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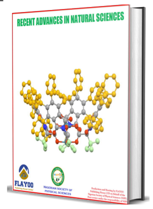

Mineral composition and Mineral Safety Index assessment of protein fractions: A comparative study of five legume varieties

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ABSTRACT

This study evaluated mineral concentrations, Recommended Dietary Allowance contributions, and the Mineral Safety Index (MSI) of five underutilized legume varieties at three processing stages: defatted flour (DF), protein concentrate (PC), and protein isolate (PI). Potassium was the predominant macro-mineral and increased from 654.02–804.61 mg/100 g in DF to 1216.55–1352.50 mg/100 g in PI. Calcium, phosphorus, iron, zinc, and copper also increased during isolation, reaching 654.81–705.05, 317.65–492.64, 9.94–18.95, 12.06–24.67, and 7.88–9.78 mg/100 g, respectively, in PI. In contrast, manganese decreased from 1.02–2.14 mg/100 g in DF to 0.02–0.25 mg/100 g in PI, indicating loss of soluble, loosely bound forms during aqueous fractionation. Sodium increased moderately to 280.50–291.99 mg/100 g in PI. A 100 g portion of PI provided up to 52% of the adult potassium adequate intake, 70% of the calcium Recommended Dietary Allowance, and 70% of the phosphorus Recommended Dietary Allowance. The Na/K ratio remained favourable (0.17–0.25), while the Ca/P ratio reached 2.063 in PI. MSI assessment indicated wide safety margins for DF and PC, whereas trace-element accumulation narrowed the margin in PI, particularly for copper. These legume fractions are nutrient-dense ingredients, but PI inclusion levels require careful portion control, especially in paediatric formulations.

1. INTRODUCTION

Driven by the prohibitive costs and environmental footprint of animal-based diets, prioritizing indigenous, underutilized legumes represents a critical strategy for sustainable food production [1–3]. As population pressures intensify toward 2050, the transition to plant-derived proteins is no longer merely a dietary trend but an immediate requirement for stabilizing domestic food security against global supply volatility [4, 5]. Within local agricultural systems, underutilized varieties of common beans are primary candidates for regional food fortification because of their high nutritional density, nitrogen-fixing capabilities, and adaptability to local cultivation practices [6]. In sub-Saharan Africa, particularly North-Central Nigeria, legumes are vital dietary staples. However, their wider application is often hindered by a complex matrix of starch, dietary fibre, and antinutritional factors [7]. These components can interfere with the bioavailability of essential minerals and cause undesirable physiological effects, such as flatulence associated with fermentable

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oligosaccharides.

Systematic processing is required to unlock the full industrial and nutritional potential of these crops. This study focuses on five varieties of major underutilized Nigerian legumes processed into defatted flour (DF), protein concentrate (PC), and protein isolate (PI). Industrial processing, particularly isoelectric precipitation, does not merely concentrate protein; it also reconfigures the mineral matrix. Although the proximate and amino acid profiles of these beans are relatively well documented, data on the Mineral Safety Index (MSI) of the resulting fractions remain limited [8, 9]. The MSI is a toxicological tool that compares observed mineral intake with the Tolerable Upper Intake Level (UL). Determining whether refining maintains a wide safety buffer or shifts mineral concentrations toward toxic thresholds is essential for food safety, particularly in medical nutrition. Comparing the properties across processing stages may provide a scientific basis for converting underutilized Nigerian beans into high-purity, clean-label ingredients. Such high-potency materials could support the development of infant formulas and meat analogues, reduce reliance on imported proteins, and enhance the economic value of local agricultural output [10]. Accordingly, this study comparatively assesses five varieties, with particular emphasis on mineral safety thresholds during the transition from DF to PC and PI.

2. MATERIALS AND METHODS

2.1. COLLECTION AND IDENTIFICATION OF SAMPLES

Five varieties of underutilized legumes (Adzuki bean, Alava pinto bean, Black pinto bean, Honey bean, and Red kidney bean) were obtained from local farmers in Rukuba, Jos North, Plateau State, Nigeria, and identified at the International Institute of Tropical Agriculture (IITA), Ibadan. The dried seeds were sorted and cleaned to remove extraneous matter (stones, chaff, broken seeds, and insects), soaked in water, and dehulled. The dehulled beans were oven-dried at 60 °C for 24 h in an air oven (Gallenkamp, UK), milled using an attrition mill, and passed through a 75 μm mesh to obtain fine flour. The flour was defatted in a Soxhlet apparatus for 4 h using hexane. The defatted samples were dried and pulverized to a powder, packaged in low-density polyethylene bags, sealed, and refrigerated until analysis.

2.2. PREPARATION OF PROTEIN CONCENTRATES AND ISOLATES

Protein concentrates and protein isolates were prepared according to the process schemes shown in Figures 1 and 2, respectively [11].

2.3. MINERAL ANALYSIS

The mineral profile was determined after dry-ashing the samples in a muffle furnace at 500 °C to constant weight. The resulting white ash was digested by boiling with 5 mL of concentrated HCl, filtered, and quantitatively transferred to a volumetric flask. The solution was made up to volume with deionized water to maintain a uniform 0.1 M HCl matrix. Procedural reagent blanks were processed concurrently with all sample sets to zero the instruments and subtract background signals. Sodium and potassium concentrations were quantified by flame photometry (Model 405, Corning, UK) against standard curves (R2 $\geq$ 0.999) generated from analytical-grade NaCl and KCl standards. Phosphorus was determined colorimetrically using the vanadomolybdate method [12].

