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Analysis of Soluble Protein Profiles in Selected Sudanese Legumes

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Abstract

This study examines the protein content and protein fractions of six selected Sudanese legumes: *Lupinus albus, Trigonella foenum-graecum, Vicia faba, Lablab purpureus, Phaseolus vulgaris,* and *Cajanus cajan.* The total protein content varied between 20.3% and 42.5%, with Lupinus albus showing the highest value. Protein fractions were dominated by globulins (28.6–50.9%) and albumins (14.5–28.1%), while prolamins (1.6–3.8%) and glutelins (5.6–17.6%) were lower. Globulins were the highest in Lablab purpureus and albumins in Phaseolus vulgaris. Environmental and genetic factors were found to significantly influence the protein content and fractions. These findings offer insights into the nutritional value of Sudanese legumes, supporting their potential role in improving food security.

Keywords: Legume Species, n-hexane Extraction , AOAC Method , Plant Biochemistry , Seed Nutritional Content

Introduction

Legumes are a rich source of plant-based protein, which is essential for human and animal nutrition, especially in regions where access to animal protein is limited. By analyzing the protein content of various legume species, the study provides valuable insights into their potential as high-protein food sources. For instance, Lupinus albus showed the highest protein content at 42.5%, making it a particularly valuable crop for addressing protein deficiency in diets.In addition, understanding the distribution of protein fractions, such as globulins and albumins, is crucial as these fractions influence digestibility, bioavailability, and functionality of proteins. Globulins, which were the predominant protein fraction across the legumes studied, are important for building body tissues and supporting metabolic functions. The high globulin content, especially in Lablab purpureus and Phaseolus vulgaris, suggests that these legumes may offer significant health benefits when included in diets.Furthermore, this research emphasizes the importance of genetic and environmental factors in determining protein content. This variability suggests that through proper cultivation practices and genetic selection, it is possible to enhance the protein content of these legumes even further, increasing their nutritional value.In terms of food security, these legumes offer a sustainable and accessible source of protein that can be cultivated in Sudanese agricultural environments. Their high protein content can help alleviate malnutrition, particularly in rural areas where food diversity and quality are often limited. By promoting the cultivation and consumption of these legumes, policymakers and agricultural stakeholders can contribute to improving food security and reducing dependence on imported or expensive protein sources. Overall, these findings underscore the role of legumes in enhancing dietary quality, providing affordable nutrition, and promoting agricultural sustainability.In the study, the following protein fractions from the selected Sudanese legumes were identified: globulins, albumins, prolamins, and glutelins. Each of these protein fractions can exhibit various therapeutic properties.

1. Globulins

Globulins are the dominant protein fraction in the selected legumes (e.g., Lablab purpureus, Phaseolus vulgaris, and Vicia faba). They are bioactive proteins and play a role in numerous physiological processes:

Globulins can scavenge free radicals, reducing oxidative stress and preventing cell damage. This activity may help in preventing chronic diseases like cardiovascular diseases, cancer, and neurodegenerative conditions. Some globulins function as immunoglobulins (antibodies), which are

vital for immune responses, protecting the body from infections and diseases.Globulins have been shown to reduce cholesterol levels by promoting the excretion of bile acids, which can help in preventing atherosclerosis and heart disease.Globulins can modulate inflammatory pathways, potentially offering benefits in managing inflammatory disorders such as arthritis.

2. Albumins

Albumins were found in significant amounts in species like Phaseolus vulgaris, Cajanus cajan, and Lupinus albus. Albumins have diverse biological and therapeutic properties: Albumins can act as antioxidants, neutralizing harmful free radicals and protecting cells from oxidative damage. They bind to and transport various molecules, including drugs, hormones, and toxins, making them important in detoxification processes.Albumins play a key role in tissue repair and regeneration, supporting faster wound healing. Albumins help regulate osmotic pressure and blood volume, contributing to normal blood pressure levels.

3. Glutelins

Glutelins, found in legumes like Lupinus albus and Lablab purpureus, are plant storage proteins that offer several health benefits: Although glutelins are not as digestible as other protein fractions, they can support gut health by promoting healthy digestion and providing a slow release of amino acids.Glutelins contain essential amino acids required for muscle growth and repair, which is beneficial for maintaining muscle mass and preventing muscle wasting.Glutelins are less allergenic than some other proteins, making them suitable for individuals with certain food allergies.

