

Original Article

Development and Nutritional Characterization of Pumpkin Seed- and Kidney Bean-Based Bars for Protein-Energy Malnutrition in Children

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ABSTRACT

Background: Protein-energy malnutrition remains an important cause of impaired growth, increased susceptibility to infection, and adverse developmental outcomes in children. Nutrient-dense foods formulated from locally available plant ingredients may provide candidates for future nutritional interventions, provided their composition, safety, acceptability, and biological suitability are adequately characterized. **Objective:** To develop pumpkin-seed- and kidney-bean-based food bars and characterize their proximate composition, phenolic and flavonoid contents, and selected mineral concentrations. **Methods:** Four bar formulations were prepared using different quantities of kidney bean powder, pumpkin seed powder, honey, coconut oil, and dark chocolate. Moisture, crude protein, crude fat, crude fiber, ash, total phenolic content, total flavonoid content, iron, calcium, zinc, and magnesium were determined using laboratory procedures described in the study. Nitrogen-free extract was recalculated by difference from the reported proximate means. **Results:** Protein increased across the reported formulations from 27.60% in T1 to 45.38% in T4, while fat decreased from 25.48% to 21.48%. Fiber increased from 2.06% to 5.04% and ash from 1.73% to 2.11%. Corrected nitrogen-free extract values were 40.87%, 34.37%, 28.99%, and 24.05% for T1-T4. Total phenolic content ranged from 19.79 to 59.80 mg GAE/100 g and total flavonoid content from 20.18 to 30.98 mg QE/100 g. T4 had the highest reported iron, calcium, zinc, and magnesium concentrations. **Conclusion:** Pumpkin-seed- and kidney-bean-based bars with high measured protein and mineral content were successfully formulated. T4 had the most nutrient-dense reported profile for several measured parameters, but therapeutic efficacy for childhood protein-energy malnutrition was not evaluated. **Keywords:** Protein-Energy Malnutrition; Pumpkin Seeds; Kidney Beans; Protein Bar; Functional Food; Minerals; Phenolic Compounds.

INTRODUCTION

Protein-energy malnutrition encompasses inadequate intake or utilization of dietary energy and protein and remains an important contributor to disease burden, impaired growth, and vulnerability to infection, particularly in children. Global analyses continue to demonstrate a substantial burden of protein-energy malnutrition despite improvements in food availability and child-health interventions (1). Childhood undernutrition can manifest as wasting, stunting, underweight, nutritional oedema, or combinations of these conditions. Although these phenotypes have different clinical and

epidemiological implications, inadequate dietary quality, recurrent infection, food insecurity, poverty, poor maternal nutrition, and limited healthcare access frequently interact in affected populations (1).

Pakistan continues to experience a substantial burden of childhood undernutrition. Recent national evidence shows persistently low dietary diversity among young children, with variation by maternal education, household wealth, residence, diarrhoeal illness, and related social determinants, reinforcing the multifactorial nature of nutritional vulnerability in Pakistan (2).

The consequences extend beyond anthropometric deficits. Children with inadequate nutrition may have reduced immune competence, delayed physical growth, impaired neurodevelopment, lower educational attainment, and greater vulnerability to infectious illness (3–5). Severe forms require structured medical and nutritional management and cannot be treated simply by offering an ordinary snack product (6,7).

Development of locally sourced nutrient-dense foods is nevertheless an important area of food and nutrition research. Previous work has demonstrated the feasibility of developing legume-based ready-to-use foods using chickpea, mung bean, peanut, milk-derived ingredients, vegetable fat, vitamins, and minerals, although products intended for therapeutic use require rigorous nutritional, microbiological, stability, and clinical validation (6,7).

Pumpkin seeds are of interest as a food ingredient because they contain protein, lipid, minerals, fiber, and phytochemicals and have been investigated as ingredients in nutritionally enhanced foods and food-bar formulations (8,9). Their relatively high nutrient density makes them potentially useful when developing compact foods in which nutrient delivery per serving is important (8,9).

Kidney beans (*Phaseolus vulgaris*) are also nutrient-dense plant foods. They provide protein, complex carbohydrate, dietary fiber, minerals, and bioactive compounds (10). Beans have established nutritional value but also contain antinutritional factors and lectins whose activity can be modified substantially by soaking, heating, germination, fermentation, and other processing procedures (10,11).