Calcium and magnesium were determined using an atomic absorption spectrophotometer (Perkin-Elmer Model 403, Norwalk, CT, USA) equipped with element-specific hollow-cathode lamps under optimized air-acetylene flame conditions. Lanthanum chloride (0.5 % w/v) was incorporated into all sample and working-standard matrices used for Ca and Mg analysis to eliminate chemical interference from refractory phosphate complexes. Instrumental detection limits for the target transition elements were established according to the Techtron protocol [13] and ranged from 0.008–0.05 mg/L. The optimum analytical calibration range was 0.1–0.5 absorbance units, with strict linearity maintained.

Accuracy and quality-control validation were assessed using a certified reference material (CRM; NIST SRM 1573a tomato leaves) and matrix spike-recovery tests, which yielded recoveries of 96.5–102.2 %. All measurements were performed in triplicate (n = 3), with coefficients of variation below 2.50 %, and the results were expressed as mg/100 g of sample dry weight. The calculated MSI for the target elements was determined to assess the dietary safety margin of the legume protein fractions. Values were computed relative to toxicological maximum thresholds according to the method of Hathcock [14]:

$$\text{Calculated MSI} = \frac{\text{Tolerable Upper Intake Level (UL)}}{\text{Mineral content per serving}} \times 100, \qquad (1)$$

where the Tolerable Upper Intake Level (UL) is the maximum daily nutrient intake unlikely to pose a risk of adverse health effects, and mineral content per serving is calculated using standard industry or demographic serving sizes. An MSI value approaching or equal to 100 indicates that a single serving provides the entire safe physiological threshold.

2.4. STATISTICAL ANALYSIS

To evaluate the simultaneous effects of genetic variation and technological processing, the experimental data were subjected to two-way analysis of variance (ANOVA). The statistical model assessed the main effects of legume variety and processing stage and their interaction on the nutritional and mineral properties of the fractions. Before analysis, normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene tests, respectively. Post hoc comparisons were used to identify significant differences among varieties within a fixed processing stage and among processing stages within each cultivar. Mean separation was performed using Duncan's multiple range test (DMRT) at a significance threshold of $p < 0.05$. All statistical analyses were performed using SPSS version 22.0 for Windows (IBM, USA).

3. RESULTS AND DISCUSSION

3.1. MINERAL CONTENT

The mineral profiles of the DF, PC, and PI are presented in Tables 1–9 for potassium, calcium, sodium, magnesium, phosphorus, iron, zinc, manganese, and copper, respectively. Tables 10 and 11 present the Na/K and Ca/P ratios, respectively. ANOVA confirmed a significant and consistent pattern of mineral enhance-

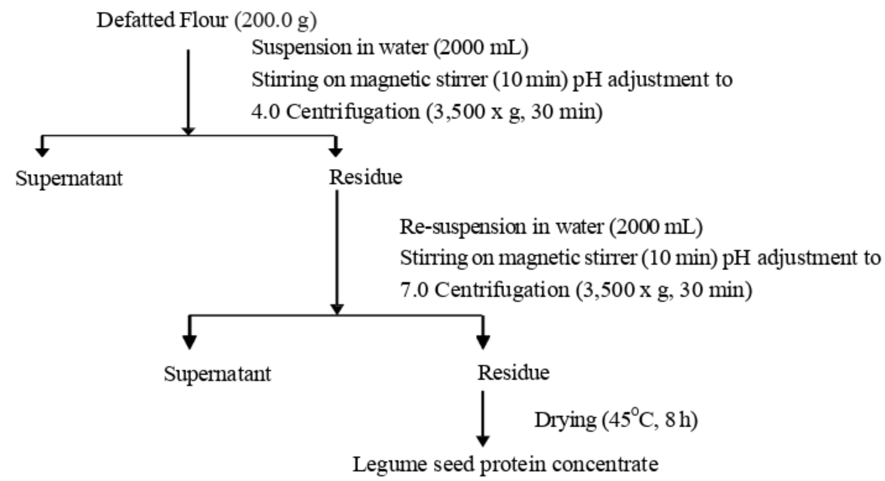

Figure 1. Scheme for the production of protein concentrates [11].