4. Prolamins

Prolamins are the least abundant protein fraction in the selected legumes, but they have unique properties: Prolamins have been shown to promote gut health by maintaining the integrity of the intestinal barrier, potentially reducing the risk of gut-related conditions like leaky gut syndrome.Some prolamins exhibit antimicrobial properties, which could help in preventing infections by inhibiting the growth of harmful bacteria and fungi.The protein fractions from the legumes studied offer a range of therapeutic benefits: Through cholesterol reduction and antioxidant activity, globulins and albumins can promote heart health. Globulins play a crucial role in boosting the immune system, helping to prevent infections and inflammation. These proteins aid in detoxification, blood pressure regulation, and overall metabolic balance. Glutelins and albumins provide essential amino acids needed for tissue regeneration and muscle maintenance. Prolamins and glutelins support gut integrity and healthy digestion, reducing the risk of digestive disorders.By

incorporating these legumes into diets, individuals may not only benefit from their nutritional value but also harness their therapeutic potential in preventing and managing various health conditions.

Vicia faba L.

Faba bean is a significant protein source in both human and animal diets in the Mediterranean and Sudan. Its protein content ranges between 23% and 38%. Duke reported a protein range of 27.7% to 32.5%, with high nutritional value. Faba bean proteins primarily consist of globulins (79%), albumins (7%), and glutelins (6%). Other studies have found slightly varying compositions of these protein fractions.

Phaseolus vulgaris L.

Kidney bean, a key legume in Europe, has a crude protein content of 22-25%. Studies report values ranging from 24% to 25.9%. Protein fractions include albumins (28.4%), globulins (35-50%), and prolamins (2-5.4%).

Lupinus albus

Lupin seeds have high protein content, ranging from 31% to 45% (Petterson et al., 1997). Globulins make up 40-60%, while albumins and prolamins account for 8-20% and 1.3-5%, respectively (Chango, 1995; Mario et al., 2003).

Lablab purpureus

The crude protein content of this legume ranges from 23% to 47% (Murphy, 1998; Kamatchi et al., 2010). Protein fractions include albumins (31.44%) and globulins (63.32%), with lower levels of prolamins (2.49%) and glutelins (2.75%) (Millward, 2004).

Cajanus cajan L.

Pigeon pea is a key crop in Africa, with protein content between 18% and 25% (Visitpanich et al., 1985; Nwokolo, 1987). ICRISAT has developed high-protein varieties with up to 32.5% protein, enriched with sulfur-containing amino acids (Rao et al., 2002). According to Oloyo (2004), the protein fractions in pigeon pea include prolamins (8.3%), albumins (8.4%), globulins (55.9%), and glutelins (10.2%). Norton et al. (1985) identified globulins as the predominant storage proteins in pigeon pea seeds, making up approximately 70% of the total protein. They were followed by glutelins (10-12%), albumins (10-20%), and prolamins (10-15%).

Trigonella foenum-graecum L.

Fenugreek is noted for its high protein content. Rao et al. (1986) reported a protein level of about 25.5%, while Mir et al. (2004) found it to be 21.7%. Niknam et al. (2003) recorded a protein content of 29.0%, and Petersandor (2004) reported levels between 30% and 33%. Studies on fenugreek's protein fractions by Sauvaire (1984) indicated that globulins constitute approximately 25% and albumins about 20%. Abdelaal and Rahma (1989) found the fractions to be 27.2% globulins, 43.8% albumins, 17.2% glutelins, and 7.4% prolamins, while Kamar (1977) reported an albumin content of around 30%.

Materials and Methods

Collection of Plant Material

Healthy mature seeds from six selected legume species were collected as follows: seeds of Phaseolus vulgaris and Lablab purpureus were sourced from the Shambat Agricultural Research Station, while seeds of Trigonella foenum-graecum, Vicia faba, Cajanus cajan, and Lupinus albus were purchased from the local market in Khartoum. The seeds were thoroughly washed to remove dust and any foreign materials.

Preparation of Seed Flour

The seeds were finely ground using a laboratory mill to pass through a No. 0.008 screen. Each species' seed flour was defatted by extraction with N-hexane at room temperature, with a flour-to-solvent ratio of 10:1. The mixture was stirred for 16 hours using a magnetic shaker to eliminate any residual oil. Subsequently, the flour was filtered through Whatman paper, air-dried, and stored in a cool, dry place at room temperature.