Combining seeds and legumes may therefore offer a practical food-development strategy by integrating complementary sources of protein, lipid, carbohydrate, fiber, and minerals (8,10–12). Product quality, however, depends not only on ingredient selection but also on processing, ingredient ratios, moisture control, palatability, food safety, and storage stability (9,11,12).

Food-bar development has received increasing attention because bars can provide a compact matrix for combining cereal, pulse, nut, seed, fruit, and other nutrient-rich ingredients. High-energy protein bars have been characterized using proximate, antioxidant, physical, and sensory analyses, illustrating the importance of evaluating multiple dimensions of quality rather than protein concentration alone (9,13).

The nutritional objective becomes more demanding when a product is proposed for children with malnutrition. Protein quantity alone is insufficient. Protein quality, amino-acid profile, digestibility, total energy density, essential fatty acids, micronutrient balance, antinutritional factors, microbiological safety, serving size, age-specific acceptability, and clinical tolerance all require consideration (7,12,14).

The present laboratory study therefore developed four food-bar formulations containing pumpkin seed powder and kidney bean powder together with honey, coconut oil, and dark chocolate. The study aimed to characterize their proximate composition, selected phytochemical characteristics, and mineral content as an initial food-development step relevant to future nutritional research.

MATERIALS AND METHODS

An experimental laboratory food-product development study was conducted at Superior University, Lahore, Pakistan. Pumpkin seeds, kidney beans, honey, coconut oil, dark chocolate, and other food ingredients were obtained locally.

Pumpkin seeds and kidney beans were processed into powders or flours before product preparation. The powders were combined according to four formulations. The final formulation table documented kidney bean powder, pumpkin seed powder, honey, coconut oil, and dark chocolate as the principal quantified ingredients.

The ingredients were blended to obtain a homogeneous mixture and formed into bars.

Proximate analyses included moisture, crude protein, crude fat, crude fiber, ash, and nitrogen-free extract. Laboratory methods were described with reference to standard AOAC procedures (15).

Moisture was determined using approximately 5 g of sample in pre-weighed dishes. Samples were dried in a hot-air oven (LDO-150N) at approximately $100\pm5^{\circ}\text{C}$ for about 24 hours to constant weight, cooled in a desiccator, and reweighed.

Crude protein was determined using the Kjeldahl method with a Behr laboratory Kjeldahl apparatus. Approximately 5 g of sample was digested using concentrated sulfuric acid and a digestion tablet. After dilution, the digested material was alkalized with 40% sodium hydroxide and distilled. Ammonia was collected in boric acid with indicator and titrated using 0.1 N sulfuric acid. Nitrogen percentage was multiplied by 6.25 to estimate crude protein.

Table 1. Composition of the Four Bar Formulations

Ingredient, g	T1	T2	T3	T4
Kidney bean powder	50	40	60	45
Pumpkin seed powder	50	60	40	55
Honey	21	18	24	20
Coconut oil	13.6	15	12	14
Dark chocolate	28.4	30	26	29
Total formulation mass, g	163.0	163.0	162.0	163.0

Crude fat was determined by Soxhlet extraction using dried sample and hexane as the extraction solvent. Samples underwent repeated siphoning, followed by solvent evaporation, cooling in a desiccator, and gravimetric determination of extracted lipid.

Crude fiber was determined on defatted material. Approximately 3 g of sample underwent sequential digestion with 1.25% sulfuric acid and 1.25% sodium hydroxide, filtration, washing, oven drying, and final ignition in a muffle furnace at approximately 550°C.

Ash was determined by incineration of approximately 3 g of sample in a muffle furnace at 550–600°C until a stable grey residue was obtained.

Nitrogen-free extract was defined by the study as:

$NFE(\%) = 100 - [\text{moisture}(\%) + \text{crude protein}(\%) + \text{crude fat}(\%) + \text{crude fiber}(\%) + \text{ash}(\%)]$.

Because the NFE values printed in the original results were incompatible with this equation, NFE was recalculated directly from the reported mean proximate components.

Total phenolic content was assessed by the Folin-Ciocalteu method using gallic acid calibration, an established spectrophotometric approach for estimating total phenolic content (16). Sample extract was reacted with diluted Folin-Ciocalteu reagent and sodium carbonate and incubated before absorbance measurement at 765 nm. Results were expressed as gallic acid equivalents.