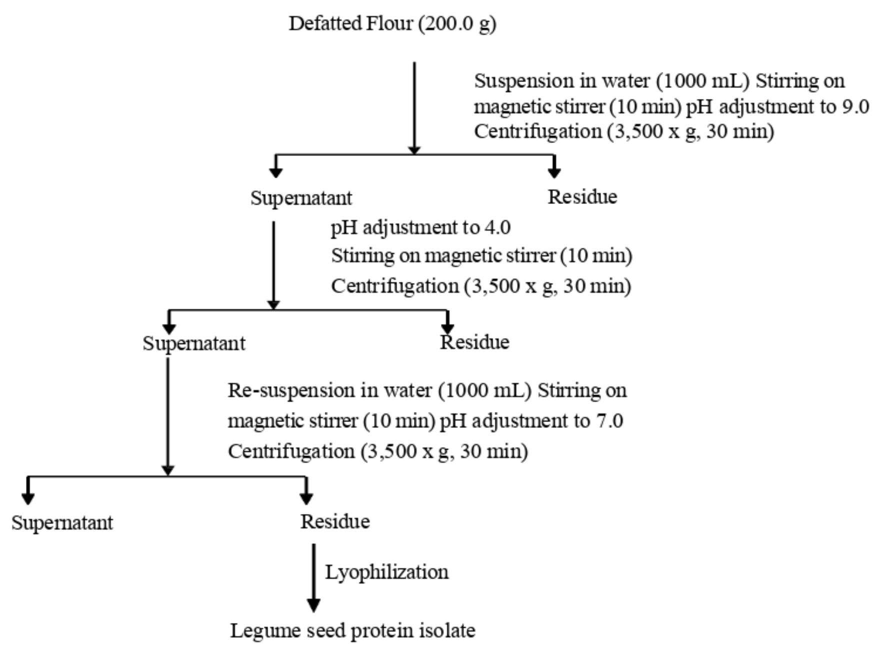

Figure 2. Scheme for the production of protein isolates [11].

Table 1. Potassium content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2,30)$	p-value
Honey bean	744.500 ± 0.010^a	943.807 ± 0.031^b	1216.547 ± 0.031^c	33529907.059	$< 0.001^*$
Black pinto bean	753.637 ± 0.025^a	955.647 ± 0.025^b	1328.503 ± 0.015^c	50781914.704	$< 0.001^*$
Alava pinto bean	654.020 ± 0.026^a	952.437 ± 0.021^b	1253.887 ± 0.051^c	53713861.621	$< 0.001^*$
Red kidney bean	755.520 ± 0.026^a	907.233 ± 0.042^b	1338.340 ± 0.046^c	54588029.783	$< 0.001^*$
Adzuki bean	804.613 ± 0.142^a	905.433 ± 0.208^b	1352.503 ± 0.015^c	50773743.748	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	1509655.142	$< 0.001^*$		
Processes	(2, 30)	237891031.601	$< 0.001^*$		
Legume × Processes	(8, 30)	1374106.329	$< 0.001^*$		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

ment across the refining processes, from DF to PI. The concentration effect was pronounced, although marked variety-specific differences were observed.

Potassium was the predominant macro-mineral in all evaluated legume fractions and showed progressive concentration during processing. In the DF, potassium values ranged from

Table 2. Calcium content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2,30)$	*p*-value
Honey bean	298.443 ± 0.350^a	423.543 ± 0.035^b	654.810 ± 0.056^c	2344450.061	$< 0.001^*$
Black pinto bean	222.207 ± 0.26^a	420.047 ± 0.045^b	703.207 ± 0.040^c	4191854.727	$< 0.001^*$
Alava pinto bean	313.963 ± 0.277^a	413.547 ± 0.035^b	698.673 ± 0.051^c	2859457.265	$< 0.001^*$
Red kidney bean	268.210 ± 0.394^a	406.803 ± 0.085^b	701.547 ± 0.031^c	3512672.929	$< 0.001^*$
Adzuki bean	315.223 ± 0.424^a	425.797 ± 0.035^b	705.057 ± 0.035^c	2894920.758	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	40,066.533	$< 0.001^*$		
Processes	(2, 30)	15,589,367.715	$< 0.001^*$		
Legume × Processes	(8, 30)	53,497.006	$< 0.001^*$		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

Table 3. Sodium content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2,30)$	*p*-value
Honey bean	168.413 ± 0.015^a	228.527 ± 0.031^b	288.847 ± 0.031^c	14107112.911	$< 0.001^*$
Black pinto bean	175.623 ± 0.021^a	215.613 ± 0.042^b	280.500 ± 0.020^c	10898944.769	$< 0.001^*$
Alava pinto bean	172.420 ± 0.020^a	222.450 ± 0.030^b	290.310 ± 0.030^c	13620626.326	$< 0.001^*$
Red kidney bean	188.040 ± 0.036^a	238.070 ± 0.020^b	291.987 ± 0.015^c	10513995.403	$< 0.001^*$
Adzuki bean	160.513 ± 0.025^a	220.560 ± 0.030^b	291.053 ± 0.035^c	16609541.383	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	462032.281	$< 0.001^*$		
Processes	(2, 30)	65129727.167	$< 0.001^*$		
Legume × Processes	(8, 30)	155123.406	$< 0.001^*$		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

Table 4. Magnesium content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2,30)$	*p*-value
Honey bean	139.513 ± 0.042^a	154.600 ± 0.040^b	163.503 ± 0.021^c	376021.430	$< 0.001^*$
Black pinto bean	135.340 ± 0.053^a	150.790 ± 0.026^b	162.243 ± 0.042^c	466052.850	$< 0.001^*$
Alava pinto bean	137.423 ± 0.021^a	152.657 ± 0.021^b	156.223 ± 0.042^c	254921.402	$< 0.001^*$
Red kidney bean	137.523 ± 0.032^a	154.337 ± 0.040^b	167.547 ± 0.040^c	578945.805	$< 0.001^*$
Adzuki bean	140.723 ± 0.021^a	154.497 ± 0.015^b	165.187 ± 0.032^c	384560.578	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	36939.236	$< 0.001^*$		
Processes	(2, 30)	2006086.718	$< 0.001^*$		
Legume × Processes	(8, 30)	13603.837	$< 0.001^*$		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

