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Micronutrient Dynamics in Green Crop Processing: Profiling Mineral Retention in LPC and PCR from Tropical Legumes

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ABSTRACT

This study investigated the mineral retention efficiency of green crop fractionation (GCF) in producing leaf protein concentrate (LPC) for human nutrition and pressed crop residue (PCR) for animal feed. A study was conducted to determine the levels of magnesium (Mg), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) in four leguminous plants: *Vigna unguiculata* L., *Lablab purpureus* L., *Cajanus cajan* L., and *Vigna radiata* L. Results indicated superior iron retention in LPC, particularly from *Vigna radiata* (L.) (7.45 mg/g). Significant iron content was also observed in PCR, with *Vigna radiata* (L.) PCR retaining 5.55 mg/g. Copper distribution in LPC was relatively uniform across species (mean = 0.70 ± 0.06 mg/g), while its concentration in PCR varied, notably high in *Lablab purpureus* (L.) (0.87 mg/g). Zinc and magnesium generally exhibited higher retention in LPC compared to PCR. These findings highlight *Vigna radiata* (L.) as a promising candidate for iron biofortification and underscore species-specific mineral partitioning between LPC and PCR. Such insights are important when creating strategies to combat micronutrient malnutrition and optimize the utilization of agricultural by-products.

INTRODUCTION

Protein and micronutrient deficiencies represent significant global health challenges, affecting over 2 billion people worldwide. In response, Pirie (1960, 1971, 1978) developed the green crop fractionation (GCF) process, a method designed to enhance the efficient utilization of plant nutrients and address widespread protein and mineral insufficiency. The GCF process involves pressing a pulp derived from freshly harvested green leaves. According to Jadhav and Mungikar (2001), the resultant juice produces leaf protein concentration (LPC) when heated beyond 90°C, which promotes protein coagulation. The fibrous residue, known as pressed crop residue (PCR), can be utilized as animal feed, particularly when ensiled (Mungikar and Joshi, 1976). LPC is recognized as a nutrient-dense product, rich in protein, minerals, and vitamins, making it a valuable component for human diets. Micronutrient malnutrition, including iron deficiency anemia and zinc deficiency, remains prevalent. Legumes, being affordable and nutrient-dense staples, offer a sustainable approach to mitigating these deficiencies. Compared to cowpea *Vigna unguiculata* (L.) cowpea and *Cajanus cajan* (L.) pigeon pea, which have previously been well studied, green gram (*Vigna radiata* (L.) and (*Lablab purpureus* (L.) Dolichos deserve greater investigation due to their high mineral bioavailability. Humans require a balanced intake of macro- and micronutrients for essential physiological processes (Berdanier et al., 2016). Nutritionally enhanced plant varieties provide a cost-effective means of delivering bioavailable nutrients to both urban and rural populations, with protein and mineral consumption being the most economical strategy to combat widespread malnutrition in sub-Saharan Africa (SSA). In South Africa, deficiencies in vitamin A and zinc contribute significantly to "hidden hunger," impacting public health and contributing to imbalanced body weight and growth. Consequently, improving the nutritional value of cowpeas is critical for reducing these deficiencies. Mineral elements such as iron, zinc, and manganese are commonly supplemented in cereal-based diets in South Africa to support pregnant women and children suffering from micronutrient deficiencies (Schönfeldt and Gibson, 2009). Additionally, the country's smallholder farmers mostly depend on grain, immature pods, and cowpea leaves to satisfy their protein and mineral needs (Belane and Dakora 2012; Gerrano et al. 2015). LPC, notably, contains high-quality protein comparable to animal products, possessing essential amino acids and reduced allergenicity, thus accommodating diverse dietary needs (Singh et al., 2014; Balfany et al., 2023). It is also abundant in micronutrients, including vitamins A, B6, B9, E, and K, and essential minerals like iron, zinc, and magnesium, which are vital for addressing nutritional deficiencies (Davys et al., 2010). This study aims to determine species-specific nutritional profiles, assess and contrast the mineral content of *Lablab purpureus*, *Vigna radiata, Cajanus cajan,* and *Vigna unguiculata* evaluate their potential contributions to recommended dietary allowances (RDAs), and propose strategies for enhancing mineral retention through agronomic practices.