Chemical Analysis of Plant Material

Determination of Crude Protein

The crude protein content was determined following the AOAC (1984) method. The procedure involved the following steps:

A 0.2 g sample was mixed with 0.4 g of a catalyst mixture (potassium sulfate and cupric sulfate in a 10:1 weight ratio) and 7 mL of concentrated nitrogen-free sulfuric acid in a 100 mL Kjeldahl flask. The mixture was digested for two hours, cooled, diluted, and transferred to a distillation apparatus. Then, 15 mL of 40% NaOH solution was added, and the mixture was heated and distilled. Fifty mL of the distillate were collected in a 100

mL conical flask. The ammonia released was absorbed in 10 mL of a 2% boric acid solution containing 3-4 drops of a universal indicator (methyl red and bromocresol green). The trapped ammonia was titrated against 0.02 N HCl. The percentage of protein (g/100 g) was calculated using the following formula to convert nitrogen into protein:

Determination of Water-Soluble Proteins (Albumins)

The albumin protein fraction was determined for each sample based on the method described by Landry and Moureaux (1970). A 2.5 g sample of defatted flour was taken for total protein fractionation. Two volumes of distilled water (50 mL each) were added, and the mixture was shaken for 30 minutes using a mechanical shaker. The solution was then centrifuged for 20 minutes to separate the insoluble components from the liquid. The extraction liquor was brought to a final volume of 100 mL with distilled water. Ten mL of the extraction liquor were set aside for protein estimation using the micro-Kjeldahl method. The percentage of albumin was calculated using the following formula

Determination of Salt-Soluble Proteins (Globulins)

The insoluble residue from the albumin extraction was re-extracted with 50 mL of 0.5 N NaCl solution at a 2:1 volume ratio for 30 minutes with continuous shaking. Afterward, the mixture was centrifuged for 20 minutes to separate the insoluble part. The extracted liquid was collected in a 100 mL volumetric flask, and 10 mL of this liquor was used for estimating soluble proteins via the micro-Kjeldahl method.

Determination of Alcohol-Soluble Proteins (Prolamins)

The insoluble fraction remaining after extracting the salt-soluble proteins was re-extracted with 50 mL of 60% ethanol at a 2:1 volume ratio. The mixture was centrifuged for 20 minutes to separate the insoluble portion. The resulting liquid was collected in a 100 mL volumetric flask, and 10 mL of this liquor were taken for soluble protein estimation using the micro-Kjeldahl method. Total prolamins were calculated using the following equation

Determination of Alkali-Soluble Proteins (Glutelins)

The insoluble residue remaining after prolamin extraction was re-extracted with 50 mL of 0.2% NaOH solution at a 2:1 volume ratio for 30 minutes with continuous shaking. The insoluble material was separated by centrifugation for 20 minutes. The resulting peptized liquor was collected

in a 100 mL volumetric flask, and 10 mL of liquor were set aside for nitrogen determination. The percentage of alkali-soluble proteins was calculated from total proteins as follows:

Results and Discussion :

1. Synonymy and Taxonomy:
2. Each plant has a set of synonyms due to historical classification by different botanists, showing taxonomy evolution as knowledge expanded. For example, Dolichos lablab has undergone several renaming processes as more was learned about the species and its classification. These changes often reflect differences in regional understanding or advancements in botany.
3. Morphological Features:
4. - Growth Habits:
5. - Most species in the text are herbaceous annuals, although some can become short-lived perennials (Lablab purpureus, Cajanus cajan). The growth forms range from trailing vines (Lablab purpureus) to erect shrubs (Cajanus cajan), showing diversity within leguminous species in terms of adaptability to different environments.

Leaves:

- The majority of these plants have trifoliate or pinnate leaves, a common characteristic in legumes. This leaf structure plays a crucial role in photosynthesis and efficient nutrient cycling.
- Inflorescence and Flowers:
- The described plants generally have racemose inflorescences (flower clusters) that bear distinctively colored flowers (white, blue, yellow, purple), serving as both an identifying characteristic and an attraction for pollinators.
- Fruits and Seeds:
- The pods vary in size, shape, and seed count. For instance, Phaseolus vulgaris has cylindrical or flat pods with 4-6 seeds per pod, whereas Vicia faba has leathery pods with up to 8 seeds. Seed size and color also vary, with black, red, or yellow seeds described. The variety in seed and pod morphology reflects adaptations to different environments and ecological roles.