Table 5. Phosphorus content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2,30)$	*p*-value
Honey bean	179.837 ± 0.035^a	260.633 ± 0.035^b	317.650 ± 0.030^c	18163.530	$< 0.001^*$
Black pinto bean	185.387 ± 0.076^a	305.340 ± 0.020^b	415.650 ± 0.030^c	50237.981	$< 0.001^*$
Alava pinto bean	177.820 ± 0.020^a	290.513 ± 0.042^b	400.863 ± 0.038^c	47111.100	$< 0.001^*$
Red kidney bean	189.623 ± 0.032^a	280.440 ± 0.040^b	492.637 ± 0.040^c	91597.052	$< 0.001^*$
Adzuki bean	185.323 ± 3.444^a	285.527 ± 0.031^b	485.900 ± 0.020^c	88721.053	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	8785.264	$< 0.001^*$		
Processes	(2, 30)	272502.445	$< 0.001^*$		
Legume × Processes	(8, 30)	5832.067	$< 0.001^*$		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

654.020 mg/100 g in Alava pinto bean to 804.613 mg/100 g in Adzuki bean. Following processing into PC, the values increased to 905.433–955.647 mg/100 g, while the PI had the highest overall concentrations, reaching 1216.547–1352.503 mg/100 g. This upward trend from DF to PI indicates that potassium ions remained bound to, or co-precipitated with, the protein matrix during isolation rather than being completely lost to the supernatant during aqueous processing. The observed concentrations are comparable to those reported for lima beans (887.10–1102.10 mg/100 g) [15], indicating robust mineral profiles in these legume varieties.

When evaluated against nutritional benchmarks, a 100 g serving of these fractions, particularly the PI, provides a substantial proportion of the adult adequate intake for potassium, which

Table 6. Iron content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2, 30)$	p-value
Honey bean	3.320 ± 0.020^a	6.867 ± 0.032^b	14.780 ± 0.072^c	96011.446	$< 0.001^*$
Black pinto bean	4.657 ± 0.025^a	8.433 ± 0.035^b	12.500 ± 0.030^c	42916.829	$< 0.001^*$
Alava pinto bean	4.583 ± 0.021^a	6.227 ± 0.015^b	9.943 ± 0.040^c	21032.738	$< 0.001^*$
Red kidney bean	5.627 ± 0.015^a	7.600 ± 0.020^b	13.803 ± 0.025^c	50779.928	$< 0.001^*$
Adzuki bean	5.233 ± 0.025^a	6.550 ± 0.036^b	18.947 ± 0.035^c	159669.329	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	12092.404	< 0.001		
Processes	(2, 30)	324827.486	< 0.001		
Legume × Processes	(8, 30)	11395.696	< 0.001		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

Table 7. Zinc content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2, 30)$	p-value
Honey bean	6.773 ± 0.021^a	13.537 ± 0.025^b	14.647 ± 0.035^c	267.317	$< 0.001^*$
Black pinto bean	5.647 ± 0.015^a	12.330 ± 0.020^b	12.057 ± 0.031^b	210.562	$< 0.001^*$
Alava pinto bean	7.227 ± 0.015^a	16.037 ± 0.025^b	20.060 ± 0.020^c	634.160	$< 0.001^*$
Red kidney bean	7.660 ± 0.020^a	15.623 ± 0.015^b	24.667 ± 0.025^c	1065.750	$< 0.001^*$
Adzuki bean	5.337 ± 1.746^a	16.533 ± 0.021^b	23.500 ± 0.010^c	1235.964	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	278.837	$< 0.001^*$		
Processes	(2, 30)	2958.880	$< 0.001^*$		
Legume × Processes	(8, 30)	113.718	$< 0.001^*$		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

Table 8. Manganese content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2, 30)$	p-value
Honey bean	1.253 ± 0.025^c	0.790 ± 0.026^b	0.253 ± 0.025^a	1310.484	$< 0.001^*$
Black pinto bean	1.020 ± 0.020^c	0.537 ± 0.025^b	0.027 ± 0.021^a	1291.066	$< 0.001^*$
Alava pinto bean	2.140 ± 0.026^c	0.503 ± 0.015^b	0.157 ± 0.031^a	5871.337	$< 0.001^*$
Red kidney bean	1.347 ± 0.035^c	0.217 ± 0.015^b	0.033 ± 0.025^a	2647.112	$< 0.001^*$
Adzuki bean	1.300 ± 0.020^c	0.400 ± 0.020^b	0.250 ± 0.020^a	1687.500	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	460.694	$< 0.001^*$		
Processes	(2, 30)	11242.992	$< 0.001^*$		
Legume × Processes	(8, 30)	391.127	$< 0.001^*$		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

