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Nutritional potentiality of an Underutilized Food legume: Lima bean

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Abstract: Pulses have been used as a staple food for millenniums, termed as power stores of energy. Limited protein supply makes a new protein hunt to minimize protein deficiency. The sources of protein are decreasing with the increased world population. Traditional pulse diets are an excellent source of vegetable protein, vitamins, minerals, and amino acids. Pulses have been used for cosmetic and therapeutic uses for their medicinal properties. The exploitation of underutilized wild legumes is an important approach to combat protein malnutrition in developing countries. Considering the current challenges to food security as a planet exploring the cultivation of new domestic underutilized legumes such as lima beans, Horse gram, and wing bean. Lima beans are commonly called butter beans. Originate in Mexico and spread globally. Two types of Lima bean seeds were collected directly from the fields of Karjan village, Vadodara district, Gujarat state. The collected seeds were screened for physical properties and nutritional components. This study describes the physical properties, nutritional, anti-nutritional components, and utilization of lima bean seeds. This attempt provides up-to-date information on lima beans sourced from various literature databases. The butter beans have numerous vital ingredients that boost our health, development, and immunity. Utilization has been worldwide even though underutilized crops. Underutilized legumes like lima beans could enhance livelihoods in various ways by improving nutrition, guaranteeing food security, establishing new markets, reducing over-dependence on common major beans, stabilizing the ecosystems, and replacing crop systems under stress conditions. There is a huge need for the commercialization of agricultural products such as lima beans to improve food security, and crop productivity and reduce poverty in society.

Keywords: Lima beans. Underutilized legume, Nutritional components, Protein, Anti-nutritional factors.

Introduction: Pulses are an excellent source of dietary protein legumes are grown agriculturally primarily for human consumption and livestock, forage. Limited supplies are deficient with the increased global population Animal protein like eggs, meat, and milk are inadequate to the demand of the world population. This protein deficiency makes the new hunt for alternative sources of protein food, as a substitute source for traditional underutilized vegetable protein crops. They may be restricted to some areas based on their production and consumption and the crop distributed globally (Ebert, A.W., 2014). The under-exploited lesser known native traditional plant possesses a great potential source of food (Ezeagu I.E., Ibegbu M,D., 2010). Several legumes were underutilized viz., lima bean, moth bean, wing bean etc.

Lima bean (*Phaseolus lunatus L*.) belongs to the family Fabaceae, commonly known as sieve bean, double bean, and butter bean, and is grown for its edible seeds. This family is large, containing more than 18,000 species of shrubs, herbs, trees, and climbers. Among all of them, only a few are used in the human diet (Janet Adeyinka Adebo., 2023). Plants are perennial, and annual tropical plants appear like bush and climbing forms. Flowers are zygomorphic, bisexual, and pentamerous. The pods are wide, flat, and slightly curved. The seeds are medium to large-sized white or green beans (Featherstone, S.2016). Seeds vary in terms of color, eye appearance, size, and shape, while their size is usually smaller than that of the common bean (*Phaseolus vulgaris*) (Briya , E, J. et al., 2019; Beyra A., et al., 2004). Domesticated and wild lima beans have been discovered in a wide range of climatic conditions [Baudoin, J.P.et al., 2004; Cerda- Hurtado, I, M., 2018]. Leaves trifoliate with ovate leaflets. Flowers are white or violated (Baudet, J., 1977). Lima beans originated in Mexico and were distributed to America, and Africa and spread globally. It is a minor growing legume globally. The minor legumes are considered to be neglected, underutilized, and less exploited. Butter bean crops flourished well and were resistant to a wide range of soils above pH level 6. Lima beans also restore soil fertility by Nitrogen fixing (Ibeabuchi, J.C, et al.,2017). So many crops are globally neglected at the regional level and national levels. They contribute remarkable food supply during certain periods (Aolemla Pongener and Chitta Ranjan Deb., 2021). The exploitation of underutilized wild legumes is an important approach to combat protein malnutrition in developing countries (Sai kumari D and Dr. Neeti saxena ., 2019). Considering the current challenges to food security as a planet exploring the cultivation of new domestic underutilized legumes such as lima beans could help the over-dependence of such common beans as soybeans, Cowpea, Pigeon pea, etc. Such an over-dependence can have negative ecological, nutritional, and agronomic impacts. As few scientific reviews exist on lima bean nutritional composition and utilization processes. This study provides up-to-date information about this crop and cultivation improvement.

