TECHNICAL BRIEF

Protein quality and bioavailability of indigenous crop-based ingredients and plant-based meat products in India

Evidence on the influence of crop choice, processing, and product format



Note: This image has been edited using AI.



Protein quality and bioavailability of indigenous crop-based ingredients and plant-based meat products in India

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FOREWORD

Over the years, the demand for dietary proteins have significantly increased due to the growing population, increased health and nutrition awareness among the consumers, urbanisation and rise of disposable incomes. Meeting the growing protein demand through animal proteins is environmentally not sustainable on the long run. Thus, there is an urgent need for the identification of sustainable alternative protein sources. Further, as India shifts its focus from food security to nutrition security, there is a need to prioritise nutritional quality over quantity. At the same time, advancing nutrition security must go hand in hand with building sustainable food systems that harness the nation's rich diversity of indigenous crops.

India is the world's largest producer of millets and pulses, with a rich diversity of crops that have long been integral to the country's food systems. Yet, many of these crops remain underexplored as sources of value-added protein ingredients. With the growing demand for alternative proteins and plant-based diet, the smart protein sector is constantly looking for nutritious, functional and resilient ingredients. Characterisation of the protein quality and functionality of India's native millets and pulses, coupled with appropriate processing and product development, could unlock opportunities for crop valorisation, domestic protein-ingredient production and diversified food applications. This is particularly relevant given the plant-based food sector's reliance on a relatively narrow range of protein sources and India's substantial dependence on imported protein isolates and concentrates.

An important consideration in this endeavour is that protein content alone does not determine nutritional quality. Amino-acid composition, digestibility and availability for absorption are equally important and can be influenced substantially by processing and the food matrix. Generating robust scientific evidence on these interactions is therefore essential for translating promising indigenous crops into nutritionally meaningful ingredients for alternative protein applications.

In this context, the present technical brief, ***Protein quality and bioavailability of indigenous crop-based ingredients and plant-based meat products in India*, presents findings** from a collaborative study undertaken by CSIR–Institute of Himalayan Bioresource Technology (CSIR-IHBT) and the Good Food Institute India. The study examines protein quality at different stages of the plant-based value chain, from selected indigenous millets and pulses and commercial plant-based meat products. By linking evidence on native ingredients, processing effects and nutritional quality, this work demonstrates that food processing can facilitate retention or even enhancement of nutrient digestibility and absorption.

Such collaborative efforts are important for translating scientific knowledge into solutions of relevance to industry and society. I hope this technical brief will stimulate further research, technology development and evidence-informed innovation, and contribute to building a diversified, nutritionally robust and self-reliant protein ecosystem for India.

Sudesh

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About the study partners

CSIR–Institute of Himalayan Bioresource Technology (CSIR-IHBT), a premier laboratory of the Council of Scientific and Industrial Research (CSIR), is located in Palampur in the ecologically rich Western Himalayas. Guided by its vision to advance the bioeconomy through sustainable use of Himalayan bioresources, IHBT offers an interdisciplinary research environment spanning medicinal and aromatic plants, plant biotechnology and molecular biology, natural product chemistry, microbial biotechnology, high-altitude agriculture, nutraceuticals, and bioresource valorisation. IHBT has developed several value-added food, nutraceutical, and wellness technologies from tea and Himalayan bioresources. The institute also focuses on isolating new bioactive metabolites from Himalayan medicinal plants, and developing green extraction systems for phytopharmaceuticals.

The Good Food Institute India (GFI India)* is the leading expert on India's emerging smart protein sector—the ecosystem of plant-based, cultivated, and fermentation-derived meat, eggs, and dairy. Drawing on India's unique strengths—including indigenous crops, a strong agrarian economy, low-cost technologies and infrastructure, an abundant talent pool, and biomanufacturing prowess—GFI India provides strategic market intelligence, ecosystem development support, and sector-focused advisory to institutional, academic, business, and civil society stakeholders across the smart protein value chain. Our work is also informed by the global research, technical expertise, and best practices shared by our international network of organisations across the U.S., Brazil, Europe, Israel, Japan, and APAC.

At GFI India, we're dedicated to positioning India as a global leader in the rapidly evolving smart protein sector.

*GFI India represents the brand name used by Alternative Protein Solutions Private Limited (APSPL).

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Executive summary

Protein quantity claims have become increasingly common across the packaged food industry. But do they paint the full picture? And do we need a more meaningful differentiator to assess nutritional value? The opportunity lies in moving beyond protein content to protein quality. India's plant-based sector can benefit from a shift in consumer-facing nutrition communication, from ***'x g of protein'*** **to** ***'x g of complete, digestible and bioavailable protein.'*** Benchmarking taste, texture, and cost parity of alternative proteins must be built on the foundation of more than just the equivalence of nutritional quantity, but quality as well. Future innovation in plant-based meat must prioritise protein quality and bioavailability, supported by robust evidence on how ingredient selection, processing, and cooking influence these metrics.

As the world's largest producer of millets and pulses, India has a significant opportunity to build a diverse and domestic protein ingredient ecosystem for the plant-based alternative protein sector. Realising this potential requires evidence-based crop selection. Further, the nutritional value of these crops must be assessed beyond the ingredient level, within the complex matrices of foods as consumed. At present, the Indian market comprises 200+ plant-based meat products across 20+ major formats, predominantly ready-to-cook. Back-of-pack labels declare protein content per 100 g or serving, but do not indicate how much of that protein becomes available for utilisation by the body after cooking and digestion.

This study assesses the protein quality of both ingredients and end-products, using measures of protein solubility, *in vitro* bioaccessibility and bioavailability. It also tests the hypothesis that **processing does not inherently compromise protein quality**. Specifically, it evaluates: (1) which biological or thermal processing approaches best enhance protein quality in millets and pulses before further processing; (2) the effects of dry versus wet fractionation on protein quality parameters; and (3) the effects of cooking on protein bioavailability across commercial plant-based meat formats, including chunks, keema, kebabs, patties and nuggets.

The key findings are as follows:

- **Protein content ≠ Protein digestibility ≠ Protein bioavailability.** Processing can substantially alter protein solubility, digestion, and the fraction potentially available for absorption, without corresponding changes in total protein content.
- **Processing effects are crop-dependent.** The optimal approach depends on the crop, the specific factors limiting protein digestion, and the mechanism by which a processing method mitigates these barriers.
- **Biological processing shows promise for millets.** Sprouting and fermentation improve the bioavailable fraction, suggesting their potential to enhance protein accessibility prior to extraction.

- **Pulses benefit from both thermal and biological processing.** Pressure cooking enhances the bioavailable fraction in chickpea and cowpea, while fermentation performs favourably across the pulses studied.

- **Extraction method must be aligned with ingredient application.** Wet fractionation results in higher protein concentration and bioavailable fraction for nutrition-focused applications, while dry fractionation better preserves solubility and can support functionality-driven, clean-label applications.

- **Cooking plant-based meat does not necessarily compromise protein quality.** In four of the five product formats tested, cooking either increases or retains the bioaccessible and bioavailable fractions of protein, with outcomes shaped by protein source, processing history and product format.

- **Plant-based meat can deliver comparable or higher bioavailable fractions of protein than animal-derived meat.** Across the product formats evaluated in this study, cooked plant-based meat products demonstrate a higher percentage bioavailability of protein than their corresponding animal-derived meat products. The use of protein concentrates that have undergone prior processing and partial structural modification may contribute towards accessibility to digestive enzymes and enhanced protein digestibility in plant-based meat products.

Overall, these findings indicate that protein quality and bioavailability are influenced by processing and product structure, rather than being determined solely by the plant- or animal-derived origin of the protein.

The findings support a shift from **protein quantity to protein quality** and from one-size-fits-all processing to **crop-, ingredient- and product-specific optimisation. Processing need not be a nutritional liability;** when appropriately tailored, it can **improve protein utilisation,** strengthen the assessment of protein quality in foods as consumed, and unlock **India's indigenous crops** for alternative protein applications.

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Background

The need to think beyond protein quantity

The protein conversation is often centred around quantity. But grams or percentage of protein is not a lone determinant of its dietary quality. According to the ICMR-National Institute of Nutrition's (NIN) Dietary Guidelines for Indians,[1] *'a diverse daily diet, including vegan diets, can readily meet protein and protein-to-energy requirements. However, protein quality remains a challenge because proteins from different sources vary in their essential amino acid composition, digestibility, and absorption.'* The average protein content of different plant-based meat products in India ranges between 9.1 and 20.8%. All product formats considered in GFI India's nutritional analysis study comply with the FSSAI's *'source of protein'* criterion and seven out of 11 formats meet the requirements for the *'high-protein'* claim. Nevertheless, analysis of amino acid composition indicates variations in sulphur-containing amino acids across product formulations. Generally, products including combinations of soy, wheat and pea proteins contain higher methionine levels than certain soy-only formulations. These findings highlight the importance of protein quality, protein source diversification and complementation.[2] Protein quality reflects the composition of its essential amino acids and their digestibility and bioavailability. Bioavailability is the fraction of a nutrient in food that the body absorbs and utilises after consumption.[3] The practical significance of protein bioavailability becomes pertinent when protein requirements need to be met from a limited number of calories. Particularly in individuals with reduced appetite or inability to masticate protein-containing foods such as meat, protein density, essential amino acid composition and bioavailability become important determinants of protein adequacy.[4]

The opportunity

Over 66% of Indian plant-based meat products rely heavily on soy or pea protein. But India currently imports nearly 90% of its plant protein ingredients, including soy protein isolates and concentrates, pea protein, gluten and vegetable protein blends.[5] Apart from limited domestic extraction and processing capacity, scientific evidence on the protein quality and functionality of India's native crops remains limited. There is a need to explore unconventional and indigenous sources of plant proteins to overcome these challenges. The effects of secondary processing on the protein quality of indigenous millets and pulses relevant to alternative protein applications also remain insufficiently characterised. Similarly, India-specific data on how cooking influences protein bioavailability in plant-based meat products, and how that compares with corresponding animal-derived products, are not available. Given that 83.3% of plant-based meat products in the Indian market are available in the ready-to-cook format, it is important to evaluate protein quality as consumed, not merely in the uncooked form.