Table 9. Copper content (mg/100 g) by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2, 30)$	p-value
Honey bean	1.653 ± 0.032^a	2.667 ± 0.025^b	7.880 ± 0.046^c	38248.325	$< 0.001^*$
Black pinto bean	2.247 ± 0.025^a	4.850 ± 0.030^b	9.787 ± 0.031^c	50253.591	$< 0.001^*$
Alava pinto bean	1.620 ± 0.010^a	3.040 ± 0.036^b	9.397 ± 0.025^c	58762.779	$< 0.001^*$
Red kidney bean	2.523 ± 0.021^a	3.863 ± 0.031^b	8.920 ± 0.020^c	38993.997	$< 0.001^*$
Adzuki bean	3.267 ± 0.025^a	4.857 ± 0.035^b	9.497 ± 0.035^c	35903.261	$< 0.001^*$
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	5427.329	$< 0.001^*$		
Processes	(2, 30)	219023.378	$< 0.001^*$		
Legume × Processes	(8, 30)	784.644	$< 0.001^*$		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

ranges from 2600 mg for adult females to 3400 mg for adult males [16]. A concentrated 100 g portion provides approximately 36–52 % of the daily requirement, positioning these isolates as potentially useful plant-based functional ingredients for addressing inadequate potassium intake. Diets rich in plant-derived potassium may also counteract the hypertensive effects of dietary sodium [17].

Calcium concentrations increased steeply across the processing sequence, closely tracking protein enrichment. Concentrations ranged from 222.207–315.223 mg/100 g in the DF, in-

Table 10. Na/K ratio by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2, 30)$	*p*-value
Honey bean	0.227 ± 0.015	0.237 ± 0.025	0.233 ± 0.031	0.186	0.831
Black pinto bean	0.217 ± 0.015	0.220 ± 0.020	0.197 ± 0.015	1.144	0.332
Alava pinto bean	0.250 ± 0.010	0.213 ± 0.015	0.230 ± 0.030	2.420	0.106
Red kidney bean	0.233 ± 0.015	0.257 ± 0.015	0.220 ± 0.020	2.473	0.101
Adzuki bean	0.177 ± 0.015[a]	0.223 ± 0.015[b]	0.233 ± 0.032[b]	6.569	0.004*
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	3.285	0.024*		
Processes	(2, 30)	0.867	0.430		
Legume × Processes	(8, 30)	2.981	0.014*		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

Table 11. Ca/P ratio by legume variety and processing method.

Legume variety	Defatted flour	Protein concentrate	Protein isolate	Simple effect of processing, $F(2, 30)$	*p*-value
Honey bean	1.600 ± 0.060[a]	1.617 ± 0.015[a]	2.063 ± 0.025[b]	265.684	< 0.001
Black pinto bean	1.200 ± 0.010[a]	1.370 ± 0.026[b]	1.677 ± 0.032[c]	224.459	< 0.001
Alava pinto bean	1.753 ± 0.038[b]	1.417 ± 0.015[a]	1.740 ± 0.030[b]	139.786	< 0.001
Red kidney bean	1.427 ± 0.031	1.450 ± 0.030	1.427 ± 0.021	0.698	0.505
Adzuki bean	1.717 ± 0.015[b]	1.480 ± 0.010[a]	1.453 ± 0.015[a]	80.812	< 0.001
Overall ANOVA	Df	F	P-value		
Legume	(4, 30)	238.281	< 0.001		
Processes	(2, 30)	208.470	< 0.001		
Legume × Processes	(8, 30)	125.742	< 0.001		

Letters appended to mean values denote significant differences between corresponding means. *Significant difference.

Table 12. Mineral safety index for defatted flours (mg/100 g).

Mineral	UL (mg)	Honey bean	Black pinto bean	Alava pinto bean	Red kidney bean	Adzuki bean	Interpretation
Calcium	2500	836.7	1123.7	795.6	930.9	792.0	Very Safe. 100 g provides 9-13 % of UL.
Copper	10	591.7	444.4	617.3	395.3	305.8	Very Safe. 100 g provides 16-33 % of UL.
Iron	45	1355.4	965.7	980.8	799.3	863.7	Very Safe. 100 g provides 7-12 % of UL.
Manganese	11	880.0	1078.4	511.6	797.1	846.2	Very Safe. 100 g provides 9-20 % of UL.
Sodium	2300	1365.3	1309.4	1334.0	1223.2	1432.5	Very Safe. 100 g provides 7-8 % of UL.
Phosphorus	4000	2223.8	2156.7	2248.8	2109.5	2182.4	Very Safe. 100 g provides 4-5 % of UL.
Zinc	40	590.0	708.0	553.3	520.8	629.9	Very Safe. 100 g provides 14-19 % of UL.

MSI: All MSI values are well above 100, indicating an excellent safety margin. UL: Tolerable Upper Intake Level.

creased to 406.803–425.797 mg/100 g in the PC, and reached 654.810–705.057 mg/100 g in the PI. Even the baseline DF values were higher than values reported for white bean (188.35 mg/100 g) and brown bean seeds (217.36 mg/100 g) [18].

This marked variation demonstrates that the extraction and isolation substantially concentrate calcium within the protein matrix. While a 100 g serving of raw or defatted flour provides only a modest fraction of adult requirements, the PI fraction satisfies approximately 55–71 % of the adult RDA (1000–1200 mg/day) established to optimize skeletal bone density [19]. For infant nutrition, where the daily allowance sits between 210–260 mg/day, these isolates serve as a concentrated mineral reservoir. However, because a 100 g dry serving is not physiologically realistic for infant physiology, these fractions must be incorporated at heavily diluted, carefully calculated inclusion rates within multi-ingredient weaning formulations rather than being viewed as a direct standalone source [20]. Conversely, for fast-growing children and adolescents whose daily requirements increase to 1300 mg/day, these fractions serve as highly valuable complementary sources rather than standalone solutions [19].