Total flavonoid content was determined using an aluminium-chloride colorimetric approach, a widely used spectrophotometric method for total flavonoid estimation (17). Sample solution was reacted with 2% aluminium chloride in ethanol and incubated at room temperature before absorbance measurement at 420 nm. Results were expressed as quercetin equivalents.

Selected mineral concentrations reported in the completed dataset included iron, calcium, zinc, and magnesium. Data were analyzed using IBM SPSS Statistics version 25. The source specified one-way analysis of variance at a significance threshold of $p < 0.05$. However, because the supplied ANOVA summary tables contained incompatible sums of squares, mean squares, degrees of freedom, and F values, the publication-facing analysis was limited to the reproducible descriptive means. No new inferential p values were generated without the original replicate-level observations.

RESULTS

Four formulations were evaluated.

Moisture was lowest in T4 at 1.94% and highest in T1 at 2.25%. Crude fat decreased from 25.48% in T1 to 21.48% in T4. Protein showed the opposite pattern, increasing from 27.60% to 45.38%. Fiber increased from 2.06% in T1 to 5.04% in T4, while ash increased from 1.73% to 2.11%. The NFE values originally printed in the manuscript could not be reconciled with the stated difference method. Recalculation from the supplied mean proximate measurements produced NFE values of 40.87% for T1, 34.37% for T2, 28.99% for T3, and 24.05% for T4. Total phenolic content increased across the reported formulations from 19.79 mg GAE/100 g in T1 to 59.80 mg GAE/100 g in T4. Total flavonoid content ranged from 20.18 to 30.98 mg QE/100 g.

Reported mineral concentrations were also highest in T4. Iron ranged from 1.62 to 2.92 mg/100 g, calcium from 38.11 to 57.72 mg/100 g, zinc from 0.629 to 0.767 mg/100 g, and magnesium from 99.09 to 161.34 mg/100 g.

Table 2. Comprehensive Nutritional, Phytochemical, and Mineral Characterization of the Four Bars

Parameter	T1	T2	T3	T4
Moisture, %	2.25	2.18	2.08	1.94
Crude fat, %	25.48	24.34	23.07	21.48
Crude protein, %	27.60	33.37	39.43	45.38
Crude fiber, %	2.06	3.74	4.39	5.04
Ash, %	1.73	1.99	2.05	2.11
NFE by stated difference method, %	40.87	34.37	28.99	24.05
Total phenolic content, mg GAE/100 g	19.79	32.97	46.52	59.80
Total flavonoid content, mg QE/100 g	20.18	23.91	27.35	30.98
Iron, mg/100 g	1.62	2.05	2.48	2.92
Calcium, mg/100 g	38.11	44.64	51.18	57.72
Zinc, mg/100 g	0.629	0.671	0.713	0.767
Magnesium, mg/100 g	99.09	121.23	145.70	161.34

Inferential ANOVA statistics are not shown because the supplied ANOVA summaries were internally inconsistent. The measurements indicate that T4 had the highest reported protein, fiber, ash, total phenolic content, total flavonoid content, iron, calcium, zinc, and magnesium concentrations, whereas T1 had the highest fat and moisture values. These patterns describe the finished formulations. They cannot be interpreted as the isolated effect of increasing pumpkin seed or kidney bean concentration because the ratios of both flours and the quantities of honey, coconut oil, and dark chocolate differed among treatments.

DISCUSSION

The present study demonstrated the feasibility of producing food bars from pumpkin seed powder and kidney bean powder combined with honey, coconut oil, and dark chocolate. The formulations differed substantially in proximate composition and selected mineral and phytochemical characteristics.

The most prominent compositional difference was the increase in measured protein from 27.60% in T1 to 45.38% in T4. This is potentially important in the context of developing compact nutrient-dense foods. Protein-energy malnutrition reflects inadequate protein and/or energy availability, and food matrices with high protein density may therefore warrant investigation as components of broader nutrition interventions (1,6,7).

The protein values should nevertheless be interpreted as crude protein estimated by the Kjeldahl nitrogen method. Crude protein concentration does not establish amino-acid adequacy, digestibility, biological value, or clinical protein utilization. Legume proteins may be nutritionally valuable, but amino-acid balance, matrix effects, and antinutritional factors can influence effective protein quality and digestibility (10,12).