02

Study design

Assessment of protein quality in native crops and prevalent plant-based meat formats in India: An *in vitro* approach

3	3	4	5
Millets	Pulses	Secondary processing approaches	Plant-based meat formats

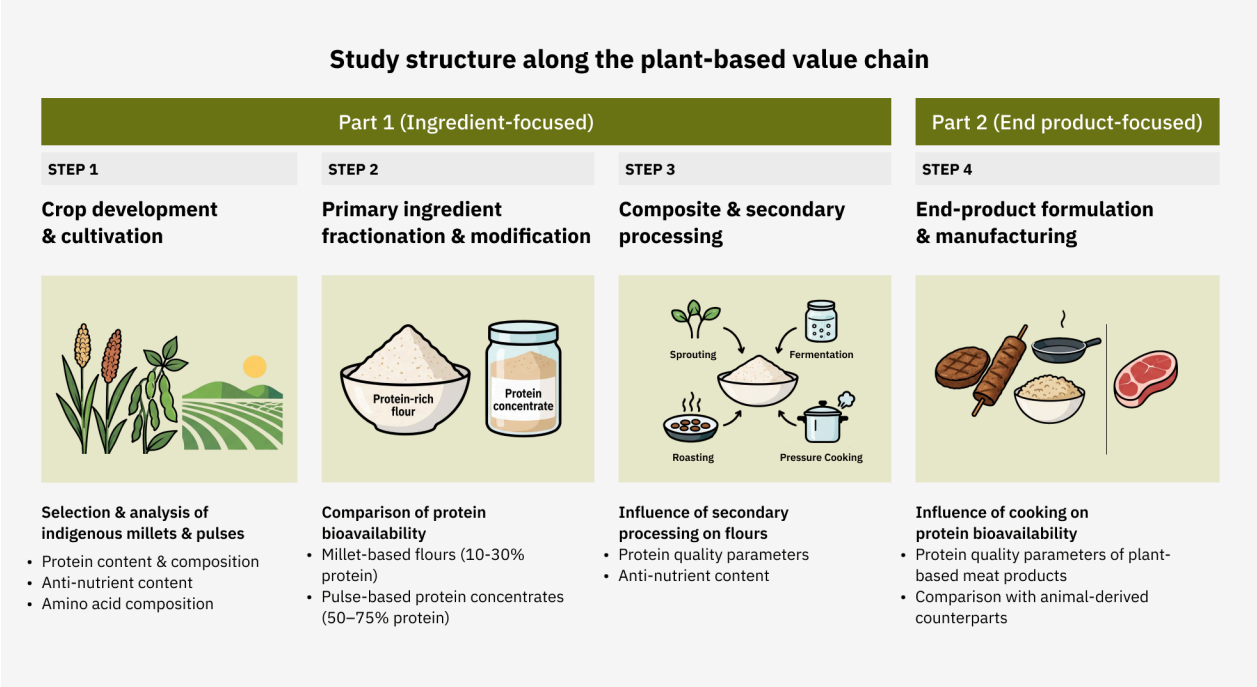
Research questions

Ingredients
Which secondary processing approach maximises protein extractability while retaining or improving protein quality?
How does processing interact with anti-nutritional factors?
How does secondary processing influence the i*n vitro* bioaccessibility and bioavailability of proteins?
What is the influence of the extraction method (dry *vs.* wet) on protein quality?

End-products
How does cooking affect protein quality across commercially available plant-based meat formats?
How do product format and matrix influence the *in vitro* bioaccessibility and bioavailability of proteins?
How does the *in vitro* protein bioavailability of cooked plant-based meat products compare with that of their animal-derived counterparts?

Study approach

The study deals with two stages of the plant-based value chain. Part 1 focuses on examining the protein composition and anti-nutritional factors (ANFs) (*Annexure-1*) *of* indigenous crop-based ingredients and the effects of secondary processing and extraction methods on their protein quality. Part 2 evaluates how cooking influences protein quality of plant-based meat products and compares the results with corresponding animal-derived products.



The indigenous crops were selected based on their production relevance, protein content and composition, amino acid profile (*Annexure-1*), and functional applicability to plant-based meat products. Commercially available plant-based meat products in the Indian market were shortlisted based on findings from GFI India's nutritional analysis[2] by considering their protein content, protein source, and market representation. Processing history and degree of structural complexity were also taken into account. The standardised INFOGEST Quant protocol was followed to estimate *in vitro* protein digestibility.[6] The INFOGEST digestion protocol has been evaluated against *in vivo* true ileal digestibility data, demonstrating a high degree of agreement and predictive capacity across a range of animal- and plant-based protein sources.[7,8] Further, the digesta was subjected to centrifugal ultrafiltration through a 3 kDa molecular-weight-cut-off (MWCO) membrane. The rationale for this integrated approach is explained below.

Conventionally, nutrient bioavailability is determined by *in vivo* experimental models. Although in vivo models provide accurate information on nutrient bioavailability, their long timelines and substantial resource requirements necessitate simpler, faster, high-throughput methods. In recent years, polarised epithelial cells (i.e., differentiated Caco-2 cell monolayers) have been used to determine nutrient bioavailability. However, the major challenge in using a cell line-based assay for bioavailability

determination is the compatibility of the food matrix with cells, as well as the inherent toxicity associated with the enzymes, reagents, and bile acid salts used to simulate food digestion *in vitro*. To counter this challenge, several techniques are employed, such as chemical enzyme inhibitors, temperature-induced deactivation, dilution with media/buffer, high-speed centrifugation, dialysis, and ultrafiltration. The aforesaid techniques have certain inherent disadvantages, such as interference from enzyme inhibitors in CaCo2 cells, loss of protein structure, degradation of small peptides and amino acids during thermal deactivation, a decrease in the quantity of analytes during buffer dilution and dialysis. Among these, ultrafiltration offers an economical and biocompatible option for processing large numbers of samples without altering the structure of the intestinal digestate.[9]

Ultrafiltration can be performed with different molecular-weight-cut-offs (MWCO) ranging from 10-30 kDa, suitable for the filtration of digestive enzymes. Literature clearly demonstrates that the pore size of tight junctions joining the intestinal epithelium has an average size (radius) of 5 nm that can allow passage of molecules with an average molecular weight between 4 and 5.5 KDa[10]. In this study, to offer a simple, high-throughput method for determining protein bioavailability, the intestinal absorption was simulated by filtering the intestinal digesta using a 3 kDa membrane that mimics the intestinal barrier pore size and the paracellular transport and passive absorption of digested proteins across the intestinal epithelium.[11]

The hypothesis is that the fraction released following simulated gastrointestinal digestion using the INFOGEST Quant protocol represents the bioaccessible protein fraction, while the permeate obtained by subsequent 3 kDa ultrafiltration, containing free amino acids and low-molecular-weight peptides, can be operationally considered the potentially absorbable fraction and quantified for its protein and amino acid components. The approach distinguishes the digestible fraction generated during gastrointestinal digestion from the lower-molecular-weight fraction potentially available for intestinal absorption.

Integration of ultrafiltration with INFOGEST-Quant provides an economical approach for screening protein bioavailability across food products and alternative protein ingredients. It may also support routine comparative testing without the resource and infrastructure requirements associated with cell-based absorption models, while providing a scalable platform for evaluating the effects of crop choice, processing, extraction and product formulation on protein bioavailability.

Study materials and parameters

Study component	Coverage
Millets	• Finger millet, pearl millet, and sorghum
Pulses	• Chickpea, mung bean and cowpea
Secondary processing approaches (Conditions of processing are explained in *Annexure-2*)	• Thermal processing: Roasting and pressure cooking • Biological processing: Sprouting and fermentation
Protein extraction methods	• Dry fractionation • Wet fractionation
Plant-based meat formats	• Keema • Kebab (shami kebab, seekh kebab) • Burger patties • Chunks • Nuggets
Cooking methods (Cooking conditions are explained in *Annexure-3*)	• Keema: Boiling • Kebabs, patties & nuggets: Deep-frying • Chunks: Pan (shallow) frying (As specified in the manufacturer's back-of-pack instructions)
Parameters of protein quality (Significance of these parameters and methodologies for quantification are explained in *Annexure-4*)	• Total crude protein (TP) (% w/w) • Protein solubility index (PSI) (% w/w) • Digestible free amino acids or bioaccessible fraction (DFAA) (% w/w) • Bioavailable free amino acids or bioavailable fraction (BFAA) (% w/w) • Percentage bioavailability (%)

% w/w refers to g per 100 g of millet or pulse flour, relative to the ingredient. For plant-based meat products, % w/w refers to g per 100 g of product.

Rationale for the selection of study materials and processing approaches

1 Selection of millets and pulses

Finger millet, pearl millet, sorghum, chickpea, mung bean & cowpea

The crops were selected to represent widely cultivated or strategically relevant Indian millets and pulses with potential for domestic protein-ingredient production. Selection considered:

- **Production and supply potential:** Domestic availability, production scale, climate resilience and crop diversification.
- **Protein potential:** Pulses contain 18.6–22.7% protein, compared with 6.5–9.8% in millets.
- **Compositional diversity:** Millets and pulses show contrasting storage protein fractions and amino acid profiles, enabling investigation of crop-specific responses to processing.
- **Application relevance:** Nutritional & functional suitability for plant-based meat ingredients and protein blends.

Pulses



Chickpea | Mung bean | Cowpea

Millets



Finger millet | Pearl millet | Sorghum

2 Selection of processing approaches

Thermal & biological processing mechanisms

These approaches were selected because they are established, industrially scalable, or transferable to commercial processing systems and act through different mechanisms.

- **Thermal treatments** can disrupt cellular structures, denature proteins, and reduce heat-labile antinutritional factors.
- **Biological treatments** can activate endogenous enzymes or microbial activity, modify the grain matrix, and reduce selected antinutrients.

Comparing both categories enabled assessment of whether processing effects depend on crop composition and the dominant barriers to protein digestion.

Thermal process

- Roasting
- Pressure cooking

Biological process

- Sprouting
- Fermentation

3 Selection of plant-based formats

Kebabs/seekh kebab, keema/mince, patties, nuggets & chunks

The five formats were selected from the Indian plant-based meat market based on four criteria.

- **Market relevance:** Formats considered account for ~59% of products in the Indian market
- **Protein relevance:** Comparatively high protein content (9-21%)
- **Structural diversity:** From particulate & formed matrices to denser, anisotropic fibrous structures.
- **Processing diversity:** A wide range of formulations, processing histories, and cooking methods, each of which can influence protein quality

Indian market share



Kebabs/ seekh kebab	Keema/ mince	Patties
18.3%	**14.2%**	**12.5%**



Nuggets	Chunks
9.2%	**5.0%**

03

Key findings: Indigenous ingredients

The effect of secondary processing on protein bioavailability is crop-dependent

Ingredient-level protein quality assessment can guide the selection of protein sources, optimisation of extraction processes, and development of protein blends for alternative protein applications. Crops with similar protein content may differ significantly in digestibility and bioavailability, which can be further modified by secondary processing. Comparing ingredient-level protein quality data and understanding processing effects can therefore help manufacturers identify promising underutilised Indian crops and select between protein concentrates, isolates, and flours.