Interpretation:

1. Ecological Importance:

Leguminous plants, including these species, are well-known for nitrogen-fixing abilite -

2. Cultural and Medicinal Use:

Several species in the text (Trigonella foenum-graecum, Lupinus albus) have medicinal value. Trigonella foenum-graecum is traditionally used in herbal medicine for its potential to regulate blood sugar, while Lupinus albus has been explored for its protein content and potential health benefits. This underscores the dual role these plants play in both nutrition and traditional medicine.

3. Agricultural and Environmental Significance:

The cultivation of these leguminous plants supports sustainable agriculture by reducing the need for chemical fertilizers through their nitrogen-fixing capabilities. The fact that some are perennials or can grow in a variety of forms (erect, twining) makes them versatile in different agroecological zones. Moreover, species like Cajanus cajan and Vicia faba are staples in food security, particularly in developing regions.

4. Biodiversity Conservation:

The detailed description of species and their synonyms also emphasizes the importance of conserving plant biodiversity. Maintaining genetic diversity among these species ensures resilience in ecosystems and agricultural systems, particularly as they face pressures from pests, diseases, and changing climate conditions.

The protein contents of six selected leguminous species—Lupinus albus, Trigonella foenum-graecum, Vicia faba, Lablab purpureus, Phaseolus vulgaris, and Cajanus cajan—ranged from 20.3% to 42.5%, reflecting significant variability in protein levels among these species. These species are all commonly known for their agricultural and nutritional value, particularly as protein sources, and the study presents a detailed comparison of their protein content with prior research findings.Diagram (1).

Protein Content Ranking (in descending order):

1. Lupinus albus (42.5%)
2. Trigonella foenum-graecum (35.6%)
3. Vicia faba (33.2%)

4. Lablab purpureus (23.3%)
5. Phaseolus vulgaris (22.4%)
6. Cajanus cajan (20.3%)
1. Lupinus albus L.

Lupinus albus, commonly known as white lupin, showed the highest protein content (42.5%), making it a significant protein source among the species. The protein content agreed with the findings of Petterson et al. (1997) and Hill (1997), reinforcing its reputation as a high-protein legume. However, disagreements with Evans (1985) and Ravindran and Blair (1992) suggest that environmental factors, agricultural practices, and genetic variation could contribute to fluctuating protein levels. Lupin's high protein content makes it especially valuable in livestock feed and human nutrition, where plant-based protein sources are increasingly in demand.

2. Trigonella foenum-graecum L.

Trigonella foenum-graecum (fenugreek) ranked second, with a protein content of 35.6%. This agrees with Petersander et al. (2004) but disagrees with earlier studies like Rao (1989) and Niknam et al. (2003). The variability in protein content across studies could be due to regional differences in cultivation and environmental factors. Fenugreek's high protein content and medicinal properties, such as its anti-inflammatory and anti-diabetic activities, make it a highly versatile crop, valued for both culinary and therapeutic uses.

3. Vicia faba L.

The protein content of Vicia faba (faba bean) was consistent with findings by Hacisefergullari et al. (2006) and Santidrian et al. (1981). However, differences with Eltinay et al. (1989) and Ravindran and Blair (1992) indicate possible discrepancies in growing conditions or bean varieties. Faba beans are widely used in traditional diets and are increasingly promoted as a sustainable protein source in the global market, making consistency in protein content across regions essential for nutrition planning.

4. Lablab purpureus L.

The protein content of Lablab purpureus (hyacinth bean) aligns with studies by Deka and Sakar (1990) and Murphy (1998) but disagrees with Addison (1984). This legume's moderate protein content suggests it plays a supporting role in protein intake compared to higher-protein species, but

it is still valuable due to its adaptability in harsh environments. Lablab is often grown in tropical and subtropical regions, contributing to food security due to its resilience to drought.