Pumpkin seeds provide protein and lipid together with several minerals and phytochemicals and have increasingly been studied as functional food ingredients and in enriched snack bars (8,9). Kidney beans also provide substantial protein and fiber, but appropriate processing is important because raw or inadequately processed beans contain antinutritional factors and lectins (10,11).

This consideration is especially important when the proposed target population is children. The source states that kidney beans and pumpkin seeds were converted into flour, but the exact heat treatment used for kidney beans is not sufficiently described to determine whether potentially harmful lectins and other antinutritional factors were adequately reduced; processing conditions materially influence the reduction of these compounds and the digestibility of legume proteins (11,12).

Crude fat was highest in T1 and declined across the treatment sequence. Fat is a major contributor to energy density in foods intended for nutritional rehabilitation, and therapeutic-food formulation requires balancing energy, protein, and micronutrient delivery rather than maximizing protein alone (6,7,14). The lower fat concentration of T4 therefore represents a trade-off against its higher protein concentration. Because all formulations also differed in honey, coconut oil, dark chocolate, and flour proportions, the observed fat changes cannot be attributed specifically to either pumpkin seed or kidney bean powder. The corrected carbohydrate-by-difference results also materially change interpretation. The source originally reported progressively increasing NFE values of approximately 71–89%. Those values were mathematically impossible because the reported moisture, protein, fat, fiber, ash, and NFE fractions substantially exceeded 100%.

Using the manuscript's own difference equation, the NFE values instead decrease from 40.87% in T1 to 24.05% in T4. This is consistent with the increasing proportion of the measured composition accounted for by protein and fiber.

Fiber increased from 2.06% to 5.04%. Dietary fiber contributes to bowel function and the physicochemical characteristics of foods, although the crude-fiber method captures only part of total dietary fiber. Contemporary AOAC dietary-fiber methodology separately addresses insoluble, soluble, and low-molecular-weight fractions, underscoring why crude-fiber values should not be interpreted as total dietary-fiber content (18).

Ash increased modestly across formulations and provides a broad gravimetric indicator of mineral residue, while direct mineral analysis provides more specific information for individual elements such as iron, calcium, zinc, and magnesium (15).

The mineral findings are potentially relevant to child nutrition. T4 contained the highest reported concentrations of iron, calcium, zinc, and magnesium. Iron, zinc, calcium, and magnesium contribute to multiple processes involved in growth, immune function, hematological status, and normal structural and metabolic development (5,19).

However, concentration alone does not establish bioavailability. Beans, seeds, and other plant foods may contain phytates that chelate minerals and can reduce absorption, particularly of iron and zinc; dephytinization and phytase interventions can modify this effect (19). No phytate concentration, mineral bioavailability, or in-vitro digestion analysis was reported.

Total phenolic and flavonoid concentrations were also highest in T4. Food-bar research has demonstrated that ingredient selection and formulation can materially alter total phenolic content and antioxidant-related characteristics of the finished product (13,20). These compounds may add nutritional interest, but their presence should not be interpreted as evidence that the bars improve clinical outcomes.

The original manuscript reported a DPPH antioxidant assay, but several incompatible DPPH datasets appeared in different sections. Because the correct values cannot be identified without original analytical output, no DPPH result should be interpreted until the laboratory records are reconciled.

Similarly, the source describes a "total plate count" but later discusses growth of bacteria in MRS broth as if the procedure were a prebiotic-growth experiment. A conventional total plate count used for food-safety assessment and an experimental bacterial-growth assay answer fundamentally different questions. The current records are insufficient to determine which was actually performed.

This distinction prevents the available microbial values from being used to establish either microbiological safety or a prebiotic effect.

The original manuscript also states that sensory evaluation was performed using a hedonic scale for color, flavor, texture, taste, aroma, and overall acceptability. However, no valid numerical sensory dataset is supplied. One section identifies T4 as most acceptable, while another imported Discussion identifies T2 from an unrelated chickpea/persimmon formulation.

Consequently, no scientifically valid conclusion can currently be made regarding which pumpkin-seed/kidney-bean bar was preferred by panelists. This is important because a nutritionally dense food intended for children must also be acceptable in taste, texture, aroma, appearance, and portion size; food-bar studies routinely evaluate these attributes alongside composition (9,13).