This study demonstrates that the effects of a given processing method on protein quality vary across crops. As discussed in the background section, crude protein content is insufficient to determine a food's ability to meet metabolic requirements.[12,13] While the total protein content did not vary significantly across the four secondary processing approaches, the other protein-quality parameters exhibited varying degrees of change.

Salient signals in the data

- **Protein solubility index (PSI)** provides a practical measure of protein extractability and solubility, enabling comparison of the processing behaviour of raw materials for developing protein-rich flours, concentrates and isolates. Further, recent interlaboratory validation supports PSI as a reproducible metric across diverse plant protein materials.[14]

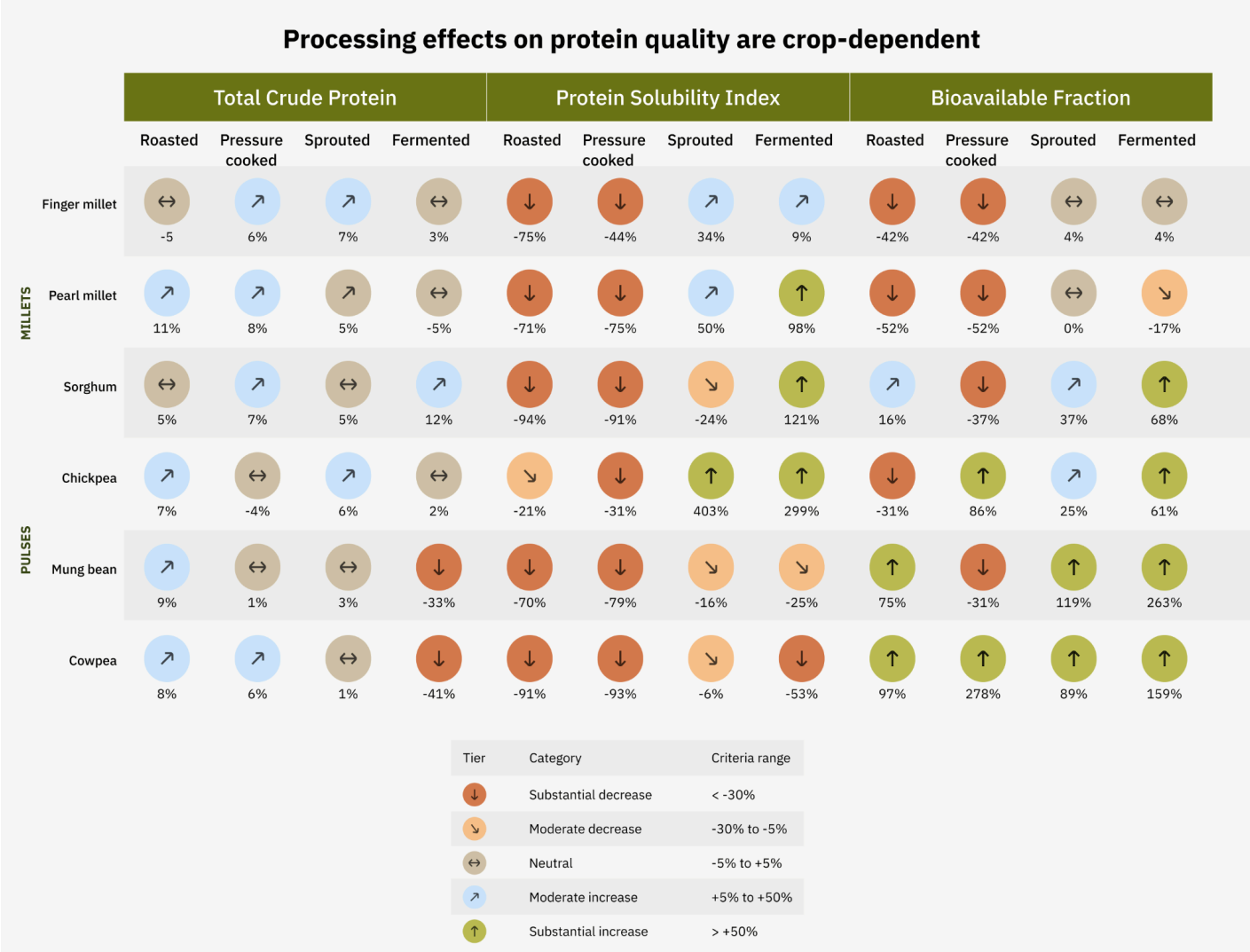
- **Finger millet:** Sprouting increased PSI by **34%**, but did not significantly increase BFAA (**4%**). This suggests that improved extractability under the PSI conditions did not necessarily translate into greater release of potentially absorbable amino acids during simulated digestion. Therefore, protein solubility and bioavailable fraction appear to represent distinct attributes that can respond differently to processing.

- **Pearl millet:** Fermentation resulted in the largest increase in PSI (**98%**) relative to the raw control, whereas sprouting maintained the BFAA fraction at control levels, highlighting the differential effects of processing approaches on protein solubility and the bioavailable fraction. Fermentation can enhance protein solubilisation through acidification, proteolysis and modification of protein-matrix interactions, while the eventual release of amino acids during digestion remains dependent on protein structure and digestive enzyme accessibility.[15,16]

- **Sorghum:** Fermentation increased the bioavailable fraction significantly (**by 68%**), suggesting its ability to enhance the release of potentially absorbable amino acids during digestion.

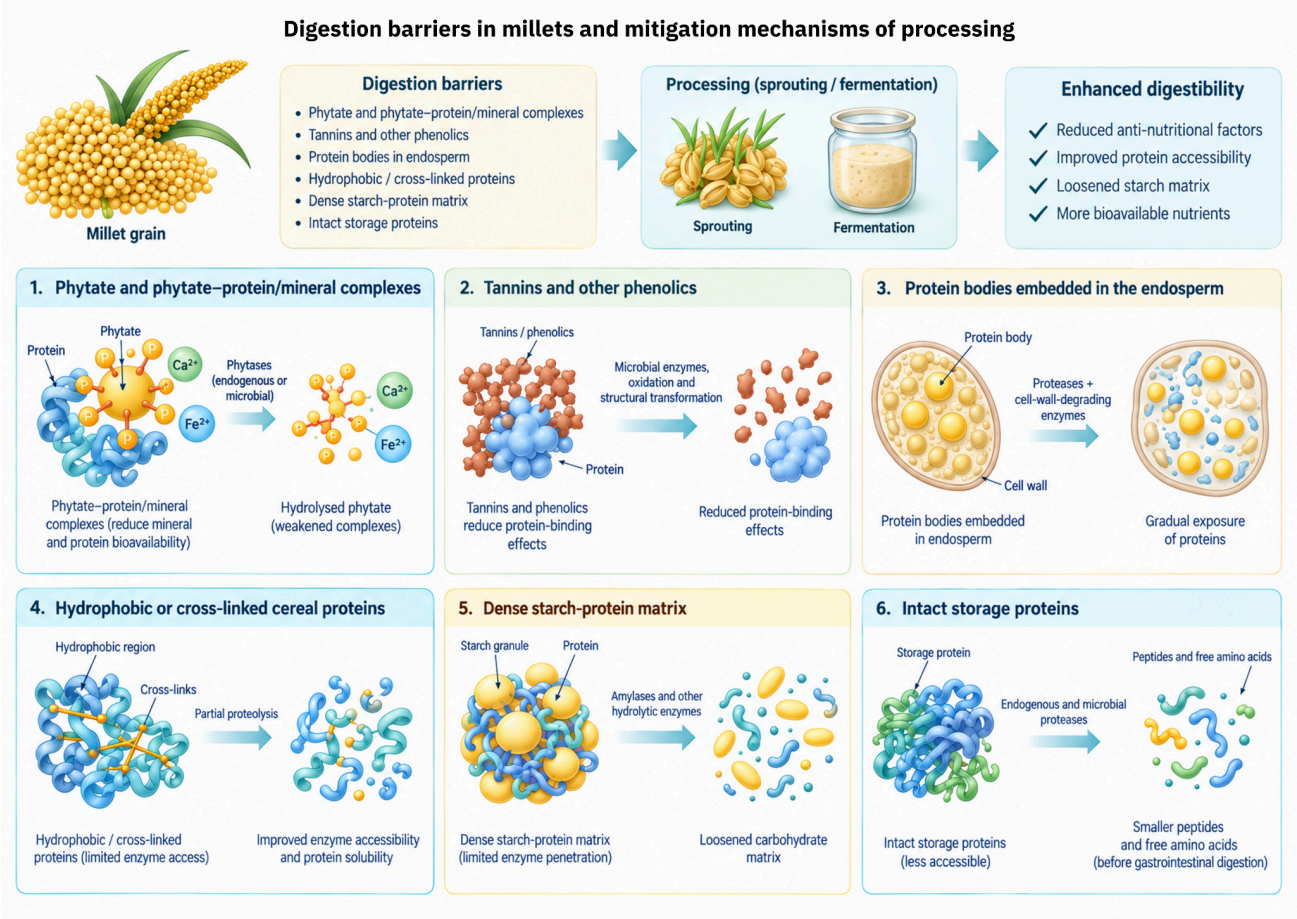
- **Pulses:** Pressure cooking led to the **highest enhancement of BFAA in chickpea (by 86%) and cowpea (by 278%)**, compared to the unprocessed control ingredient; whereas fermentation substantially improved the BFAA in mung beans **(by 262%)**. This highlights the crop-specific effects of processing on the bioavailable fraction.

Overall, processing effects were crop-dependent. While biological treatments were generally favourable for millets, pulses responded positively to both thermal and biological treatments. A possible explanation could be crop-specific interactions between the dominant digestion barriers and the ways in which processing modifies the food matrix to mitigate these barriers. In millets, the barrier to protein digestion is often the complex cereal matrix and polyphenol-phytate interactions. Whereas, in pulses, the major constraints include heat-labile protease inhibitors, lectins, compact storage proteins and resistant cotyledon structure.

