India, is an annual crop belonging to the Leguminosae or Fabaceae family, Papilionoideae subfamily and Vicieae tribe [3], [4]. Nowadays, grass pea is cultivated for stock-feed as well as human consumption in Asia (Bangladesh, China, India, Nepal and Pakistan) in the Middle East (Iraq, Iran, Afghanistan, Syria and Lebanon) [5], in North Africa (Ethiopia, Egypt, Morocco, Algeria and Libya) [6] and in Southern Europe (France, Spain and Italy) [7].

Grass pea is a much branch sub-erect, straggling or climbing herbaceous winter annual; stems are 0.6-9.0m tall and the leaves are pinnately compound with usually two leaflets (linear-lanceolate 25–150 mm long, 3-9 mm broad). The upper leaflets have modified tendrils. Flowers are solitary, axillary and are borne on peduncles 30-60 mm long; corolla 12-24 mm long, and are reddish-purple, pink, blue or white. Pods are oblong, 2.5-4.00 cm long, flat and slightly curved and each pod has 3-5 seeds that are white, grayish-brown or yellowish and usually spotted or mottled [8]. The grass pea is a valuable crop but neglected and underutilized on that has over the past decade, has bloomed back as a designer crop for adaptability to adverse conditions of both soil and water. That is to say, it can be cultivated in poor soils – heavy clay soils as well as in all soil types – acid, basic or neutral in nature. It can also be well grown in arid factors of drought flood, moderate salinity, extreme climatic environments and altitudinal variations [9]. These plants show abiotic and biotic stress resistance. Additionally, it does not necessitate any irrigation or the use of harmful fertilizers and pesticides. It is also resistant to many insect pests as compared to other legumes [10], [11], [12], [13]. We can neither notice any weeding nor any soil turning on the cultivation of this crop. Moreover, its nitrogen-fixing ability turns the crop into a promising one and in terms of highest productivity among all pulses turns it into the most profitable one. It can be cultivated to about 2.5-3 tonnes per hectare, requiring very little investments on labour and cost. It is also the cheapest amongst all other pulses of its family, too. In fact, the seeds of grass pea have relatively good protein content (rich in lysine) and a high level of polyunsaturated fatty acids [14]. However in common with other grain legumes, these seeds contain a variety of anti-nutritional factors [15]. In particular, β-diaminopropionic acid (β-ODAP), neurotoxic secondary metabolite, is a non-protein amino

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acid which causes neurolathyrism, an irreversible spastic paraparesis (paralysis) of the legs; this pathology appears when this molecule is ingested in large quantities over a three-to-four month period [16]. Neurolathyrism is characterised by nervous disorders such as hyper-irritability, muscular rigidity, weakness and paralysis of the leg muscles and convulsions leaving the patients crippled for life [17], [18]. The various cooking procedures reduce the levels of the proteinaceous anti-nutritional factors and of β-ODAP as well [18]. Grass pea flour is being used to adulterate legume flours such as Bengal gram, chick pea or dry peas at a high rate [19]. Even today, grass pea is cultivated, using essentially traditional techniques and practices, in marginal and remote areas characterized by specific climatic conditions. This renewed interest in its cultivation is very well justified by the attempt to recover the crops belonging to popular tradition with the awareness that the maintenance of biological diversity is the vital key to any future strategy for sustainable production or for agricultural mechanization [20]. However, not much data on proximate composition and anti-nutritional components of *L. sativus* seeds cultivated by farmers in India is available in literature. The availability of nutritional information both to understand the positive effects of consumption of *L. sativus* seeds on human health and also to back their possible production, marketing by rescinding the ban imposed upon it. In this framework, the present study is aimed at evaluating and establishing the proximate composition of local grass pea seeds collected from farmer's fields in some States of India (Bihar, Chattisgarh, West Bengal, Orissa and Andhra Pradesh) to promote their use not only limited to local cuisines but also adding it to the national cuisine, too. In this respect, the total content of proteins, lipids and sugars have been determined along with fibre, ash and moisture contents and the effect of treatments on their proximate composition as well.

2. MATERIALS AND METHODS:

2.1 Plant material and Sample collection:

The grass pea seed samples were procured from local farmers from the States of Bihar, Chattisgarh, West Bengal, Orissa and Andhra Pradesh, each weighing one kilogram in weight and thus, making a total of 5 samples.