Calcium is a critical structural macro-mineral required for skeletal ossification, cellular signaling, and neuromuscular function [21]. The removal of phytates and soluble oxalates during the protein isolation process is vital here, as it prevents the formation of insoluble calcium complexes in the gastrointestinal tract, thereby ensuring that a high percentage of this mineral is available for intestinal absorption [22].

Sodium concentrations exhibited a statistically significant increase ($p < 0.05$) as processing progressed from DF to protein fractions. In the DF, sodium values ranged from 160.513–

Table 13. Mineral safety index for protein concentrates (mg/100 g).

Mineral	UL (mg)	Honey bean	Black pinto bean	Alava pinto bean	Red kidney bean	Adzuki bean	Interpretation
Calcium	2500	590.3	595.2	604.5	614.6	587.4	Very Safe. 100 g provides 37-43 % of UL.
Copper	10	374.5	206.2	327.9	258.4	205.8	Very Safe. 100 g provides 20-49 % of UL.
Iron	45	654.1	533.8	722.3	592.1	686.0	Very Safe. 100 g provides 13-22 % of UL.
Manganese	11	1375.0	2037.0	2200.0	5000.0	2750.0	Extremely Safe. 100 g provides only 2-9 % of UL.
Sodium	2300	1006.5	1066.8	1033.7	966.0	1043.0	Very Safe. 100 g provides 9-10 % of UL
Phosphorus	4000	1534.6	1310.4	1376.9	1426.2	1400.8	Very Safe. 100 g provides 7-8 % of UL.
Zinc	40	295.4	324.4	249.4	256.1	241.8	Very Safe. 100 g provides 25-41 % of UL.

MSI: All MSI values are well above 100, indicating an excellent safety margin. UL: Tolerable Upper Intake Level.

Table 14. Mineral Safety Index for protein isolates (mg/100 g).

Mineral	UL (mg)	Honey bean	Black pinto bean	Alava pinto bean	Red kidney bean	Adzuki bean	Interpretation
Calcium	2500	381.8	355.6	357.8	356.4	354.5	Very Safe. 100 g provides 28-35 % of UL.
Copper	10	126.7	102.2	106.4	112.4	105.3	Slightly Safe. 100 g provides 78.9-97.8 % of UL.
Iron	45	304.9	360.0	454.5	326.1	237.5	Very Safe. 100 g provides 22-42 % of UL.
Manganese	11	4400.0	55000.0	7333.3	36666.7	4400.0	Extremely Safe. 100 g provides <0.02-0.2 % of UL.
Sodium	2300	796.4	820.0	792.3	787.8	790.2	Very Safe. 100 g provides 12 % of UL
Phosphorus	4000	1259.1	962.1	997.8	811.9	823.2	Very Safe. 100 g provides 8-12 % of UL.
Zinc	40	273.0	332.0	199.4	162.1	170.2	Safe: 100 g provides 30-62 % of UL.

MSI: All MSI values are well above 100, indicating an excellent safety margin. UL: Tolerable Upper Intake Level.

188.040 mg/100 g, values already higher than those reported for comparable legumes by James *et al.* [23]. Upon processing, sodium content rose moderately in the PC and ultimately peaked within the PI, reaching a range of 280.500–291.987 mg/100 g. This significant accumulation suggests that sodium ions interact electrostatically with the hydrophilic domains of the legume globulins and are retained during alkaline solubilization and isoelectric precipitation [24].

For an adult population where the daily intake guideline is capped at 1500 mg/day (with an upper tolerable limit of 2300 mg/day), a 100 g portion of the protein isolate contributes approximately 19 % of the daily threshold [25]. This characterizes the isolates as inherently low-sodium ingredient sources on an adult scale. Conversely, for infants whose adequate intake spans a tight boundary of 110–370 mg/day, a 100 g dry-matter serving of the isolate yields an incredibly high concentration that could satisfy or even exceed their total daily allowance [20]. Consequently, while these isolates are highly valuable for adult functional foods, their inclusion rates in infant weaning formulas must be carefully calculated and diluted to prevent excessive renal solute loads [26]. Sodium remains essential for fluid balance and blood pressure regulation [27].

Magnesium levels varied across the fractions, ranging from 135.340–140.723 mg/100 g (DF), 150.790–154.600 mg/100 g (PC), to 156.223–167.547 mg/100 g (PI). While the two-way analysis of variance confirmed significant differences ($P < 0.05$) between processing stages, the values consistently remained within a tight absolute window of 135.340 to 167.547 mg/100 g. This close proximity across stages suggests a stable matrix-retention mechanism that undergoes minimal disruption during sequential extraction [28].

A 100 g portion of the flour and its resulting protein fractions provides a substantial contribution to daily mineral requirements, fulfilling approximately 42.29–54.05 % of the magnesium RDA (310–320 mg/day) for adult females and 32.22–41.89 % for adult males (400–420 mg/day), making these legumes a robust, reliable plant-based source of dietary magnesium [29].