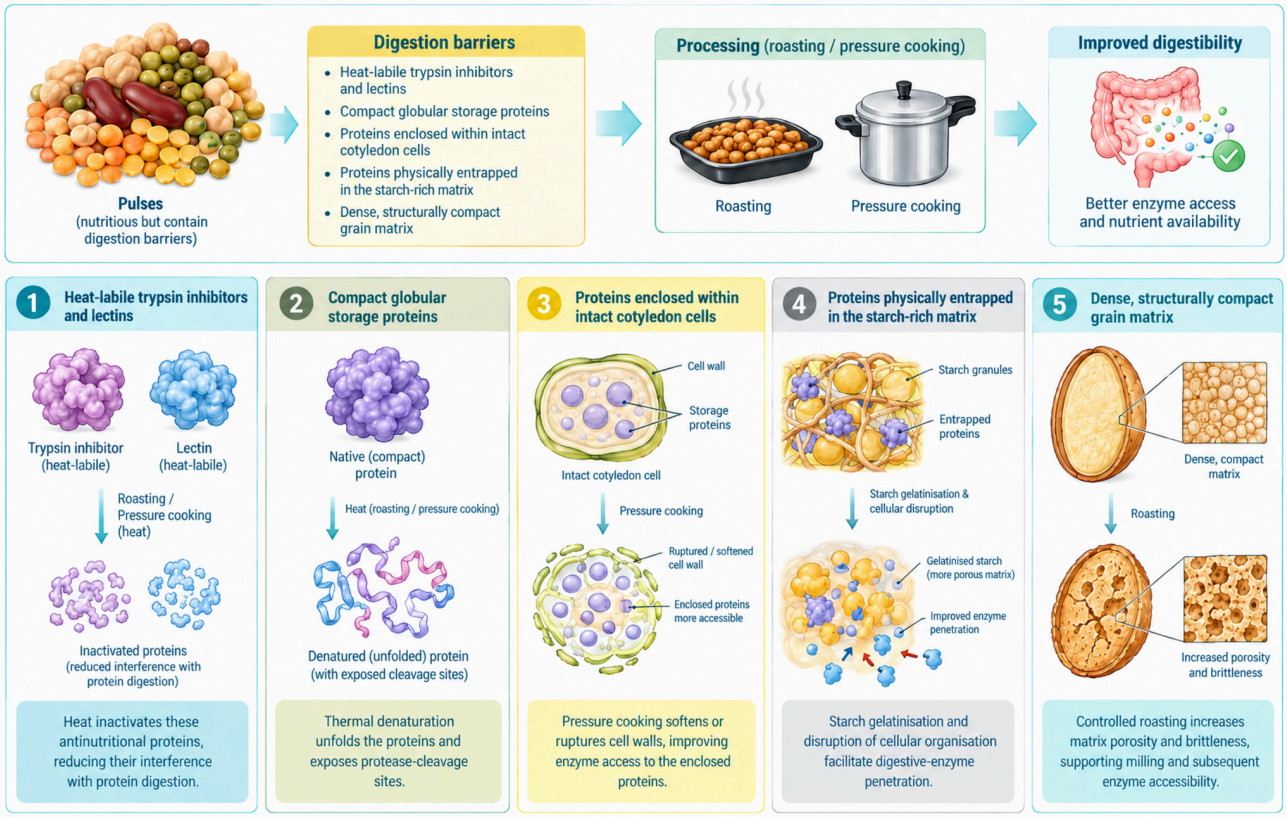


Percentage change was calculated relative to the unprocessed control as [(processed-control)/control] x 100). Positive and negative values indicate increase and decrease relative to the unprocessed control.

Thermal processing may offer some advantages for millets, as heating can soften the grain matrix and reduce some antinutrients, but its effects on millet proteins are not uniformly beneficial. For example, in sorghum, kafirins are hydrophobic prolamins located in compact protein bodies. Heating can promote disulfide cross-linking between kafirin fractions, protein aggregation, interactions between proteins and gelatinised starch and formation or strengthening of protein–polyphenol complexes. These changes can reduce protease access in cooked grains.[17,18] Excessive roasting can additionally produce Maillard reaction products and lower reactive lysine availability. Heating partially opens up the protein structure and leads to their aggregation, affecting the solubility and thus affecting protein release adversely. Biological processing may be better suited to the slow enzymatic deconstruction required by these cereal matrices. Importantly, thermal processing-induced modifications are not exclusive to plant proteins; meat proteins are also vulnerable to thermal processing-induced modifications,[19] which may reduce their solubility and alter their susceptibility to gastrointestinal enzymes during digestion.[20]



Digestion barriers in pulses and mitigation mechanisms of processing



The effects of the four secondary processing methods on ANFs are summarised below.

Effect of processing on the anti-nutritional factors of millets and pulses

Crop group	Crop	Processing method	Tannins	Phytic acid	Saponins	Trypsin inhibitors
Millets	Finger millet	Sprouting	↓↓	↓	↓↓	↓↓
		Roasting	↓	↓	↓	↓↓
		Pressure cooking	↓↓	↓	↓	↓↓↓
		Fermentation	↓	↓	↓	↓↓
	Pearl millet	Sprouting	↑	↓	↓↓	↓
		Roasting	↓	↓	↓↓	↓↓↓
		Pressure cooking	↓	↓	↑	↓
		Fermentation	↓	↓	↓	↓↓↓
	Sorghum	Sprouting	↑↑	↓	↓↓	↓↓↓
		Roasting	↑	↓	↓	↓↓↓
		Pressure cooking	↑	↓	↑↑	↓↓↓
		Fermentation	↓	↓	↑↑	↓↓↓
Pulses	Chickpea	Sprouting	↑	↓↓	↓	↓
		Roasting	↓↓	↓	↓	↓↓↓
		Pressure cooking	↓	↓	↑	↓↓↓
		Fermentation	↓↓	↓	↓	↓
	Mung bean	Sprouting	↓↓↓	↓↓	↓	↓↓
		Roasting	↓↓	↓	↓	↓↓↓
		Pressure cooking	↓	↓	↑↑	↓↓↓
		Fermentation	↓↓	↓	↓	↓
	Cowpea	Sprouting	↓	↓	↓	↓↓↓
		Roasting	↓	↓	↓	↓↓
		Pressure cooking	↓↓	↓	↑	↓↓↓
		Fermentation	↓↓	↓	↓	↓↓

↓ 0-33% decrease ↓↓ 33-66% decrease ↓↓↓ 67-100% decrease
↑ 0-33% increase ↑↑ 33-66% increase

Based on the above observations and rationale, the most appropriate secondary processing approaches to achieve maximum protein bioavailability for each crop-based source considered in this study are listed below.

Crop	Favourable processing approaches for high protein bioavailability
Finger millet	Sprouting
Pearl millet	Sprouting
Sorghum	Roasting, sprouting, and fermentation
Chickpea	Pressure cooking and fermentation
Mung bean	Sprouting and fermentation
Cowpea	Pressure cooking and fermentation

How is this data relevant for the industry?

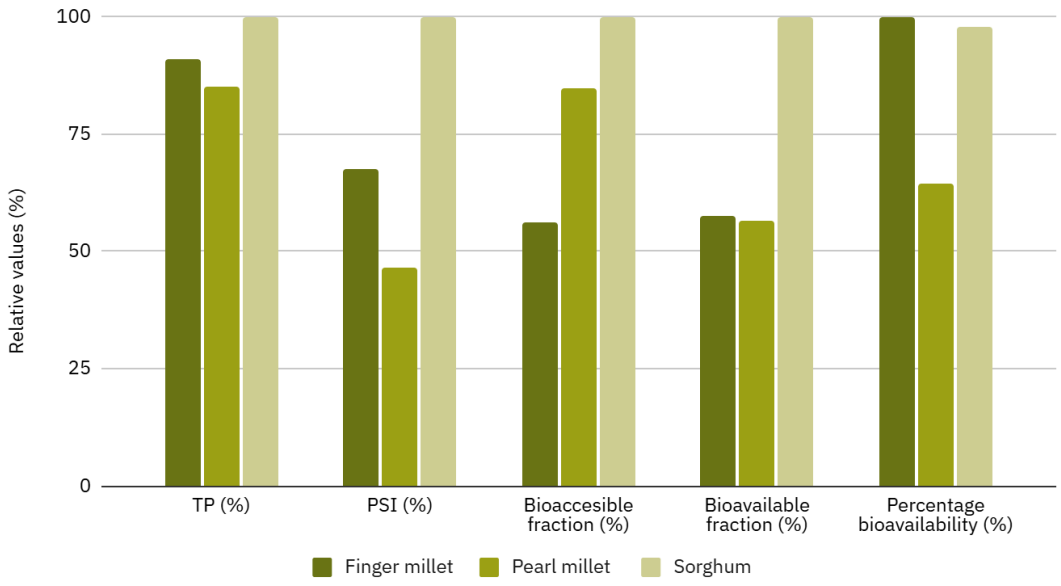
Biological processing enhances protein extractability, while targeted thermal processing can improve the availability of absorbable amino acids.

- **For millet protein manufacturing, sprouting and fermentation can be explored as pre-treatment steps** to enhance protein extractability and potentially reduce solvent requirements associated with alkaline or enzymatic extraction, without adversely affecting protein bioavailability.
- **For pulses, sprouting and pressure cooking can be used as effective pre-treatments to reduce anti-nutritional factors** and enhance protein bioavailability.
- **Where pressure cooking presents scale-up constraints, commercially scalable moist-heat treatments such as steaming can be integrated with fermentation** to reduce anti-nutritional factors and improve protein bioavailability.
- **Pressure-cooked and fermented pulses offer potential as raw materials for soluble protein ingredients,** with applications in instant plant-protein mixes, beverages, and protein-fortified foods.

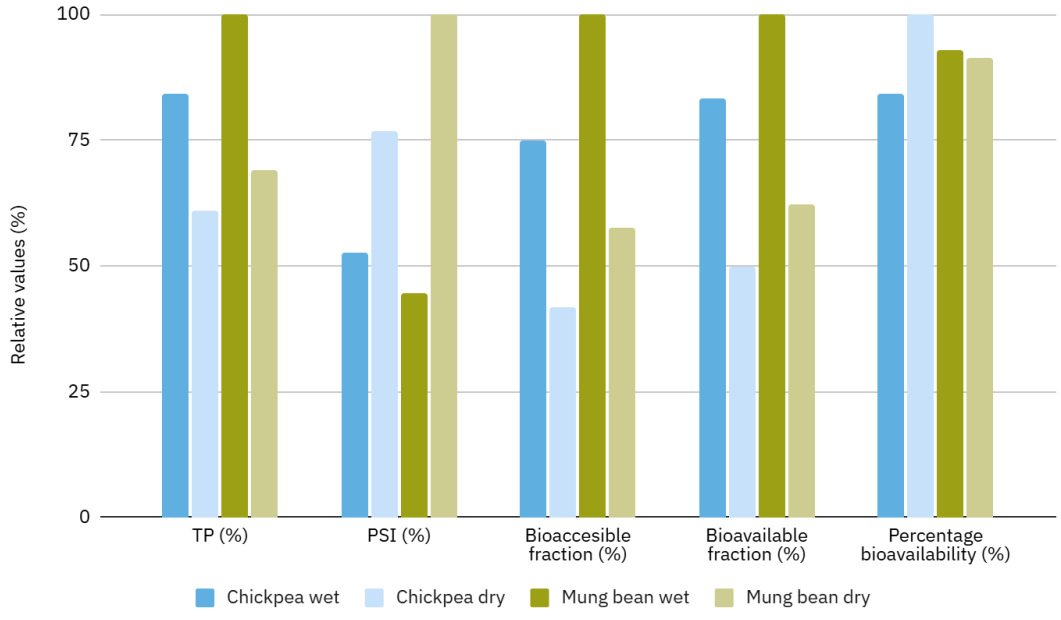
Influence of protein extraction method on protein quality

Comparative protein quality of millets and pulses obtained through dry and wet fractionation

Protein-rich millet flours from wet fractionation



Pulse protein concentrates from wet and dry fractionation



Millet-derived protein-enriched flours: Differences across crops

- **Sorghum demonstrated relatively higher values for all the protein quality parameters.** This observation could be because of its highest PSI and accessibility to digestive enzymes. However, its percentage bioavailability was similar to finger millet rather than significantly superior. Sorghum proteins, particularly kafirins, can have relatively low digestibility because of disulfide cross-linking, protein–starch associations and interactions with tannins. Processing that disrupts these structures or protein–phenolic complexes can substantially improve protease accessibility and protein bioaccessibility.[21,22] Thus, the extraction conditions used in this study may have generated a comparatively accessible sorghum protein matrix.