Materials and Methods

Plant Collection and Preparation:

Mature leaves from four leguminous plants-*Vigna unguiculata* L (Cowpea), *Vigna radiata* L. (Mung bean), *Cajanus cajan* L. (Tur) and *Lablab purpureus* L. (Dolichos) were harvested from a farm in Baramati and Undvadi village. The leaves were pulped and subjected to mechanical pressing to extract leaf juice. Leaf protein concentrate (LPC) was obtained from the extracted juice through heat-induced coagulation, as outlined in the protocol established by Reddy and Mungikar (1998). The resulting pulp, pressed crop residue (PCR), and LPC were oven-dried at 60 °C, ground into a fine powder, and stored in polyethylene bags for subsequent mineral analysis. The dry matter content (g/kg fresh weight) of the plant material was also recorded, along with the yields of PCR and LPC.

Mineral Analysis

The concentrations of copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn) in the prepared samples were quantitatively determined using atomic absorption spectroscopy (AAS).

Sample Preparation for AAS

1. Preparation of Analytical Sample:

A representative portion of each homogenized sample was prepared by either fine grinding or creating a slurry using deionized water. For certain sample types, such as fortified milk powders and infant cereals, deionized water was preheated to approximately 50 °C to facilitate homogenization. Exactly 10.0 ± 0.1 grams of the homogenized sample were accurately weighed and placed into a 100 mL Erlenmeyer flask, followed by the addition of 90.0 ± 0.1 grams of deionized water. The contents were then securely stoppered and vigorously mixed to ensure a uniform suspension suitable for analysis.

2. Test Aliquot Preparation for Digestion:

After weighing, an accurately measured test aliquot of 0.50 ± 0.01 grams of the prepared sample (or an equivalent amount based on dry weight) was transferred into a microwave digestion (MDC) vessel. For procedures involving microwave digestion optimized (MDO) methods, 1.00 ± 0.01 grams of the sample were placed in a 100 mL volumetric flask. Optimal sample sizes of 0.5 g for MDC and 1.0 g for MDO were empirically selected to ensure consistent energy release (~3 kcal) from the food matrix and to maintain nutrient recovery rates within the 90–110% efficiency range. To prevent sample adhesion during transfer, weighing paper was used inside MDC vessels or, alternatively, a Pasteur pipette was employed for accurate liquid transfer. Liquid samples were pipetted directly into the digestion vessel immediately after homogenization. In all cases, the test aliquot was weighed immediately after preparation.

3. Acid Digestion

For mineral extraction, the prepared MDC or MDO vessels were carefully filled with 5.0 ± 0.1 mL of concentrated nitric acid (HNO_3). In the case of MDO vessels, an additional 5.0 mL of hydrogen peroxide (H_2O_2) was added to aid in oxidation and matrix breakdown. The vessels were loosely capped (not tightly sealed) and allowed to pre-digest at room temperature for at least 10 minutes, or until vigorous foaming subsided, indicating initial decomposition of organic matter. After pre-digestion, the vessels were securely sealed and placed on a microwave digestion platform, ensuring uniform microwave energy distribution across all samples for complete digestion. Note (for food-grade salt analysis): Approximately 0.20 ± 0.01 grams of salt were weighed into a 100 mL volumetric flask and dissolved in deionized water. To continue the procedure, 10 mL of concentrated nitric acid was subsequently added. The solution was then made up to volume with deionized water, ensuring a minimum dilution factor of 500 to guarantee complete dissolution and appropriate matrix compatibility for AAS.