Plate 1: Lima bean plant

In this attempt physicochemical nutritional and biochemical parameters of lima beans viz., moisture content, germination percentage, protein, carbohydrate, free fatty acids, amino acid value, and reducing sugar content were studied. In addition, this study describes nutritional and anti-nutritional, health compositions and utilizations of lima beans and provides up-to-date information on lima beans sourced from various literature databases.

Materials and Methods: Large and small lima bean field seed samples are directly collected from the farmers of Karjan village, Vadodara district, Gujarat state. Screened for nutritive constituents (protein, carbohydrate, free fatty acids, amino acid value, and reducing sugar content) and physicochemical (Germination and moisture content) study were determined as per the standard methods immediately after collecting the seeds from the field (A.O.A.C., 1960).

Physicochemical properties: Germination and Moisture percentages were estimated through standard methods.

i) **Germination percentage:** one hundred seeds were employed to test the germination percentage. Fifteen seeds were placed on filter paper in each sterilized Petri dish. Sterile distilled water was added to the filter papers to moisten them. A total of three replicates were tested each time. Subsequently, the number of seeds germinated was counted and the germination percentage was calculated. A total of three replicates were tested each time.

ii) **Estimation of moisture percentage:** The moisture content of seeds was estimated by the "Dry air oven method; AOAC method" (A.O.A.C.,1960). 10 g of lima bean seedswere taken in the weighing dish of known weight and kept in the hot air oven for 5 hours at 110^0C. Then it was cooled over $CaCl_2$ in a desiccator. The weight of the seedswas taken and this process was repeated till constant weight was obtained. A total of three replicates were tested each time. The moisture percentage was calculated by the following formula.

$$\text{Percentage of Moisture} = \frac{\text{Fresh wt. of Seed} - \text{Dry wt. of seed}}{\text{Dry wt. of the seed}} \times 100$$

II. Nutritional properties:

1. **Protein content:** The protein content of the seeds was determined by Lowry et.al. 1951(Lowry O.H.et al., 1951). 500mg of seed flour of each variety of horse gram separately ground in 10ml distilled water and centrifuged at 1800 rpm for 30 mins. The proteins of the supernatants were precipitated by 30% Trichloroacetic acid (TCA) and centrifuged at 18000rpm for 15 minutes. The sediment thus obtained was washed 2-3 times with distilled water and dissolved in a known volume of 0.1 N NaOH and used for protein estimation. To 1 ml of test solution, 0.5 ml of folin-ciocalteau (FC) reagent and reagent C (4% sodium carbonate,0.2 N sodium hydroxide and 1% Copper sulfate in 5:5:2 ratio) were added. The intensity of the blue color thus developed after incubating for 15 minutes at 37^0 C was read at540nm. the amount of protein was read from a standard curve prepared using bovine albumin and calculated per gram seeds and expressed in mg of protein per gram seed.
2. **Estimation of Free fatty acid content:** the analysis was proposed by the method of Zeleny and Coleman (1938), with slight modification. Each variety of horse gram seeds was taken and ground well with mortar and pistil.10 gm of the seed flour was taken in a 250ml conical flask and 50ml Benzene was to this flour. Then the total content was on a magnetic stirrer for 30 mins and was filtered through Whatmann No.42 filter paper. 10ml of the filtrate was taken in another conical flask.10ml of the phenolphthalein was added to this solution and titrated with standard 0.0178N KOHto a slight pink colour.10ml of Benzene with the alcoholic phenolphthalein served asa blank. The acidity was expressed as mg of KOH required to neutralize 100 gm of flour on a dry weight basis by the formula:

F.A.V = T-B×s/ 100-w×100 Here, F.A.V = Fat

acidity value

T = ml of 0.0178N KOH required to titer

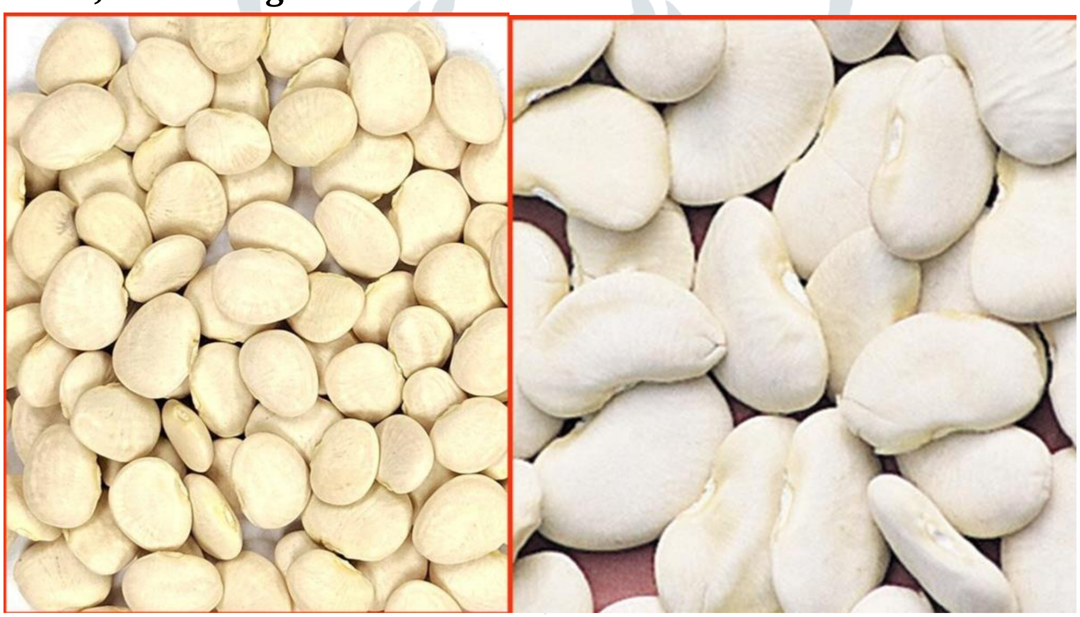
B = ml of 0.0178N KOH required to titer the blankW= G water in 100gm

of the sample.

Extract and Analysis: For the estimation of Total soluble Carbohydrates and free amino acids variety of seed extracts were used. 2gm of dry seeds were ground and extractedwith 10ml of 60% methanol, boiling for 3minutes.This was repeated 3 times, by adding each time 10ml of methanol and boiling for 3 minutes. The whole extract was left aside for 2 hrs. The extract was filtered through Whatman No.42 filter paper and the volume of the filtrate was made up to 5oml (Rambabu, P.,1994)

3. **Estimation of Carbohydrate**: Loewas (1952) employed this method. The estimation of carbohydrates as determined by using anthrone reagent. The readings were taken at 620nm in a spectrophotometer. The glucose standard was maintained as a blank
4. **Estimation of Free Amino acids:** Lee and Takhashi's (1966) method was used to estimate total amino acids. Ninhydrin-citrate- glycerol mixture was used as a reagent for developing color and read at 570nm in a spectrophotometer. Glycine is used as a blank.
5. **Reducing sugars:** Reducing sugars was estimated following the Somagys method (1952) . Copper sulfate and arseno-molbdate reagents were used in this method and the readings were taken at 520m in aspectrophotometer. The glucose standard was maintained for comparison.