- **Finger millet showed moderate bioavailable fractions but a high conversion efficiency.** Its percentage bioavailability was close to sorghum, despite substantially lower BFAA. This variation may be attributed to differences in protein fraction composition, protein–starch interactions and limited accessibility of some protein fractions to digestive enzymes. Thus, a combined interpretation of percentage bioavailability and absolute bioavailable protein is necessary. An ingredient with lower protein content can have a high fraction of its digestible protein become bioavailable while still providing less bioavailable protein per unit of ingredient.

- **Pearl millet showed the lowest values for PSI and percentage bioavailability.** The divergence between the bioaccessible fraction and the percentage bioavailability suggests that the accessibility of free amino acids alone may not determine final protein utilisation. Differences among millet species in storage-protein composition, matrix organisation, phenolics/phytate and protein–starch interactions can alter both enzymatic accessibility and downstream peptide/amino-acid availability.

- Overall, the results demonstrate that millet processing responses are strongly crop-dependent.[21,23]The contrasting patterns among finger millet, pearl millet and sorghum suggest that extraction can alter protein solubility, accessibility and digestibility differently depending on crop-specific protein composition and matrix interactions. Thus, crop selection and extraction conditions should be optimised together when developing protein-enriched cereal ingredients.

Why higher solubility doesn't always lead to higher bioavailability?

Protein solubility index is useful, but it is not a direct indicator for digestibility, bioaccessibility or bioavailability. Solubility primarily describes how much protein can disperse or dissolve under the test conditions. Bioavailability additionally depends on protein structure, susceptibility to gastrointestinal proteases, molecular size of released peptide sub-units, matrix interactions and anti-nutritional factors.

Pulse-based protein concentrates: Wet versus dry fractionation

While wet fractionation results in greater protein purification through solubilisation and precipitation, dry fractionation retains more of the native protein matrix and preserves substantially higher protein solubility. This closely agrees with published comparisons showing higher solubility in dry-fractionated pulse proteins.[23,25] Unlike wet extraction, milling and air classification avoid the extreme pH shifts, precipitation and drying steps, which can promote protein unfolding, aggregation or loss of native functionality. In both chickpea and mung bean, wet-extracted ingredients showed markedly higher protein quality than their dry-fractionated counterparts. Comparable studies have also found higher protein contents in wet-fractionated pulse ingredients and, higher *in vitro* digestibility in several crops.[23,24] While the bioaccessible and bioavailable fractions remained lower for dry-fractionated chickpea, the percentage bioavailability was higher. This shows the distinction between absolute yield and conversion efficiency. A plausible explanation is that dry fractionation retains proteins in a less structurally altered, more soluble state, allowing a greater proportion of the digested material to reach the bioavailable fraction. Published industrial-scale comparisons similarly report higher solubility and reduced structural disruption in dry-fractionated pulse ingredients. Notably, dry-fractionated chickpea has also shown particularly favourable digestibility-corrected protein quality despite lower protein purity.[24] In the case of mung, the crop-specific nature of this response is reinforced: dry fractionation more than doubled PSI, while bioavailability remained essentially similar to wet fractionation. Dry-fractionated mung bean concentrates have been reported to retain native protein composition and show competitive protein quality relative to isolates.[26]

Wet-extracted cowpea had the highest total protein (76.45%) among all ingredients assessed, but its percentage bioavailability (35.9%) was close to finger millet and sorghum. Therefore, high protein density and digestibility do not automatically translate into a higher percentage bioavailability.

From extraction to application: Matching the protein ingredient to its purpose

Protein quality is a function of both crop and processing route. The same extraction approach can produce markedly different nutritional outcomes across protein sources. Therefore, extraction should be optimised for protein quality and utilisation, rather than protein concentration alone. Wet fractionation is suited to nutrition-focused applications where higher protein concentration and absolute bioavailable protein are priorities. Dry fractionation can support functionality-driven applications where solubility is valued. Sorghum shows particular promise for protein fortification and dispersible functional foods, while pulse-derived ingredients offer flexibility across nutrition and clean-label applications.

04

Key findings: Plant-based meat products

Cooking can improve protein bioavailability, but processing history and product format matters

Understanding protein bioavailability of plant-based meat products can guide product reformulation, cooking recommendations and nutrition-focused product positioning. Importantly, such assessment reflects protein quality in the product as consumed, which cannot be reliably inferred from crude protein content or ingredient-level data alone.

Cooking enhanced the protein bioavailability in four out of five product formats considered in this study. However, the degree of influence on percentage bioavailability varied with protein sources (single source or blends), processing history of proteins (degree of refinement, extruded *vs.* formed, hydration, structuring), product matrix (degree of anisotropicity or fibrous structure), and cooking conditions.[27,28,29]

Keema

Though the increase in bioaccessible fraction was statistically insignificant after boiling, percentage bioavailability showed a steep reduction from 62% to 16%. This indicates that boiling-induced changes in the product matrix affected the peptide release during *in vitro* intestinal absorption. The plausible reasons could be leaching of free amino acids into the boiling medium, heat-induced amino acid reactions, interaction of amino acids or peptides with other formulation components, and aggregation of previously soluble protein fractions. These possibilities are reasonable given keema is a highly fragmented matrix and relatively isotropic, wherein boiling can cause further structural breakdown and promote loss or sequestration of small bioaccessible nitrogenous compounds.

Chunks

Given their extrusion-based processing and highly organised, anisotropic structure relative to the other plant-based meat formats, the findings regarding chunks are particularly pertinent. The overall positive effect on bioaccessible and bioavailable fractions of protein can be attributed to the possible disruption of the previously extruded chunk matrix caused by pan-frying. Frying is likely to have caused major structural reorganisation and the resultant matrix disruption could have exposed proteins without producing an excessively resistant aggregate. Subsequent cooking can substantially enhance enzyme accessibility when it disrupts or loosens the architecture created by extrusion. Though extrusion involves high temperature, pressure and shear, which can alter certain heat-sensitive nutrients, it can also reduce ANFs and improve protein digestibility. Therefore, the protein quality of extruded products depends on the ingredients, processing history and matrix structure. This interpretation is also consistent with published literature showing that extrusion conditions can matter more than the actual cooking method in determining the digestibility of plant-based meat products.[27]

Kebabs & patties

The Shami kebab, seekh kebabs and the patty product containing textured soy protein demonstrated the most optimistic results. The effects shift toward neutral or negative in the second patty product made with a combination of soy and wheat proteins. Formed patties and kebabs occupy an intermediate structural state. They generally contain hydrated protein, oils, starches/hydrocolloids, fibres, vegetable particulates, and binders, which are held together by relatively discontinuous networks. Deep frying these products can lead to several competing phenomena that could be positive or negative for protein quality. Positive effects include protein unfolding, disruption of particulate structures, matrix softening, and increased exposure of cleavage sites. Potentially negative consequences are aggregation, disulfide interchange, protein–polyphenol interactions, Maillard chemistry, dehydration and surface crust formation. Thus, the effect of frying on protein bioavailability is governed by whether thermal disruption exceeds thermal aggregation.

Nuggets

Nuggets containing soy granules represent a comminuted composite matrix in which hydrated protein particles are held together with other formulation components. A 1.5-fold increase in percentage bioavailability suggests that frying particularly enhanced the release or transportability of low-molecular-weight peptides and free amino acids during the intestinal absorption stage. Deep-frying may have promoted protein unfolding, softened or disrupted the particulate matrix, and increased exposure of enzymatic cleavage sites. Although frying can also induce aggregation, Maillard reactions, dehydration and crust formation, these effects did not appear to outweigh the improvements in protein digestion and intestinal availability. Thus, for this nugget product, thermal disruption and matrix reorganisation appear to have dominated over the potentially adverse effects of heat-induced aggregation.

Effect of cooking on protein digestibility and bioavailability of plant-based meat products

Parameter	Burger patty	Keema	Chunks	Seekh kebab	Nuggets
Protein source	Soy	Pea protein + soy protein isolate	Pea protein isolate + soy protein isolate	Soy	Soy granules
Cooking method	Deep frying	Boiling	Pan frying	Deep frying	Deep frying
Total crude protein	↑ 21%	↓ -4%	↓ - 3%	↑ 33%	↑ 42%
Bioaccessible fraction	↑ 34%	↔ 1%	↑ 186%	↓ -10%	↑ 69%
Percentage bioavailiability	↑ 31%	↓ -74%	↑ 138%	↑ 29%	↑ 57%

Parameter	Burger patty		Seekh kebab		Chunks	
	Brand 1	Brand 2	Brand 1	Brand 2	Brand 1	Brand 2
Protein source	Soy	Soy + wheat Protein	Soy	Soy + wheat Protein	Pea protein isolate + soy protein isolate	Pea protein isolate
Total crude protein	↑ 21%	↑ 31	↑ 33%	↑ 34%	↓ -3%	↑ 134%
Bioaccessible fraction	↑ 34%	↔ 4%	↓ -10%	↑ 43%	↑ 186%	↑ 528%
Percentage bioavailability	↑ 31%	↓ -7%	↑ 29%	↑ 9%	↑ 138%	↑ 54%

● Significant increase ● No significant change ● Significant decrease

~ % change = [(processed - raw)/raw] x 100

Processing can be a pathway to improved protein utilisation—not a limitation.
Formulation—especially protein source, processing history, and product matrix—govern the protein bioavailability.