Results

Dry Matter Content and Extraction Efficiency table 1 summarizes the dry matter content (g/kg fresh weight) of pulp, pressed crop residue (PCR), and leaf protein concentrate (LPC) obtained from the four studied leguminous species. Among them, *Vigna radiata* exhibited the highest pulp dry matter content (1437 ± 21.37 g/kg), whereas *Cajanus cajan* recorded the lowest (1056 ± 61.22 g/kg). Notably, both *Vigna radiata* (L.) and *Vigna unguiculata* (L.) yielded significantly higher dry matter in pulp compared to *Lablab purpureus (*L.) and *Cajanus cajan (L.*), reflecting distinct species-specific biomass efficiencies. These variations underscore the potential of certain legumes, particularly *Vigna* species, for enhanced green biomass utilization and fractionation efficiency in protein extraction systems. Yields of Pressed Crop Residue (PCR) and Leaf Protein Concentrate (LPC) Among the four leguminous species, *Vigna radiata* (L.) produced the highest dry matter yield from pressed crop residue (447 ± 84.33 g/kg), while *Lablab purpureus (*L.) yielded the lowest (376.6 ± 30.5 g/kg). A high standard deviation in *Vigna radiata (L.)* PCR reflects significant variability in residue yield, likely caused by sample diversity or inconsistencies in the extraction process. In terms of LPC yield, *Vigna radiata (L.)* again led with the highest value (24.33 ± 0.57 g/kg), indicating its superior potential for protein concentrate production. Notably, *Lablab purpureus* (L.) showed the minimum LPC yield, amounting to 20.66 ± 1.15 g/kg. Particularly, *Vigna radiata (L.)* also exhibited the lowest standard deviation in LPC yield, reflecting a highly consistent performance with a variability of ±1.0 g/kg. In contrast, *Cajanus cajan* (L.) reflected the most inconsistent extraction performance, likely due to process inefficiencies or sample diversity. These findings highlight *Vigna radiata* (L.) as a robust candidate for reliable and efficient biomass processing in green protein extraction systems.

Table 1. Dry matter yields of pulp, Pressed Crop Residue (PCR), and Leaf Protein Concentrate (LPC) from Four Leguminous Plants

Sr. No.	Plant Species	Dry matter content (g/kg fresh weight)		
		PCR	LPC	Pulp
1	*Vigna unguiculata*	404.3 ± 7	21.66 ± 1.52	1375.3± 55
2	*Lablab purpureus*	376.6 ± 30.5	20.66 ± 1.15	1081.6±47.08
3	*Cajanus cajan*	382.6 ±30.35	22 ± 1	1056 ± 61.22
4	*Vigna radiata*	447 ± 84.33	24.33 ± 0.57	1437 ± 21.37

Note: Data represent mean ± standard deviation (SD) of triplicate measurements. Values are expressed as g/kg fresh weight. LPC: Leaf Protein Concentrate; PCR: Pressed Crop Residue.

Mineral Composition of Leaf Protein Concentrate (LPC) As shown in Table 2 and Figure 1, the mineral composition (mg/g dry weight) of LPC derived from the four leguminous species reveals considerable interspecies variation in micronutrient retention during protein extraction. Magnesium content was highest in *Vigna unguiculata* (L.) (0.65 mg/g), suggesting strong mineral affinity within its protein matrix, while *Lablab purpureus* (L.) exhibited the lowest magnesium concentration (0.23 mg/g). In the case of manganese, *Cajanus cajan* LPC displayed the highest value (0.67 mg/g), whereas *Vigna radiata* (L.) had the lowest (0.34 mg/g), indicating species-specific uptake or binding tendencies during coagulation. *Vigna radiata* (L.) LPC stood out for its exceptionally high iron content (7.45 mg/g), making it a strong candidate for iron biofortification. However, iron concentrations across species were notably variable, suggesting differing efficiencies in iron retention during processing. Zinc levels ranged from 0.15 mg/g in *Vigna unguiculata* (L.) to 0.32 mg/g in *Vigna radiata* (L.), further reinforcing the latter's advantage in multi-micronutrient retention.

Table 2. Mineral Composition of Leaf Protein Concentrates (LPC) from Four Leguminous Species

Plant Species	Minerals(mg/g)				
	Magnesium	Manganese	Iron	Copper	Zinc

Vigna unguiculata	0.65	0.45	1.77	0.67	0.15
Lablab purpureus	0.23	0.56	0	0.67	0.27
Cajanus cajan	0.56	0.67	0.05	0.67	0.17
Vigna radiata	0.38	0.34	7.75	0.78	0.32