Plate 2: A, B Showing two varieties of lima bean seeds



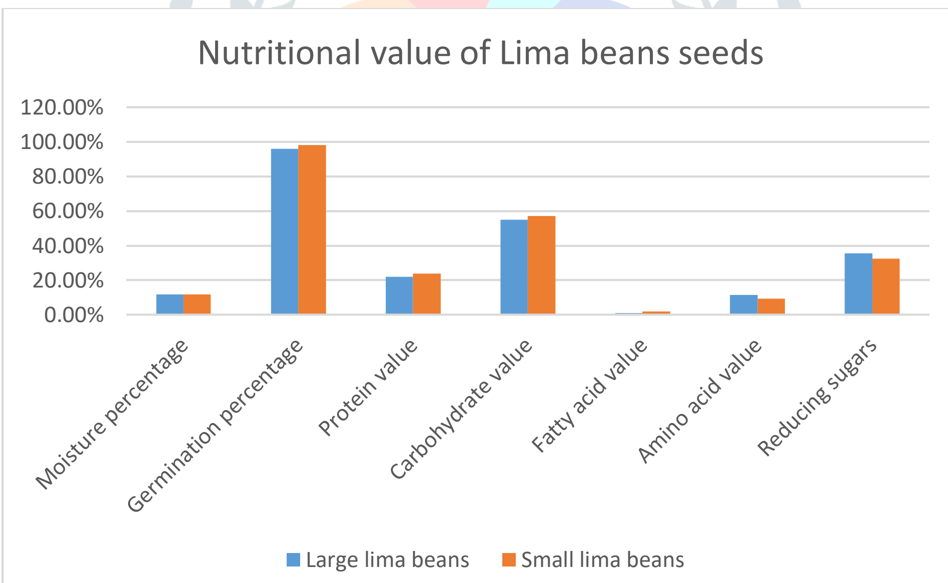
A. Small lima bean seeds B. Large lima bean seeds

Result and Discussion: The nutritional composition of lima beans shows variation based on species and climatic fluctuations directly affect the nutritional value in wild plants and crop plants. 98% in small seeds and 96% in large seeds of the germination was recorded in the collected lima bean seeds. Edna Ursulino Alves et al., 2017 observed 96%of germination in lima bean seeds treated with different concentrations of salt water treatment. 85%of germination was recorded in California lima bean seeds reported by Paul Gepts., 2013.

11.8% in large seeds and 11.7% of moisture content was recorded in the collected lima bean seeds. Aghkhani et al., 2012 reported 8.12 to 29.20%of moisture content recorded in the Christmas lima bean seeds collected from local markets in Tehran, Iran. Giambi., 2001 observed 12.41%, whereas Seidu et al., 2015 reported moisture content in raw lima beans.

Table 1: Seed constituents of two varieties of lima bean seeds.

S. No	Name of the seed	Seed Constituents	Large lima beans	Small lima beans
1	Lima bean	Moisture percentage	11.8%	11.7%
2		Germination percentage	96%	98%
3		Protein value	22%	24%
4		Carbohydrate value	55%	57%
5		Fatty acid value	1%	1.9%
6		Amino acid value	11.47%	9.5%
7		Reducing sugars	35.68%	32.6%