05

Comparative context

Plant-based meat vs. animal-derived meat

Plant-based meat is intended as an alternative to conventional meat, rather than replacing other plant-based protein foods, such as beans or lentils. Hence, while considering the nutritional quality of plant-based meat products, it is important to compare them to conventional meat products.[30] The study shows that higher total protein content in the animal-derived products does not consistently translate into higher bioavailable fraction. Instead, the comparative outcome varies with product format and the endpoint considered.

Plant-based vs. conventional burger patties

For burger patties, the animal-derived product contained more crude protein after cooking (18.62%) than either of the two plant-based patties (11.03% and 16.96%). It also had higher DFAA (1.74%) compared to the plant-based counterparts (0.91% and 1.07%). However, this advantage was not retained with the proportion of bioaccessible fraction that became bioavailable. **After cooking, percentage bioavailability was 56.63% and 57.96% for the two plant-based patties, compared with only 21.22% for the animal-derived patty.** Thus, although a greater absolute quantity of protein was digested from the animal-derived patty, a substantially greater proportion of the amino acids released during digestion was bioavailable in the plant-based products.

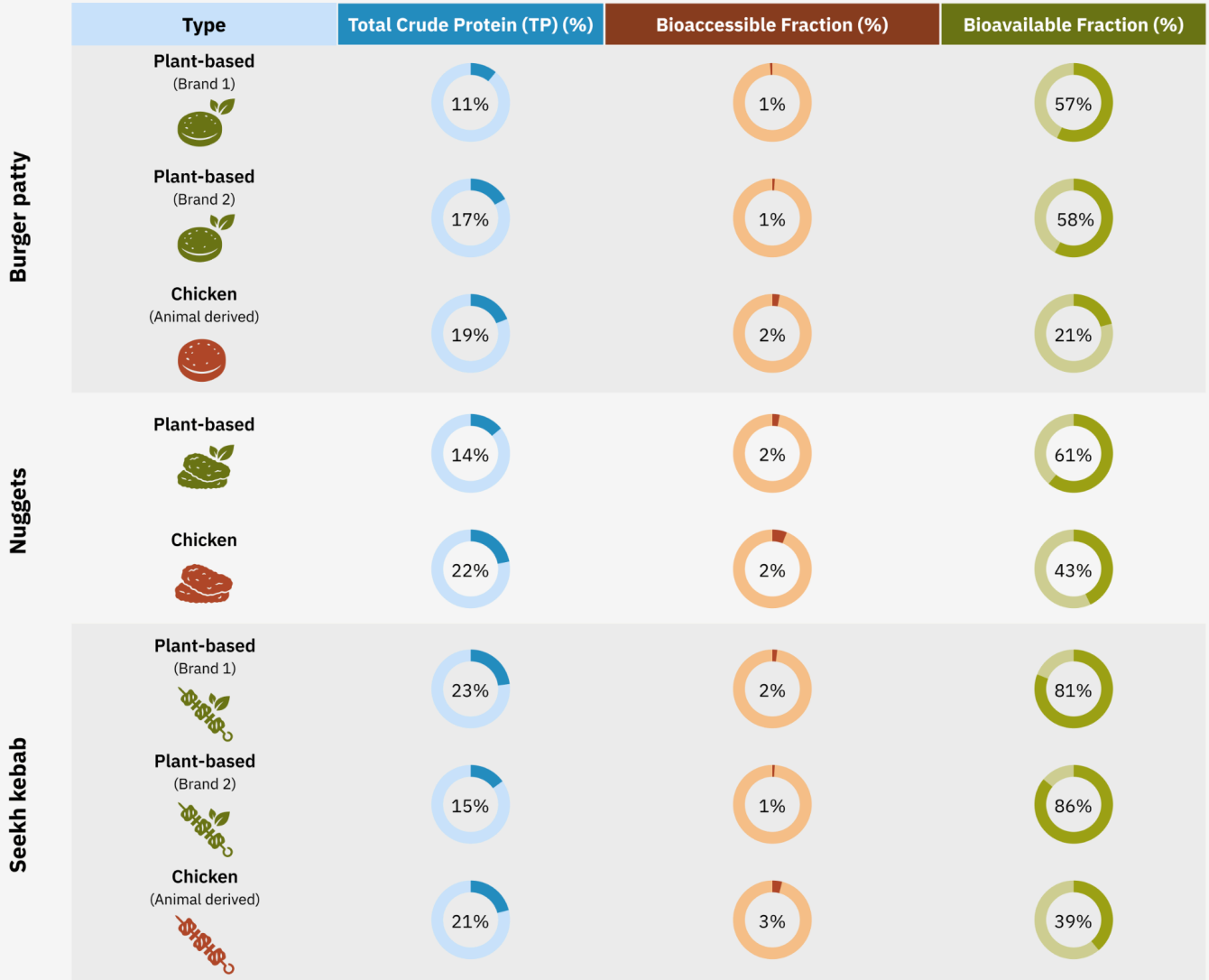
Plant-based vs. chicken nuggets

A similar distinction was observed for nuggets. Following deep-frying, the chicken nugget had higher crude protein (22.37%) than the soy-granule-based nugget (14.47%). In contrast, there was no statistically significant difference between the bioaccessible (1.52%) and bioavailable (0.93%) protein fractions in the cooked plant-based nugget and those in the chicken nugget (1.88% and 0.82%, respectively). **Notably, the percentage bioavailability of the plant-based nugget was 61.15%, compared to 43.39% for the chicken nugget**. Cooking increased the bioavailable fraction in both products, but the plant-based nugget retained the higher post-cooking value.

Plant-based vs. chicken kebabs

The comparison was more nuanced in the case of the kebabs. **The two cooked plant-based kebabs showed percentage bioavailability of 80.75% and 86.09%, compared with 38.81% for chicken kebabs**. The response to cooking was also markedly different: percentage bioavailability increased from 73.97% to 80.75% and from 66.57% to 86.09% in the two plant-based kebabs, whereas it decreased from 49.75% to 38.81% in the animal-derived product. This indicates that the effect of cooking on the proportion of the bioaccessible fraction becoming bioavailable was strongly dependent on the product matrix.

How does the protein quality of plant-based meat compare with conventional meat?



Collectively, these findings reveal an important distinction between the amount of protein digested and the subsequent bioavailability of digestion products. Across the three formats, the animal-derived products demonstrated higher protein content and effective gastrointestinal digestion. However, all five cooked plant-based products exhibited higher percentage bioavailability than their corresponding cooked animal-derived products. The findings show that higher crude protein content or even greater protein digestibility does not necessarily predict the proportion of bioaccessible fraction that becomes bioavailable.

Factors influencing differences in protein bioavailability between plant- and animal-based meats

1. Differences in the molecular profile of digestion products

Plant- and animal-derived proteins can generate different mixtures of free amino acids and peptides during gastrointestinal digestion. Their molecular size, composition, charge and hydrophobicity can influence subsequent passage through an intestinal-barrier model. Further, plant-based meat products are typically formulated using protein concentrates or isolates, which may exhibit greater protein digestibility and bioavailability than their source ingredients due to their lower fibre content and reduced levels of anti-nutritional factors. Conventional meat products, particularly fatty and certain processed formats, may contain substantial amounts of fat/saturated fat. Although dietary lipids can influence the digestion of proteins through food-matrix effects and changes in enzyme accessibility, the extent to which they contribute to differences in *in vitro* protein bioaccessibility requires further investigation. Such differences may also depend on protein structure, product formulation, and processing conditions. Thus, the plant-based products may have generated a more readily transportable pool of digestion products, even where the total quantity released was lower. This interpretation is consistent with emerging comparative studies showing that digestion behaviour cannot be predicted simply from protein origin. For example, incorporation of fava-bean protein into beef burgers increased the post-digestion availability of several free essential amino acids.[31]

2. Processing of the protein ingredients before end-product manufacturing

The soy granules, textured soy/wheat proteins and related ingredients used in plant-based meat have already undergone substantial processing, such as protein fractionation and, commonly, extrusion, before being incorporated into the final product. These operations can cause protein unfolding, disruption of native cellular structures and inactivation or reduction of some ANFs, potentially increasing enzyme accessibility and altering the peptides generated during digestion. Processing effects are particularly important for plant proteins, as protein digestibility depends not only on protein source but also on food matrix, interactions with other food components, processing and storage; thermal processing can also improve plant-protein digestibility through reduction of ANFs.[32]

3. Greater disruption of the plant-based product matrix during cooking and digestion

Cooking can unfold proteins and reorganise the surrounding matrix. In products made from hydrated textured proteins or protein granules, frying may disrupt the structured network and increase accessibility of digestive enzymes. Although the magnitude varied by format, the increased bioavailable fraction after cooking in all five plant-based products could be attributed to the above reason. This could be particularly relevant to nuggets and seekh kebabs, where the product consists of restructured or comminuted protein particles embedded within a composite formulation, rather than an intact muscle structure.

4. Cooking may affect the two matrices differently

The effect of heating on protein digestion and bioavailability depends on the processing conditions and the resulting structural changes in the food matrix. Heating can promote protein unfolding and expose enzymatic cleavage sites, whereas more extensive structural modifications, including protein aggregation, oxidation, cross-linking and Maillard reactions, can reduce accessibility to digestive enzymes and subsequent amino acid availability.[34]

The seekh-kebab comparison particularly establishes this distinction, wherein the percentage bioavailability in both plant-based products increased after cooking, whereas it decreased in the chicken-based product. This contrasting response suggests that matrix-specific structural transformations during cooking, rather than protein origin alone, may influence the accessibility of digestion products and their subsequent potential for absorption. In animal-derived meat, for example, high-temperature cooking can induce extensive oxidation, disulphide bond formation, and protein aggregation, potentially burying reactive or cleavage sites within the protein structure and reducing their accessibility to digestive enzymes. The extent and nature of these modifications depend on the processing conditions and temperature reached during cooking, with myofibrillar, sarcoplasmic, and connective tissue proteins exhibiting distinct behaviours. Processing-induced changes such as protein denaturation and Maillard reactions may also generate compounds that are less readily absorbed or utilised by the human body.[35]

The above pattern is consistent with emerging comparative literature showing that protein-quality differences between plant- and animal-based meat products are product- and endpoint-dependent. While one study reported higher protein solubilisation for beef burgers than commercial plant-based burgers,[33] the other found lower digestibility for plant-based steak but higher digestibility for plant-based cured-meat relative to their animal counterparts.[34] A more recent study reported higher overall protein digestibility for beef patties, yet greater free-amino-acid release by plant-based meat; four of six meat alternatives also had higher IVPDCAAS than beef, and one had a higher *in vitro* DIAAS.[36]

Comparative protein quality cannot be inferred solely from whether the protein is plant- or animal-derived. Rather, protein source, formulation, processing, cooking and the resulting food matrix collectively determine digestion and downstream bioavailability.