For young children, whose requirements span 80–130 mg/day, a standard serving completely covers or surpasses their daily metabolic needs [29]. For infant formulations, where baseline requirements are minimal (30–75 mg/day), these fractions represent a highly concentrated mineral reservoir. Consequently, minor inclusion rates scaled down to realistic infant portion sizes are sufficient to meet these requirements, mitigating any risk of digestive or metabolic overload [20]. Maintaining this stable magnesium profile is crucial for a wide range of metabolic functions. Magnesium acts as an essential intracellular cofactor for more than 300 enzymatic reactions, including those governing glycolysis, ATP-dependent energy transfer, and protein synthesis [30]. Furthermore, magnesium plays a fundamental structural role in bone mineralization alongside calcium and phosphorus, and is critical for stabilizing neuromuscular membranes and maintaining the electrical potential of nerve and muscle tissues [31]. The stable, resilient magnesium density demonstrated by these varieties highlights their functional value as reliable raw materials for producing fortified foods.

Phosphorus concentrations demonstrated a substantial and statistically significant increase across the processing stages, rising from a baseline of 177.820–189.623 mg/100 g in the DF to an enriched range of 317.650–492.637 mg/100 g in the PI. This pronounced accumulation indicates that phosphorus is highly concentrated alongside the protein matrix during isolation [22, 32]. Evaluating these metrics shows that a single 100 g portion of the PI satisfies an exceptional 45–70 % of the adult RDA (700 mg/day). For growing children (ages 1–8) whose daily benchmarks sit between 460 and 500 mg/day, the PI fraction serves as a high-potency standalone source, meeting up to 100 % of their daily requirements [19].

Most notably, for infant nutrition where the daily requirement spans a tight range of 100–275 mg/day, the extreme concentration of phosphorus in these isolates exceeds the infant metabolic threshold on a 100 g basis. This mandates strict dilution in final weaning foods to maintain a balanced Ca/P ratio and protect infant renal function [20]. This demonstrates that while blending with other phosphorus-rich sources is unnecessary for adult baselines, these isolates can serve as powerful, highly concentrated mineral foundations on their own. Integral to energy cycles and bone mineralization, these profiles support their structural deployment [33].

Iron concentrations exhibited a pronounced upward trajectory across the processing sequence, increasing from 3.320–5.627 mg/100 g (DF) to an enriched range of 9.943–18.947 mg/100 g (PI). This significant accumulation indicates that iron ions are strongly bound to the protein matrix, likely forming stable coordination complexes with the amino acid side chains of the legume globulins during alkaline extraction and subsequent isoelectric precipitation.

Among the evaluated varieties, Red kidney bean stands out as a promising biofortification candidate. A single 100 g serving of its defatted flour delivers a substantial trace-mineral contribution, satisfying nearly 70 % of the adult male RDA (8 mg/day) and 31 % for women of reproductive age (18 mg/day) [19]. This dense iron profile is critical in the Nigerian context, where iron-deficiency anaemia remains a prevalent microcytic anaemia and a severe public health crisis, particularly affecting growing children, adolescent girls, and pregnant women [33, 34]. Utilizing these iron-enriched fractions as functional ingredients offers a sustainable, plant-based strategy to combat systemic nutritional deficiencies in local food systems.

Zinc demonstrated a marked increase in density during the concentration and isolation stages, shifting from a baseline of 5.337–7.660 mg/100 g (DF) to 12.330–16.533 mg/100 g (PC) and culminating in a highly concentrated range of 12.057–24.667 mg/100 g within the PI. This distinct concentration spike confirms that zinc, similar to iron, exhibits high binding affinity for the storage proteins, resisting leaching during aqueous processing. When evaluated against nutritional requirements, a 100 g portion of the protein concentrates alone provides over 100 % of the daily RDA for adult females (8 mg/day) and up to 150 % for adult males (11 mg/day) [19]. Given that zinc serves as an indispensable catalytic component and structural motif for more than 300 enzymes, these refined legume fractions represent vital, high-potency raw materials for protecting systemic metabolic health and mitigating stunting in vulnerable populations [35].

In contrast to the other minerals, manganese exhibited a unique decrease across the processing chain, reversing the standard (DF $<$ PC $<$ PI) enrichment trend. Levels dropped sharply from 1.020–2.140 mg/100 g in the DF to a minimal range of 0.027–0.253 mg/100 g in the PI. This steep downward trajectory confirms that manganese ions are not structurally associated with the globulin fractions. Instead, they exist primarily in highly soluble, loosely bound forms that are easily partitioned into the liquid supernatant and discarded during the aqueous fractionation and washing phases [36].

While manganese is a vital cofactor for key antioxidant metalloenzymes, these highly processed PI fractions provide minimal contributions to adult intakes. A 100 g serving of the isolate fails to satisfy the adult Adequate Intake of 1.8–2.3 mg/day, meeting the baseline nutritional targets for infants (0.003–0.6 mg/day) only [37]. Consequently, to retain manganese for broad-spectrum nutritional fortification, the DF or PC matrices should be utilized rather than the more highly refined PI.