x-axis content name: y-axis: percentage value

The protein content (22%mg) in large seeds and 24% in small seeds was observed in the collected seed samples. Baudoin and Maquet reported 25% of protein content in wild lima beans. Pongener and Deb,2021reported 54%mg/g highest protein content observed in wild lima beans. Lourembam Chanu Bonita et al., 2020 reported 21 -26 % protein values observed in wild and cultivated seeds of *Phaseolus lunata*.55% in large beans and 57%in small beans Carbohydrate content was observed in the two varieties of lima bean seeds. Giami, 2001 observed 53% of the Nigerian lima bean seeds. Seidu et al., 2018 reported 52% of carbohydrates in the field variety of lima beans. Bello -Perez et al., 2007 reveal 37 -38% in the field variety of lima bean seeds in IARI, Nigeria. Palupi et al., 2022 described 41% carbohydrate from Malong, East Jawer Province, Indonesia. In large beans and 1.9%in small beans fatty acid content was observed in the field variety of lima bean seeds. Ryan et al., 2007 found 0.9% total oil fat in a variety of lima beans. Marimuthu et al., 2014 disclose 19 fatty acids through the GC-MS technique in dry lima beans. Lourembam Chanu Bonitha et al., 2020 discovered 0.32-2% fat in lima bean seeds. Farinde et al., 2018 observed 2.97 % fat content in cooked seeds of lima beans. 11.47% in large beans and 9.7%/100mg in small beans Amino acid content was found in the collected lima bean seeds. Whereas Shar-Gohery ., 2021 observed 7.97g/100g amino acid content in Field variety lima bean seeds, Agricultural Research Centre of Giza, Egypt. 6.85% to 7.25g/100g of amino acid content observed in the proteins of lima bean reported by Seidu et al., 2018. 35.68% in large beans and 32.6%in small beans observed in two varieties of field lima beans. Pongener et al., 2021 revealed that 35.68mg of reducing sugar was found in wild beans of *Phaseolus lunata.* The same results were observed in the field variety of *phaseolus lunata* described by Sparvoli et al., 2001. Ishaya et al., 2020 discovered 26.97mg/100g reducing sugars in lima bean seeds. Amino acids like Leucine, Isoleucine, Phenylalanine, arginine, and Methionine were high in lima beans when compared to cowpea and soybean. They help in metabolic activities and mental health improvement (Aletor et al., al 1989; Janet Adeyinka Adebo., 2023). The minerals and vitamins are also high in lima bean seed which acts as cofactors in enzymatic reactions, and metabolic and catabolic reactions such as manganese, Calcium, Phosphorous, Sodium, Magnesium, Copper, and Zinc which are needed in very low concentrations. Piergivonni et, al.2012 stated that the nutritional composition of lima beans shows variation based on species, and climatic fluctuations directly affect the nutritional value of wild plants and crop plants.

Table 2: Nutritional compositions (%) of Lima bean seeds were observed in previous database literature.

S. No	Fat	Protein	Carbohydrate	Fiber	Reference
1	2.19	8.61	72.56	2.00	Adebayo., 2014
2.	1.3-2.3	21.6-24.0	60.0-63.1	-	Bello-Parez et.al,2007
3	3.77	24.07	-	5.10	Chel-Guerrero et,al.2002
4	1.31-1.86	17.53-22.69	50.68-54.92	6.24-7.84	Chukwunyere and Abtew., 2018.
5	0.21	23.17	71.14	18.40	Ezeagu and Ibegbu., 2010
6	3.03	26.02	60.78	7.12	EI-Gohery., 2021
7	2.9-3.05	24.90-25.01	50.44-51.64	1.98-2.07	Fasoyiro et,al.2006
8	2.03	24.98	-	22.72	Granito et.al, 2007
9	2,78-2.94	23.98-24.76	53.17-54.40	5.59-6.09	Gemede and Birhanu., 2020
10	1.51	21.20	68.51	4.20	Iheanacho., 2010
11	2.8	24.6	57.3	6.8	Jayalaxmi et.al,2016
12	1.60-1.62	24.65-25.55	49.42- 52.71	4.98- 5.00	Obiakor.,2009
13	3.12	14.23	57.70	16.50	Ogungbemi et al.2022
14	1.68	22.72	57.31	4.27	Oraka and Okoye., 2017
15	5.93	24.46	54.43	2.51	Oke et.al.2013

Anti-nutritional Factors(ANFs): The lima bean contains anti-nutrients such as Tannins, Thio-glucosides, Trypsin inhibitors, Oxalate, and Cyaninde producing glucosides. They inhibit the absorption of minerals, and nutritive substances and impact digestibility leading to pernicious effects on the consumers. Anti-nutrient factors present in all legumes act as metabolites in the plants and can affect the organism positively or negatively (Bolade et, al. 2017 Awulachu .,2022). Anti-nutritional factors levels of tannin, Phytic acid, oxalate, trypsin inhibitor, and hydrogen cyanide ranges were presented in the table according to the literature. Inadequate levels of anti-nutritional factors can cause deleterious effects (Egbe., 1990, Adegbehingbe.,2014). Cyanide contents that affect respiratory organs, Phytates form kidney stones, some of those factors can cause some detrimental effects on different organs on their functions (Gemede and Ratta., 2014). Inactivation or removal of ANFs will increase the value of food and its nutrition. Roasting, Soaking, De-hulling, Fermentation, and boiling or cooking methods can remove ANFs permanently and increase digestibility, and absorption in the intestine.