06

From evidence to action

Recommendations for ingredient and product manufacturers

Advancing protein optimisation from a compositional target to a whole-product design strategy is imperative. Protein quality must be considered across ingredient selection, processing, formulation, structuring and final preparation, rather than assessed primarily through protein content in the finished product.



Positioning protein quality as an early-stage design criterion

Nutritional design targets could incorporate amino-acid quality, digestibility and bioavailability alongside protein content, allowing these parameters to inform product development rather than serve only as downstream validation measures. In parallel, consideration of cost, sensory performance, ingredient availability, processing yield, shelf-life and manufacturing compatibility alongside protein-quality targets could help translate nutritional improvements into products that are affordable, scalable, and acceptable to consumers.



Developing crop-specific ingredient processing pathways

A crop-to-process optimisation framework can account for differences among protein sources while considering extraction efficiency, reduction of ANFs, and protein accessibility. Protein solubility index (PSI) can be considered as a metric for extractability of plant protein ingredients. Dry-fractionated proteins can be considered for clean-label and functionality-led applications where preserving native protein functionality, particularly solubility and dispersibility, is prioritised alongside less intensive processing. Wet-extracted proteins may be more suitable for nutrition-led applications where higher protein concentration and greater absolute amounts of digestible and bioavailable protein are key formulation targets.



Designing for the product as consumed

Considering cooking and preparation methods (cooking time, temperature, and cooking medium) consumers are most likely to follow in product development and validation would help account for their influence on nutritional performance. Such evidence could also inform formulation optimisation and consumer preparation guidance.



Integrating protein-quality metrics into ingredient specifications

Validated protein-quality metrics, such as essential amino acid (EAA) scores and measures of protein digestibility or bioavailability, could be considered for inclusion in the Certificate of Analysis (CoA) of protein ingredients, alongside conventional compositional and functional specifications. This could provide a more consistent basis for ingredient qualification and formulation benchmarking, particularly where products make protein-quality or nutritional claims such as ‘complete protein’ or high BCAA content.

Takeaways for government initiatives and public-nutrition intervention programmes

A shift in the protein-quality approach from measurement towards application is imperative. Public investment can help build the evidence, infrastructure and standards needed to translate India's indigenous crop diversity into nutritionally robust protein ingredients and foods.



Strengthening an indigenous protein-ingredient ecosystem

Greater public R&D and translational funding could support crop screening, protein fractionation, processing optimisation and scale-up of Indian millets, pulses and other underutilised protein-rich crops, linking agricultural diversity with domestic ingredient manufacturing and nutritious food products. Formulations that combine multiple protein sources can help achieve a more balanced essential amino acid profile and, consequently, higher overall protein quality could be prioritised.



Building standardised protein-quality assessment capabilities

Support for harmonised protocols, reference materials, inter-laboratory validation and testing infrastructure could strengthen the assessment of protein digestibility and bioavailability in ingredients and complex foods, enabling more comparable evidence across academia, industry and public programmes.



Positioning nutritional quality as an outcome of food-innovation funding

Publicly funded alternative-protein and crop-processing programmes could consider protein quality, anti-nutritional factors and nutritional performance after processing alongside conventional outcomes such as yield, functionality and sensory performance.



Strengthening the evidence base for public-nutrition applications

Targeted studies could evaluate promising indigenous crop-based ingredients and products under realistic recipes, cooking conditions and serving contexts, with human validation where appropriate. Evidence on protein quality, micronutrient contribution, nutrient retention, digestibility and bioavailability, alongside affordability and acceptability, could help establish which products and formulations are suitable for different public-nutrition applications.



Developing evidence-based procurement frameworks for protein-rich foods

As the evidence base matures, procurement frameworks could incorporate protein quality and nutritional value alongside protein quantity, safety, cost, acceptability and operational feasibility when setting specifications for foods procured for institutional and public-nutrition programmes. This could

help translate emerging nutrition evidence into practical purchasing and programme-design criteria.



Recognising the role of processing and food matrices in protein quality

Protein quality assessment and food-innovation programmes could account for the effects of formulation, processing, and cooking conditions on digestibility and bioavailability, recognising that the same protein source can perform differently depending on the resulting food matrix. Doing so, strengthens the case for evaluating protein ingredients and products across multiple endpoints, including digestibility and bioavailability rather than protein content alone, while supporting manufacturers in optimising processing equipment and operating conditions for nutritional outcomes.



Development of a guidance document for plant-sourced protein ingredients and protein-rich products

Public institutions could support the development of an appropriate guidance document translating general regulatory requirements into practical guidance for manufacturers. This may span guidance on fractionation, fermentation, extrusion, allergen management, sampling and testing methodologies, protein quality assessment and optimisation and batch consistency.



Including digestibility or protein quality within the ‘rich/high’ protein claims by food business organisations

Under the existing FSSAI Advertising and Claims Regulations, the eligibility to make a *‘high in protein or protein-rich’* or *‘source of protein’* claim is principally determined by the quantity of protein relative to the applicable recommended dietary allowance. An assessment for digestibility or protein quality through the most relevant method could be added to the eligibility criteria, ensuring that a *‘high in protein’* claim is well rounded and translates nutritionally for consumers.

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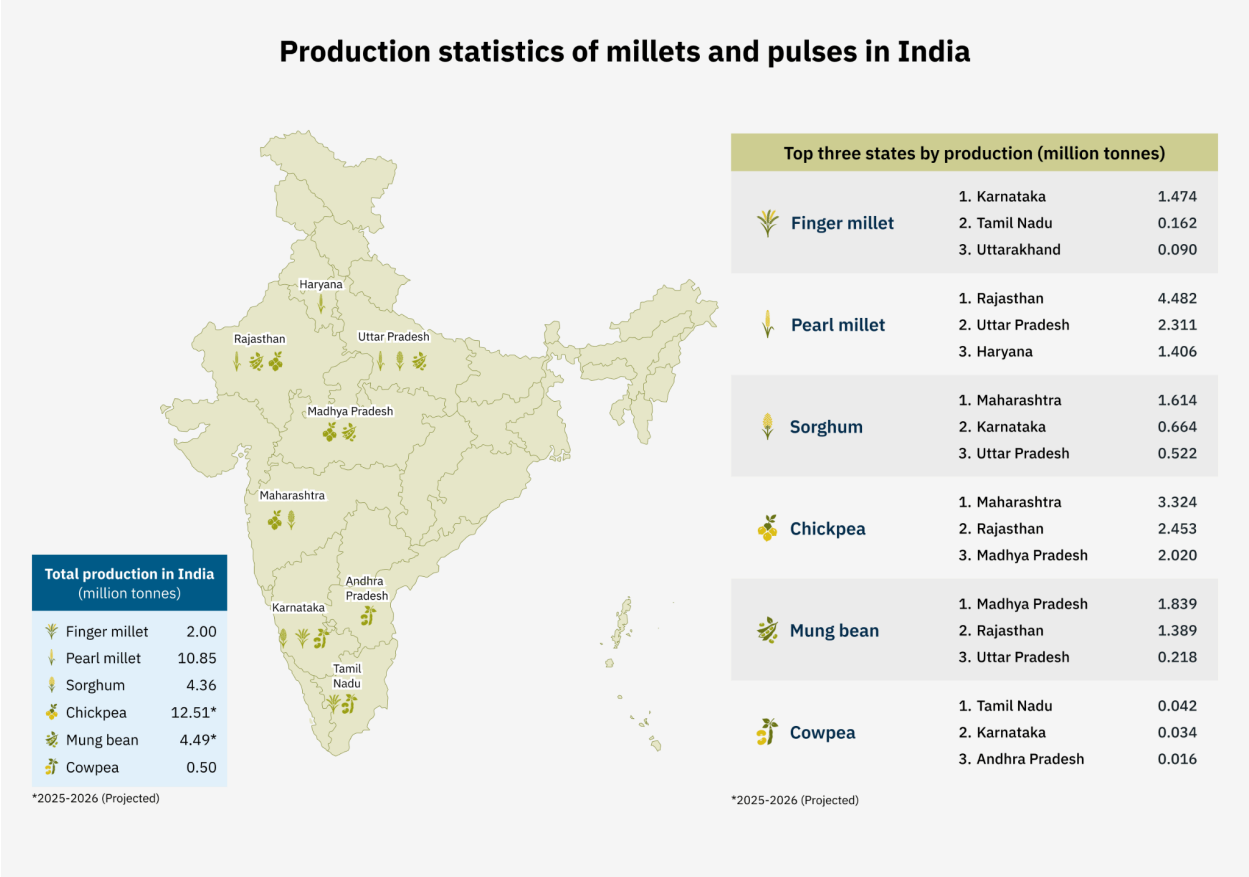
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Glossary of terms

Term	Explanation
Alternative proteins (or) smart proteins	Products that can reliably and predictably diversify the consumption of animal-derived meat, eggs, and dairy because they replicate the sensorial and cultural experience and offer several advantages in the supply chain for producers.
Plant-based alternative proteins	The term refers to products which are solely made of ingredients derived from protein-rich crops such as pulses (pea, chickpea, mung bean), oilseeds (soy), cereals (wheat, rice), nuts (almond, cashew), and others (millets, jackfruit, potato). This category includes plant-based meat, eggs, dairy, and seafood that serve as alternatives to their corresponding animal-derived products.
Total crude protein (TP)	Total protein content, typically estimated from total nitrogen using an appropriate nitrogen-to-protein conversion factor.
Protein solubility index (PSI)	Proportion of the total protein that is soluble under the specified extraction conditions.
Digestible free amino acids or bioaccessible fraction (DFAA)	Total free amino acids released and present following gastrointestinal digestion.
Bioavailable free amino acids or bioavailable fraction (BFAA)	Portion of digestion-released free amino acids recovered in the fraction designated as potentially absorbable/bioavailable by the analytical model.
Wet fractionation	Wet fractionation extracts protein based on the variations in solubility at different pHs of the extraction medium.
Dry fractionation	Protein extraction by dry fractionation is governed by differences in the size, density, and tribocharging properties of particles.
Anti-nutritional factors (ANFs)	Anti-nutritional factors (ANFs), also known as antinutrients, are natural or synthetic chemical compounds that reduce the body's ability to absorb, digest, or utilize essential nutrients

Annexures

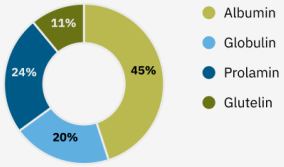
Annexure 1: Protein content, composition, anti-nutritional factors and amino acid profile of millets and pulses considered in this study



Reference: Directorate of Economics & Statistics, Department of Agriculture and Farmers Welfare, Government of India (2025-26)