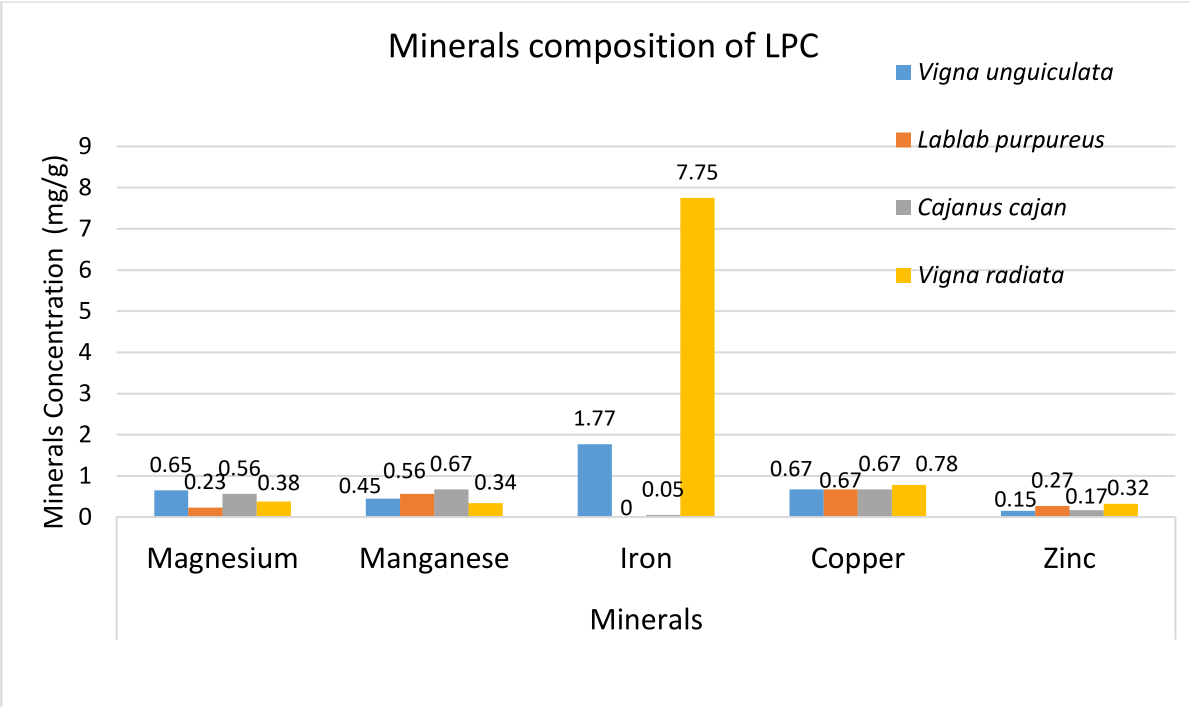

Figure 1: Mineral composition of leaf protein concentration from four leguminous plants

The mineral profile of PCR from four leguminous plants, as detailed in Table 3 and revealed in Figure 2, shows distinct species-specific patterns in micronutrient retention (mg/g dry weight). *Vigna unguiculata* (L.) exhibited the highest magnesium concentration (0.37 mg/g), while *Vigna radiata* (L.) contained the lowest (0.18 mg/g), indicating variation in magnesium translocation or retention within plant tissues. For manganese, *Lablab purpureus* (L.) PCR showed the highest concentration (0.47 mg/g), contrasting sharply with the lowest level in *Vigna radiata* (L.) (0.18 mg/g), reflecting differential accumulation capacities among species. Notably, *Vigna radiata* recorded the highest iron content in PCR (5.55 mg/g), suggesting its strong potential for iron recovery from fibrous residue. Iron content data for *Lablab purpureus* (L.) PCR were not available (NA), limiting comparative assessment for this species. Copper levels were especially elevated in *Lablab purpureus* (0.87 mg/g), making it a notable source of this trace element in residual biomass. In contrast, *Cajanus cajan* (L.) PCR showed the lowest copper concentration (0.23 mg/g). For zinc, concentrations ranged from 0.13 mg/g in *Cajanus cajan* (L.) to 0.26 mg/g in *Lablab purpureus* (L.), highlighting moderate but species-dependent variation.