5. Phaseolus vulgaris L.

Phaseolus vulgaris (common bean) had a protein content of 22.4%, which agrees with Weder et al. (1981) and Broughton et al. (2003) but differs from Jose et al. (1999) and Marzo et al. (2003). This variation highlights the importance of genetic diversity and environmental conditions. The common bean is a staple food globally and a crucial source of plant-based protein in many developing regions, but the relatively lower protein content compared to lupin or fenugreek suggests it is often used alongside other protein sources.

6. Cajanus cajan L.

Cajanus cajan (pigeon pea) showed the lowest protein content (20.3%) in this study. This result agrees with Vistipanich et al. (1989) and Ravindran and Blair (1992), although it disagreed with Evans (1958). Despite its lower protein content, pigeon pea is a drought-resistant legume and provides a critical protein source in arid and semi-arid regions, contributing to food security and nutritional stability. Its use in traditional and subsistence agriculture makes it a vital crop in resource-poor areas.

Discussion

The protein content variation across species highlights the diverse nutritional roles legumes can play. Lupinus albus, with the highest protein content, stands out as a promising crop for both human and animal nutrition, particularly in regions where protein-rich food sources are scarce. Trigonella foenum-graecum and Vicia faba also contribute significantly to dietary protein, making them valuable components in balanced diets.On the lower end, Cajanus cajan and Phaseolus vulgaris, while still important protein sources, may require supplementation or be consumed alongside other higher-protein crops to meet dietary requirements. Despite this, their resilience and adaptability to various environmental conditions make them important crops for sustainable agriculture.

Comparisons with Literature

The study's findings largely align with previous research but also reveal some discrepancies. These differences could be attributed to:

- Geographical location and growing conditions, which can significantly affect the protein content of legumes.
- Varietal differences: different cultivars of the same species may exhibit variations in protein content.
- Cultivation practices and environmental stresses (such as drought, soil quality, and temperature), which play key roles in nutrient accumulation.

Agronomic improvements and breeding programs could focus on optimizing the protein content of species, particularly those with lower levels like Cajanus cajan and Phaseolus vulgaris, to maximize nutritional contributions in regions where they are staple foods.

Conclusion

The data on protein content provide valuable insights into the nutritional potential of legume species. Each species has a role in providing plant-based protein, whether as a primary source (as in Lupinus albus and Trigonella foenum-graecum) or in combination with other foods. Understanding factors that influence protein content across different studies is crucial for agronomists, nutritionists, and policy-makers to develop strategies for enhancing food security and nutrition through sustainable legume cultivation.