2.2 Sample Preparation:

The samples were screened to eliminate defective and poor quality units so as to obtain uniformity and packed in polyethylene bags. For the analysis, samples were cleaned with RO-treated water, drained and gently rubbed dry using a paper towel. These dried seeds were later ground in a laboratory model mill and passed through 60 BSS mesh sieve for obtaining flour of homogenous size. This flour was then transferred into 500 ml. polypropylene bottles (Tarsons Product Pvt. Ltd.), covered with silver foil and stored at freezing temperatures until use.

2.3 Chemical Analysis:

Grass pea samples were analysed for proximate composition by A.O.A.C. (1990) methods [21].

2.3.1 Moisture, ash and crude fibre composition:

Moisture content: 10 g of the sample (pulse powder) was taken in a previously weighed suitable dish and dried in an oven maintained at 100-104 ^{0}C. Cooled the dish in the dessicator and weighed again. The difference in weight was due to loss of moisture. The drying process was repeated until successive weighings differ by less than 0.1% of original mass of the sample [21].

Total ash content: About 4-6 g of dehydrated sample was taken into previously weighed, cleaned, dried porcelain dish and heated over Bunsen flame for charring. Transferred the crucible into a muffle furnace and left at 550 ^{0}C for 4-5 hours until a light gray ash is obtained. If the residue was black, the residue was moistened and repeated the ashing process. The crucibles were cooled in a dessicator and weighed. Total ash was expressed as weight percentage of original food taken. The process was repeated until the difference between two weights was less than 1mg [21].

Crude fibre content: 2 g of dried and powdered food sample was extracted with ether and the residue alongwith 0.5 g asbestos was transferred to the digestion flask for digestion firstly with conc. Sulphuric acid and then with NaOH solution (1.25 g/100 ml) [21]. In between these two treatments the sample is filtered and washed in water till the washings are neutral. After the digestion the residue is washed with 10% potassium sulphate solution, filtered with the Gooch crucible, washed with alcohol, dried and then weighed. The losses in weight for the amount of sample taken in grams X 100 gives percentage crude fiber.

2.3.2 Macronutrient composition:

Total protein content: Protein was estimated by Macro-Kjedahl method [21]. This method involves estimation of total nitrogen content of the foods and converting the percentage of nitrogen to food protein. (% Protein = *f* X % Nitrogen), where *f* is the conversion factor. In brief, transferred carefully 1-2g of test material accurately weighed into the Kjeldahl flask, added 10g of anhydrous sodium sulphate, and 0.2-0.3 g of copper sulphate and 20 ml of conc. Sulphuric acid. Placed the flask in the inclined position and heated for digestion at 425 ^{0}C for about 30 min. to 1 hr. until the mixture becomes both pale green or colorless and clear. Cooled the contents and then diluted by adding 200 ml of water carefully, few pumice stones to prevent bumping and subjected to distillation. The conical flask containing 25 ml of 0.1 N of boric acid solution is placed at the condensor outlet. Dispensed 25 ml alkali (40% NaOH) solution through the wall of the flask so as to form a layer below the acid.

The contents of the flask were mixed by shaking and distilled until whole ammonia has passed over the standard boric acid for about 4 hours. The burner is then shut, detached the flask from the condenser, rinsed the condenser with water into the beaker and washed the dip tube until all traces of the condensate are transferred to the beaker. 2-3 drops of methyl red indicator was added to ammonium borate formed during distillation process and then titrated with either 0.1 M sulphuric acid or hydrochloric acid to purplish-gray end point. Blank determinations were run using all reagents in same quantities but without the food sample material. (% N = 0.14 X A / weight of sample, titration).

Ether extract: The seed meal was defatted by Soxhlet extraction method, which involved extracting of fat from the dried food with minimum exposure to high temperature. A non-polar organic solvent, petroleum ether was used for continuous extraction of fat for about 1 hour. A known weight of food was placed in porous thimble and the extraction solvent placed in a dried, weighed distillation flask.

The solvent was heated, volatilised, collected, condensed which then falls on the porous thimble, where the solvent mixes with food, extracts the fat out and then siphons it back to the original distillation flask. The process was repeated for about 1-2 hours continuously until the whole fat is assumed to be in the solvent in distillation flask. The solvent was evaporated and left over residue weighed and accounted for the fat content [21].

Nitrogen free extract: Total carbohydrate in seed meal was calculated by the difference method by subtracting the sum of the values of moisture, protein, fat, ash and crude fiber (per 100 g) from 100.

2.3.3 Calorific value:

The calorific value of different seed meals was calculated based on the knowledge of composition of the food. The calculations were based on using calorific values of 4 Kcal per gram of protein or carbohydrate and 9 Kcal per gram of fat.