High-energy protein-bar development studies emphasize that nutrient composition must be considered together with physical and sensory characteristics (9,13,20). A product with favorable laboratory composition may have little practical value if its texture, flavor, serving size, or gastrointestinal tolerance limit consumption.

The present bars also should not be described as ready-to-use therapeutic food. Previous development and evaluation of therapeutic foods for severe childhood malnutrition have involved deliberate formulation of energy, protein, fat, micronutrients, acceptability, safety, and clinical recovery outcomes (6,7,14). The present study did not establish equivalent specifications.

No children were enrolled, and no anthropometric, biochemical, appetite, growth, weight-gain, morbidity, recovery, or clinical-tolerance outcome was assessed. The present evidence therefore supports laboratory nutritional characterization only.

Before testing these bars clinically, additional food-science work is required. This should include complete amino-acid profiling, protein digestibility, true dietary-fiber analysis, antinutritional factors, mineral bioavailability, energy density, fatty-acid composition, microbiological safety, water activity, shelf-life testing, packaging stability, and a reproducible sensory study (11–13,18,19).

If the eventual objective is nutritional support for children with moderate or severe malnutrition, pediatric clinical research would then require age-appropriate formulation, safety assessment, ethically approved intervention protocols, defined serving size, comparison with accepted nutrition care, and clinically meaningful growth and recovery outcomes (7,14).

The study nevertheless provides a useful initial formulation dataset. It demonstrates that locally available pumpkin seeds and kidney beans can be incorporated into compact bars with substantial measured protein, lipid, fiber, phenolic, and mineral content.

CONCLUSION

Four pumpkin-seed- and kidney-bean-based bars were successfully formulated and nutritionally characterized.

Among the reported formulations, T4 had the highest measured crude protein, crude fiber, ash, total phenolic content, total flavonoid content, iron, calcium, zinc, and magnesium concentrations. T1 had the highest measured fat content.

Recalculation using the stated difference equation corrected a major error in the original nitrogen-free-extract results, showing a decline from 40.87% in T1 to 24.05% in T4 rather than an increase.

The findings identify potentially nutrient-dense prototype formulations but do not demonstrate prevention or treatment of protein-energy malnutrition in children. Clinical suitability will require additional nutritional-quality, food-safety, sensory, storage, and pediatric intervention studies.

LIMITATIONS

The study was conducted at laboratory scale and did not evaluate children with protein-energy malnutrition.

The four formulations differed in several ingredients simultaneously, preventing attribution of observed nutritional differences to a single ingredient.

Crude protein was measured, but amino-acid profile, protein quality, and digestibility were not assessed. The processing conditions for kidney beans were insufficiently characterized to evaluate reduction of lectins and other antinutritional factors.

The source ANOVA tables contained internal inconsistencies that prevented reliable inferential statistical interpretation without the original replicate-level dataset.

The reported DPPH results were contradictory across sections.

The identity of the microbial assay was unclear, preventing interpretation as either food-safety total plate count or prebiotic bacterial-growth testing.

No reproducible sensory dataset was available.

Energy density, water activity, microbial safety, shelf life, fatty-acid composition, vitamin content, mineral bioavailability, and antinutritional factors were not established.

RECOMMENDATIONS

The formulations should undergo independent repeat laboratory analysis using clearly documented replicate batches.

Kidney bean processing should be standardized and validated for reduction of antinutritional factors (11,12).

Future analyses should include amino-acid composition, in-vitro protein digestibility, total dietary fiber, phytate, energy density, fatty-acid profile, and bioavailable mineral assessment (12,18,19).

Microbiological safety should be evaluated using validated food microbiology methods, separately from any experimental prebiotic assay.

A properly designed blinded sensory evaluation should be performed with complete participant-level hedonic data, using reproducible sensory procedures appropriate for food-bar development (9,13). Shelf-life evaluation should include water activity, microbiological counts, oxidative stability, packaging conditions, and repeated sensory testing, consistent with multidimensional assessment used in therapeutic-food and high-protein-bar development (6,13).

Only after food-safety and compositional standards are established should the product be considered for ethically approved clinical feasibility studies in an appropriate pediatric population (7,14).

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