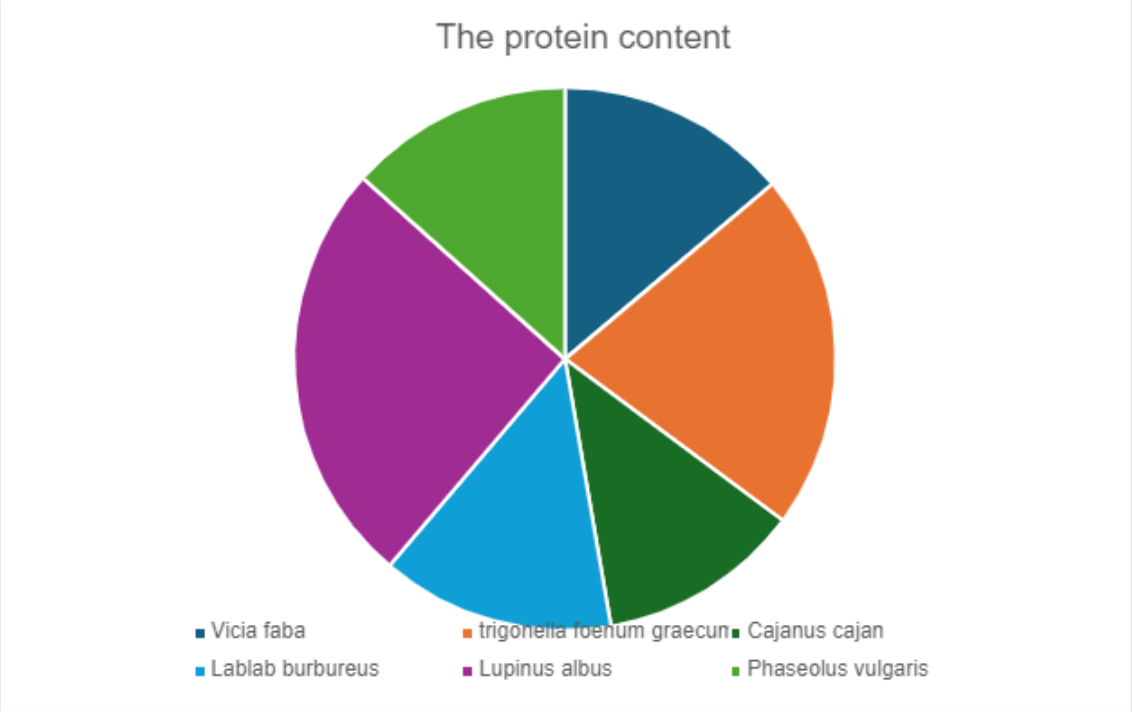


Diagram (1).

Analysis and Interpretation

The study on protein fractions of six legume species shows that globulins and albumins are predominant protein types, with lesser amounts of glutelins and prolamins. These fractions serve as important functional and nutritional components in legumes.

Globulins are the dominant protein fraction, ranging from 28.6% to 50.9%. The highest concentration was observed in Lablab purpureus (50.9%), while Lupinus albus had the lowest (28.6%) Diagram (2).

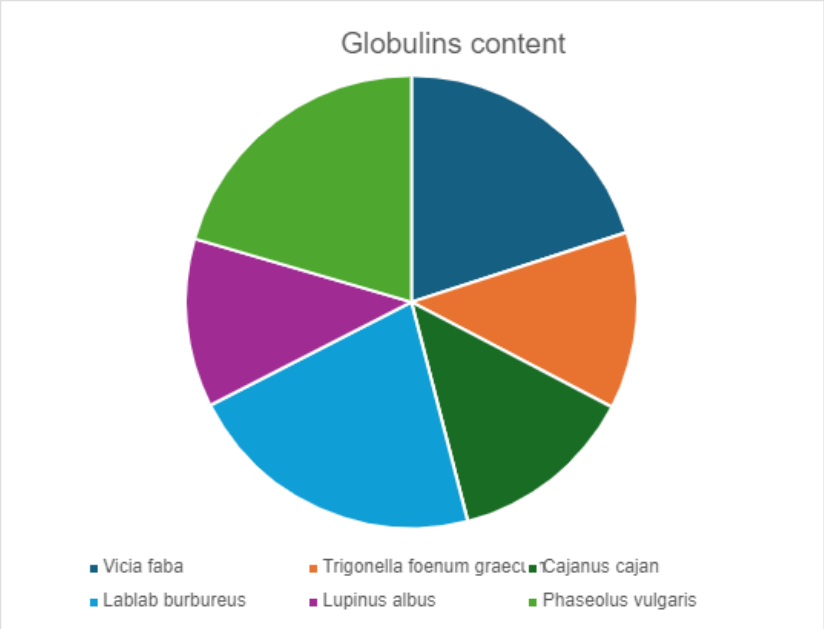


Diagram (2).

Albumins also constitute a significant portion, ranging from 14.5% to 28.1%, with Phaseolus vulgaris having the highest (28.1%) and Trigonella foenum-graecum the lowest (14.5%).Diagram (3).