Table 3. Anti-nutritional factors (mg/g) of lima beans were reported in the previous database literature.

S. No	Alkaloid	HCN	Heamagglutinin HU/g	Oxalate	Saponin	Tannin	Trypsin inhibitor Activity(Tiu/ML)	Phytic acid	Reference
1	-	-	-	5.42	-	4.99	-	4.15	Adebabayo.,2014
2	-	5.68	-	1.61	15	0.01	-	0.02	Agegbenhingbe2014
3	2.3	-	-	3.22	3.8	0.34	-	24.48	Agegbenhingbe and Daramola.,2019
4	-	0.05	61.4	-	-	0.02	29.3	8.6	Bolade et al.,2017
5	-	-	-	-	-	5.58	4.28mg/g	8.57	EI-Gohery2021
6	-	-	-	0.69	-	-	29.7(Tiu/mg)	0,08	Ezeagu and Ibegbu 2010
7	-	-	-	-	-	59.2-78.9	20.40-26.98(Tiu/mg)	36.7-47.2	Fasoyira et al.2006
8	-	-	-	0.04-0.05	-	0.29-1.16	-	0.91-1.11	Gemede and Birhanu., 202-
9	-	-	-	0.13	0.26	0.1	16.5(Tiu/mg)	3.36	Jayalakshmi et al.2016
10	-	0.10	-	2.27	-	0.72	-	0.66	N'zi et al.2021
11	-	-	-	-	0.45	1.39	-	0.19	Ogungbemiet al., 2022
12	-	-	-	-	-	0.11	-	0.22	Oke et al.2013
13	12.3	-	-	2.7	162.9	9.8	184.1	6.5	Okara and Okoye., 2017
14	-	0.03	-	-	16.84	-	36.07	11.57	Palupi et al. 2022
15	-	0.01	-	-	-	-	-	10.36	Palupi et al.2021

Utilization of Lima bean: Lima bean is consumed in many parts of the world as an underutilized legume a low awareness of the health and nutrition benefits of this crop. Generally, lima beans are planted as a cover crop in between the crops. It has a starchy flavor. Most of the beans were cooked in salads, stews, Cakes, soups, and Casseroles (Sood et, al. 2017). Mostly fresh pods are used as green vegetables, manure, and fodder if dried as a pulse. Usually, they can be cooked, fried, roasted, soaked, fermented, and canned before consumption, and make different types of dishes based on the region. Lima bean starch is mixed with other cereals and makes bread, extruded, and different soups.

The Yoruba tribe in Nigeria processes the seeds into cakes, porridge, and puddings, while the Japanese cook the dry seeds as "nimame"(a boiled bean sweetened with sugar). In Java, lima beans are regularly eaten as a side dish with rice, and the young pods are cooked or steamed (Lim., 2012). The leaves and pods of lima beans are used in traditional medicinal systems. Underutilized legumes like lima beans could enhance livelihoods in various ways by improving nutrition, guaranteeing food security, establishing new markets, reducing over-dependence on common major beans, stabilizing the ecosystems, and replacing crop systems under stress conditions. There is a huge need for the commercialization of agricultural products such as lima beans to improve food security, and crop productivity and reduce poverty in society.

Conclusion: The present study observed the physical properties, nutritional and anti-nutritional factors of lima beans. However, it has anti-nutritional components lima beans have potential nutritious factors that contain high protein content, abundant carbohydrates, and essential amino acids, Various mineral elements that promote our health and development. They are not only used as food but also used in traditional medicinal systems. The commercialization of lima beans into value-added products further improves the livelihood of farmers, and food industries and assists household consumers. It helps in the domestication and diversification of dietary products. This study emphasizes that there is a need for the commercialization of lima beans into value-added products that enhance productivity, and utilization also improves the economy. These underutilized legumes could solve food security problems and stabilize the ecosystem through crop replacements.

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