2.4 Effect of treatments on the proximate composition:

In response to the presence of some anti-nutritional factors in grass pea seeds, the following factors were given:

- **Soaking:** 100 g sample soaked overnight (8-9 hrs.) in water under room temperature.
- **Boiling**: 100g sample boiled in sufficient water until the pulse seed is easily pressed soft by hand/spoon/ladle.
- **Soaking prior to 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.
- **Autoclaving:** Sample autoclaved for one hour at 0.1 kg / cm^3.

3. RESULTS and DISCUSSION:

The results of proximate analysis are presented in Table 1.

Table 1: Proximate composition of *Lathyrus sativus* seeds in some States of India

L. Sativus samples / Parameters (%)	Bihar	Chattisgarh	W. Bengal	Orissa	Andhra Pradesh
Moisture	8.38	8.93	10.45	8.47	9.44
Protein	22.79	28.26	25.15	27.43	26.93
Ash	3.31	3.00	3.30	2.29	2.92
Ether extract	1.50	0.68	1.08	1.11	1.25
Fiber	4.95	5.83	15.22	7.31	5.59
Nitrogen free extract	56.27	58.25	61.18	59.86	70.91
Energy (Kcal)	330	342	355	359	403

There is slight significant change in the protein content by area of cultivation. The moisture content of the seeds did not vary much so also the ether extract concentrations from one State to the other. But, the difference in the crude fibre and nitrogen free extract concentrations. However, crude fibre and nitrogen free extract content was significantly different by the area of cultivation of *L. sativus* but in contrast, the moisture, ash and ether extract did not vary significantly by the area of cultivation. Thus, we can say that there is slightly significant change in the moisture, protein and also the calorific values in accordance to the area of cultivation.

The data on the effect of treatments on the proximate composition of the *L. sativus* seeds in various States of India are given in Tables 2,3,4,5 and 6 for Bihar, Chattisgarh, West Bengal, Orissa and Andhra Pradesh respectively.

To give an overall view on the 5 tables (Table 2 - Table 6) depicting the effect of various treatments on the proximate composition of grass pea seeds of various States of India, we can note very clearly that all the tables showed almost similar changes when the treatments were applied onto the samples. That is to say, the moisture content increased considerably in soaked & boiled seeds rather than the soaked only and boiled only seeds but the minimum increase was noticed in autoclaved seeds. The percentage of pulse protein gradually decreased in the treated seeds – the lowest noted in the autoclaved seeds. This could be due to the water-soluble proteins present in the seeds which got leached during the process into the water used for the said treatment. The values of protein for grass pea were as high as 23.50 per cent and even more as reported [22], but as far as soaked, boiled and autoclaved seeds were concerned, the loss of pulse protein content was high. Such results were reported [23]. The ash and ether extract values in raw grass pea seeds were very close to 3.10 % and 1.10 % respectively as described by [24]. The values of ash, ether extract and fiber fluctuated – decreasing in case of soaked samples, increasing in boiled seeds, decreasing a bit in soaked & boiled samples and eventually decreasing in autoclaved samples (for ash and fiber) and increasing in autoclaved

samples (in case of ether extract). The difference in soaked samples might be due to physical errors and the difference in autoclaved ones might be due to the effect of heat on the samples. Nitrogen free extract includes soluble sugars as well as carbohydrates. It is the index for the estimation of the content of carbohydrates in the samples. Generally, the extent of nitrogen free extract depends upon the contribution of all other vital nutrients like protein, fat, ash, fiber, etc. and then subtracting all these from 100. The increase in this factor might be due to the other overall nutrients being lesser in comparison to the raw/untreated samples. So does the energy/calorific values of the treated samples which boosted highest in the autoclaved samples as compared to soaked & boiled, boiled only, soaked only and raw samples. This great difference in the energy values might be due to the levels of pulse protein, fat and carbohydrate changing due to the treatments given to the samples.

4. CONCLUSION:

Although the seeds of *L. sativus* have been consumed for centuries as a pulse, this rich crop should be intensively cultivated in India. There is little information on the nutritional value of this pulse seed as well. The data obtained from this study is a clear indicator to show the high potential of *L. sativus* in the provision of high protein, carbohydrates and minerals for human consumption. This pulse can be consumed in limited quantity without much effect on human health. Moreover, the crop can be called as an eco-friendly crop as it can be cultivated in all ecosystems, reduces soil erosion and enhances soil productivity and quality and also recovery of unproductive fields. Thus, it is high time we unveil the richness of this crop for both environmental as well as human welfare.

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