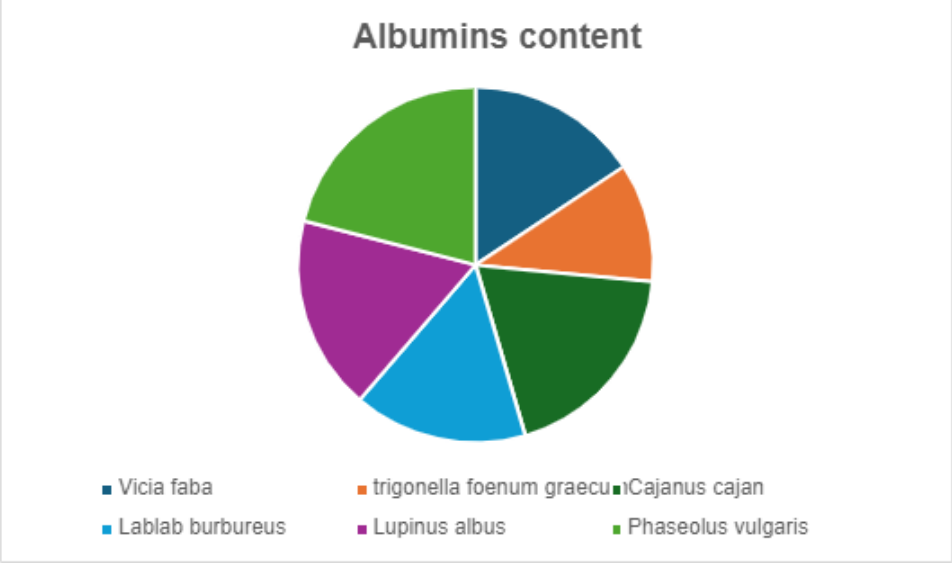


Diagram (3).

Glutelins are present in smaller amounts, with the highest concentration found in Lupinus albus (17.6%) and the lowest in Phaseolus vulgaris (5.6%).Diagram (4).

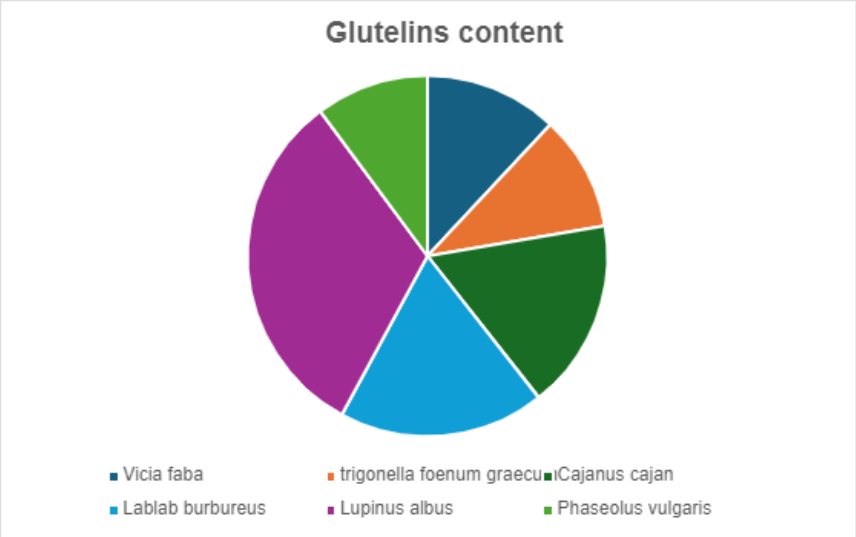


Diagram (4).

Prolamins, the smallest fraction, varied from 1.6% to 3.8%. Cajanus cajan had the highest (3.8%), while Vicia faba and Phaseolus vulgaris had the lowest content (16).Diagram (5).