Copper values range from 1.620–3.267 mg/100 g in DF, 2.667–4.857 mg/100 g in PC, and 7.880–9.787 mg/100 g in PI. Despite this notable concentration effect, the calculated MSI across all varieties and processing stages remained well within the designated safe threshold. This high safety margin indicates that the resulting protein matrices are unlikely to pose a copper-related toxicity risk at the assessed serving level and can be safely utilized as functional ingredients in food formulations without the risk of exceeding mineral safety thresholds.

The Na/K ratio remained stable and favourable (< 0.60), ranging from 0.177–0.257 across all stages. A ratio less than one is recommended to support blood-pressure control [38]. Similarly, the Ca/P ratio, a marker of bone health, was consistently above 1.0 (reaching 2.063 in PI), which facilitates increased calcium absorption in the small intestine.

The protein isolation process culminated in a highly concentrated mineral profile. Adzuki and Red kidney bean isolates demonstrated superior trace mineral potency. The retention of low Na/K and superior Ca/P ratios confirms that these isolates are not merely protein-dense ingredients but high-quality mineral vehicles with potential to support cardiovascular and skeletal health [35, 39].

The results of the MSI for the defatted flours and processing stages are summarized in Tables 12, 13, and 14. In this assessment, a reference value of 100 serves as the critical safety threshold; values significantly exceeding 100 represent a robust safety margin, while values descending toward this limit indicate a narrowing window for safe consumption. The transition across the processing chain reveals a distinct variation in mineral kinetics. The results show that progressive purification concentrates these elements within the protein isolate matrix, thereby narrowing their calculated safety margins.

The five legume varieties exhibited wide safety margins in the DF. Every calculated MSI was substantially greater than 100, indicating that a 100 g serving contributed only a fraction of the Tolerable Upper Intake Level. As the DF was refined into PC and PI, MSI values decreased as nutrient density increased, except for manganese, whose concentration decreased. During isolation, inorganic salts were partitioned between the protein matrix and the supernatant.

Calcium MSI values drop significantly from 836.7–1123.7 (DF) to 354.5–381.8 (PI). This indicates a high-density protein source that remains comfortably distant from the UL (2500 mg), effectively eliminating concerns regarding hypercalcemia.

Sodium and phosphorus MSI profiles remain relatively stable and safe across all stages: DF: 7–8 % (Na) and 4–5 % (P) of UL; PC: 9–10 % (Na) and 7–8 % (P) of UL; PI: 12 % (Na) and 8–12 % (P) of UL. This stability confirms that the concentration process does not lead to an over-accumulation of electrolytes that may disrupt renal osmotic balance.

Copper and zinc maintain functional safety margins across the fractions: The MSI for copper ranges from 305.8–617.3 (DF), 205.8–374.5 (PC), and 102.2–126.7 (PI). While 100 g of DF poses no risk of toxicity, the Black Pinto isolate provides approximately 98 % of the copper UL, essentially reaching the maximum safe daily threshold from a single food item. While the MSI confirms a wide safety cushion for most minerals, a critical exception occurs with copper in the PI. Consequently, these fractions cannot be classified as globally safe without restriction; their inclusion rates in food formulations must be carefully regulated. The MSI for zinc moves from 520.8 – 708.0 (DF) to 162.1 – 332.0 (PI). Even at these high concentrations, the risk of zinc interfering with copper absorption is minimized, remaining within safe parameters for adult populations [21].

A highly favourable situation appears in the manganese MSI, particularly for the Red kidney and Adzuki varieties. Although absolute manganese levels decrease during processing, the reported MSI values indicate that these fractions maintain a wide safety margin relative to the upper tolerable intake level (UL 11 mg). Consequently, these legume fractions represent safe, stable ingredients for nutritional applications, including paediatric formulations where manganese clearance is typically slower. As noted by Soetan *et al.* [40], while Mn is essential for skeletal structure, ensuring ingestion remains well within these established safe boundaries avoids any risks associated with chronic overexposure [41].

4. CONCLUSION

The transition along the protein-extraction chain shifts these underutilized legumes from simple macronutrient sources to concentrated mineral-delivery vehicles. While defatted flours or concentrates are required to retain manganese, isolation concentrates potassium, calcium, phosphorus, iron, and zinc alongside the protein matrix while maintaining a low sodium concentration. A 100 g serving of the resulting PI meets more than half of several adult mineral benchmarks and satisfies the reported thresholds for older children, making these fractions candidates for high-density nutrition. The concurrent retention of a Na/K ratio below 0.26 and a Ca/P ratio reaching 2.063 supports the potential use of the isolates as functional ingredients for cardiovascular and skeletal health. The MSI assessment indicates that DF and PC provide the widest safety margins for general weaning formulations, while PI remains within the reported physiological thresholds for most microelements. However, because of the high retention and narrow safety margin observed for copper in the isolates, commercial utilization requires controlled inclusion rates and targeted varietal blending. Similarly, because the mineral densities exceed baseline infant requirements on a 100 g basis, use in paediatric nutrition should be restricted to carefully calculated inclusion levels or blending in infant weaning formulations to avoid excessive mineral or renal solute loads. These strategies may provide a safe, mineral-dense raw material for addressing nutrient deficiencies while limiting the risk of trace-mineral overload.

DATA AVAILABILITY

Data will be made available upon request from the corresponding author.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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