Table 3. Mineral Composition of Pressed Crop Residue (PCR) from Four Leguminous Species

Plant Species	**Minerals(mg/g)**				
	Magnesium	**Manganese**	**Iron**	**Copper**	**Zinc**
Vigna unguiculata	0.37	0.45	0.89	0.28	0.15
Lablab purpureus	0.27	0.47	NA	1.03	5.55
Cajanus cajan	0.2	5.55	1.03	0.23	0.13
Vigna radiata	0.18	0.18	5.55	0.48	0.21

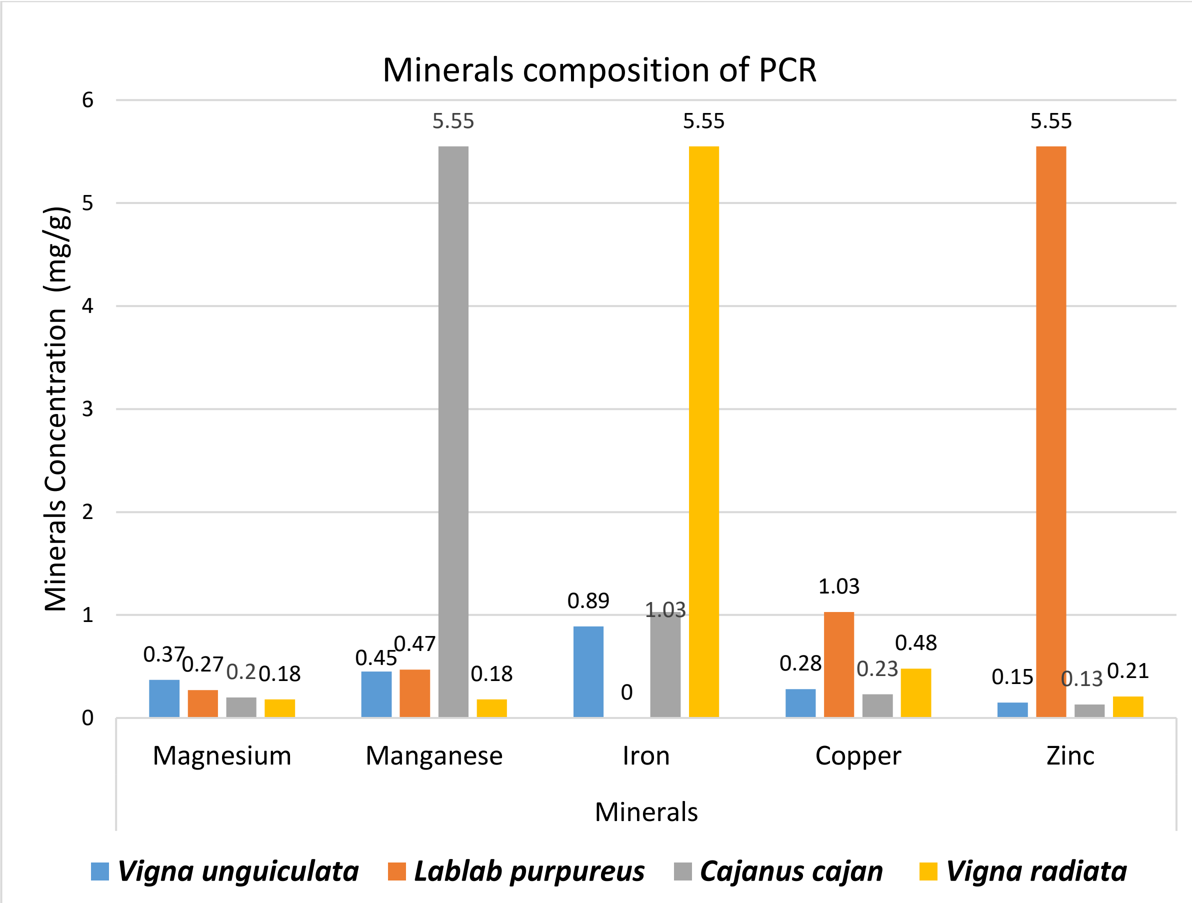

Figure 2: Mineral Composition of Pressed Crop Residue from Four Leguminous Plants

CONCLUSION

This study highlights distinct species-specific variations in dry matter yield and mineral retention among four leguminous plants, underscoring their potential for sustainable protein and feed applications. *Vigna radiata* (L.) excelled in both biomass yield and iron-zinc enrichment, making it ideal for human dietary use. *Vigna unguiculata* (L.) showed high magnesium levels, suitable for mineral-rich biomass processing. Despite lower yields, *Lablab purpureus* (L.) exhibited high copper and manganese in PCR, valuable for livestock feed. *Cajanus cajan*, (L.) though less productive, showed promise for manganese-focused applications. These findings support species-specific strategies to optimize green biomass for nutritional and agro-industrial purposes.

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