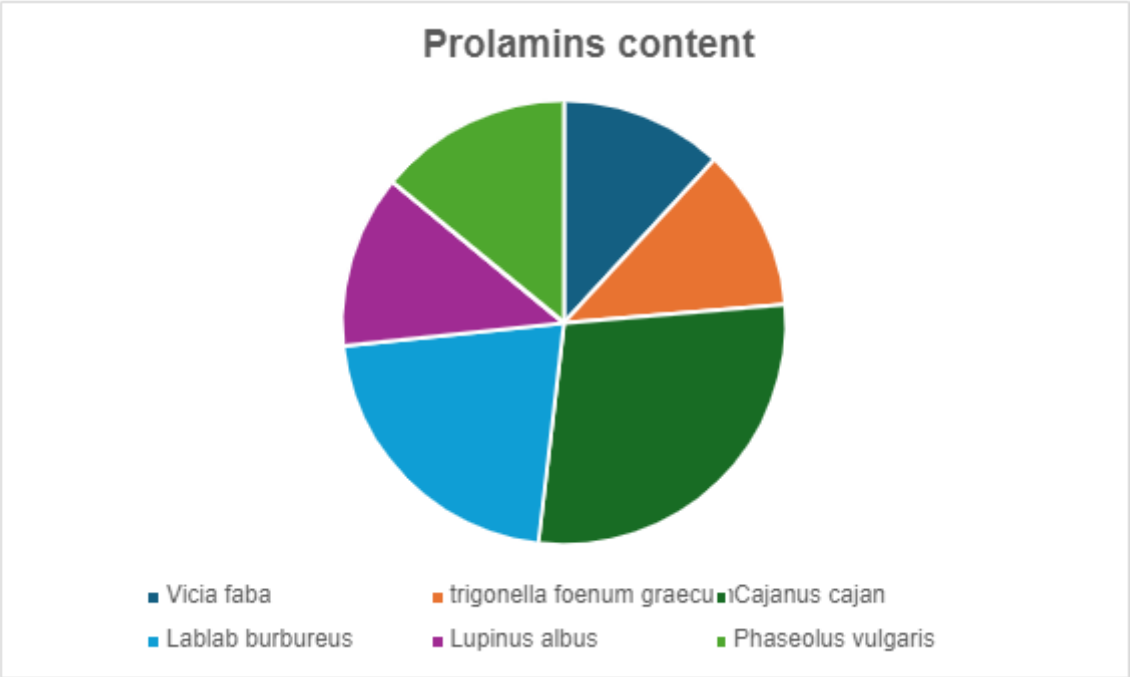


Diagram (5).

The study shows that globulins are the major storage proteins across all species, consistent with findings by Jansman (1996). This suggests that globulins play a critical role in seed storage, germination, and nutritional value for human consumption. The high proportion of albumins also indicates their importance in metabolism and defense mechanisms in plants.

Comparison with Previous Studies

The protein fraction distribution agrees or disagrees with various researchers, indicating variability due to genetic, environmental, and methodological factors:- Vicia faba protein fractions match those reported by Cubero and Moreno (1988), suggesting consistency in globulin and albumin distribution. However, discrepancies with Haciseferogullari et al. (2003) and others highlight possible environmental or genotypic influences.- Phaseolus vulgaris fractions were consistent with Taylor et al. (2008) for globulins and prolamins, and Soral (2005) for globulins and albumins. Discrepancies with other studies may stem from differences in growth conditions or protein extraction techniques.- Lupinus albus shows agreement with Mario et al. (2003) regarding prolamin content but disagrees with Chango (1995), which may indicate different environmental conditions affecting protein synthesis.

Lablab purpureus aligned with Millward (2004) and Kamatchi (2010) for prolamins but differed in other protein fractions, further suggesting environmental or varietal differences.

Cajanus cajan results were consistent with Oloyo (2004) for glutelins but diverged for other fractions, which might be influenced by genetic variability or cultivation practices.

Trigonella foenum-graecum results conflicted with previous studies (Sauvaire, 1984; Kamar, 1977), indicating that genetic diversity or differences in cultivation environments play a major role.

Factors Influencing Protein Content and Fractions

The variation in protein content and fractions among the species can be attributed to both genetic and environmental factors, as suggested by Swanson (1990) and Owusu-Ansah and Muccurdy (1991). Factors such as temperature, water availability, and disease are critical:

1. Environmental conditions like temperature (Karjalainen and Korel, 1987) and water stress (Foroud et al., 1993) influence protein synthesis and accumulation. For example, high temperatures during flowering and seed filling increase the protein content through their effect on nitrogen metabolism.
2. Genotypic variation also plays a role. Studies by Mathews and Arthur (1985) and Burstin et al. (2007) emphasize the significant influence of genetic makeup on legume seed protein content.
3. Diseases (Garry et al., 1996) could negatively impact protein content by altering the nitrogen-carbon ratio in the seed, further complicating the variability in protein composition.
4. Phosphoenol pyruvate carboxylase over-expression, as reported by Saxena et al. (2002), can enhance seed protein content, suggesting potential for biotechnological interventions to improve legume protein yield.

Discussion and Implications

The results emphasize the importance of understanding both genetic and environmental factors in protein fraction variation among legume species. Given that legumes are a crucial source of plant-based proteins, the dominance of globulins highlights their nutritional and functional significance in food processing, with potential applications in the development of protein-rich foods and supplements.

Furthermore, the variability in albumins, glutelins, and prolamins suggests that different legumes can be targeted for specific nutritional or industrial applications based on their protein composition. For instance, the high globulin content in Lablab purpureus and Phaseolus vulgaris could make these species ideal for developing protein isolates, while species like Lupinus albus may offer benefits due to their higher glutelin content.

The study also points to the need for continued research into genetic and environmental factors that influence protein composition. Understanding these influences could allow for the development of more resilient legume varieties with enhanced protein content, particularly in the context of climate change and food security.

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