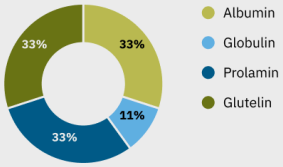
Protein composition as per Osborne classification

Finger millet



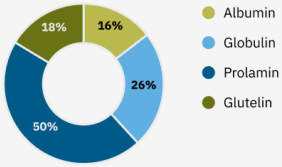
Total protein: 8.58 ± 0.35 g/100g grain

Pearl millet



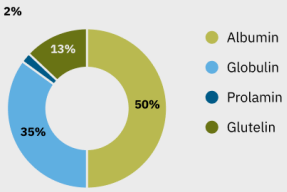
Total protein: 8.82 ± 0.21 g/100g grain

Sorghum



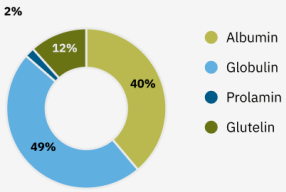
Total protein: 9.80 ± 0.32 g/100g grain

Chickpea



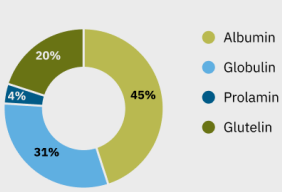
Total protein: 18.61 ± 0.68 g/100g grain

Mung bean



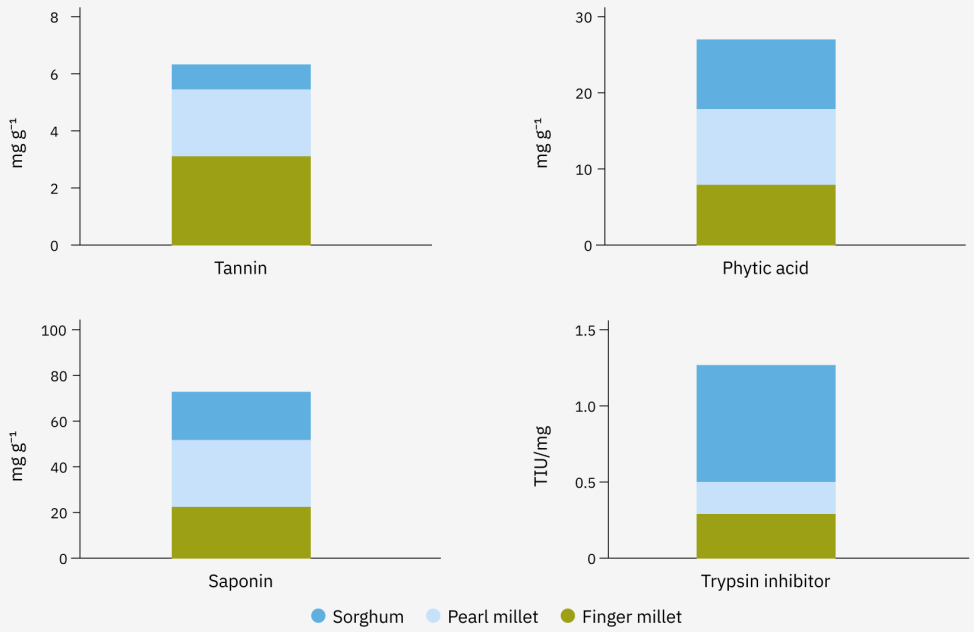
Total protein: 22.70 ± 0.14 g/100g grain

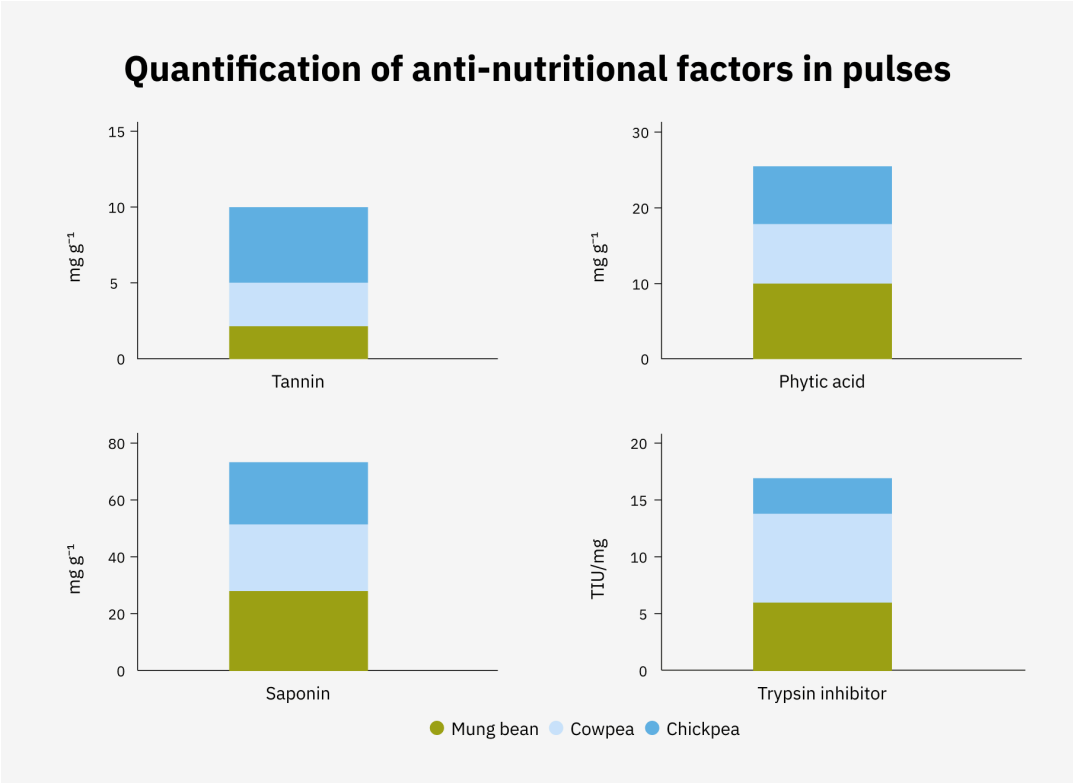
Cowpea



Total protein: 21.52 ± 0.24 g/100g grain

Quantification of anti-nutritional factors in millets





Amino acid profile of millets and pulses *versus* soy protein isolate and animal-derived proteins

Generally, millets and pulses considered in this study exceeded FAO's recommendations on amino acid requirements for children and adults for histidine, isoleucine, threonine, tryptophan and aromatic amino acids. However, all the six crops were lower in their leucine than egg, milk, whey protein and soy protein isolate. Millets showed comparatively favourable lysine, valine and sulphur-containing amino acid profiles, while pulses were more variable: cowpea provided relatively high lysine, whereas mung bean was lower in lysine, valine and sulphur-containing amino acids. This broad pattern agrees with published evidence that millet–legume combinations improve amino acid balance.[37] Nevertheless, mung bean and cowpea proteins have shown amino acid profiles comparable to soy and suitable functional properties for plant-based meat applications.[38] This diversification could address the limitation associated with several predominantly soy-, pea- or jackfruit-based Indian meat alternatives, which contained low sulphur-containing amino acids and fulfilled only part of adult essential-amino-acid requirements. Combining lysine-rich pulses with millets contributing relatively more sulphur-containing amino acids could improve amino acid balance, reduce dependence on soy and pea, and support nutritionally stronger plant-based meat formulations. However, plant protein blends should be optimised based on careful consideration of digestibility, antinutrients, processing effects and product functionality.

Essential amino acids	Amino acid requirements recommended by FAO (mg/g protein/day)[#]		Reference protein sources[*]				Indigenous millets[$]			Indigenous pulses[$]		
	Children (3-10 years)	Adults (>18 years)	Raw egg	Whole milk	Soy protein isolate	100% whey protein	Finger millet	Pearl millet	Sorghum	Chickpea	Cowpea	Mung bean
Histidine	16	15	22.82	23.2	26.05	16.96	46.76	57.08	50.3	46.49	48.21	36.38
Isoleucine	31	30	49.68	51.35	48.13	55.06	41.32	46.6	50.3	55.45	49.58	44.36
Leucine	61	59	84.68	82.43	76.78	104.05	38.06	44.27	43.26	39.21	35.81	35.05
Lysine	48	45	67.1	43.47	60.36	91.01	64.16	89.7	82.49	45.93	59.68	38.6
Threonine	25	23	47.9	44.28	35.56	67.09	57.64	60.57	50.3	41.45	42.7	40.81
Tryptophan	6.6	6	13.39	23.2	12.68	15.95	115.27	73.39	80.48	82.34	72.54	64.33
Valine	40	39	59.19	59.46	46.43	56.08	46.76	46.6	44.27	37.53	35.81	28.39
Sulphur containing amino acids (SAA)	24	22	64.76	23.2	12.8	22.03	36.97	31.45	26.16	24.65	16.99	17.3
Aromatic amino acids (AAA)	41	38	94.52	92.34	88.45	57.97	95.7	88.53	73.44	152.36	126.25	110.91

Essential amino acid scoring of selected millets and pulses vs. FAO recommendations

- [#]Source: Dietary protein quality evaluation in human nutrition, Report of an FAO Expert Consultation, 92(1-66), 26369006, Food and Agriculture Organization (FAO), Rome, Italy, 2013.
- [*]Source: USDA Agricultural Research Service, FoodData Central (https://fdc.nal.usda.gov/; accessed 20 October 2026).
- [$]Essential amino acid scores for indigenous millets and pulses were calculated using data generated in the present study.

Annexure 2: Conditions for secondary processing of millets and pulses

	Roasting		Pressure Cooking		Sprouting		Fermentation	
Sample	**Temperature (℃)**	**Time (min)**	**Temperature (℃)**	**Time (min)**	**Temperature (℃)**	**Time (h)**	**Temperature (℃)**	**Time (h)**
Finger millet	170±5	3-4	240 ± 5	10-11	26 ± 1	40-42	28 ± 1	72
Pearl millet	170±5	6-7	240 ± 5	11-12	26 ± 1	22-24	28 ± 1	72
Sorghum	170±5	5-6	240 ± 5	15-17	26 ± 1	40-42	28 ± 1	72
Chickpea	170±5	18-20	240 ± 5	11-13	26 ± 1	40-42	28 ± 1	72
Mung bean	170±5	4-5	240 ± 5	6-7	26 ± 1	22-24	28 ± 1	72
Cowpea	170±5	2-3	240 ± 5	9-10	26 ± 1	40-42	28 ± 1	72

Annexure 3: Cooking conditions for plant-based meat products

Product format	Method of cooking	Cooking temperature (°C)
Keema	Boiling	90-95
Chunks	Pan-frying	175-190
Shami kebab	Deep-frying	185-195
Seekh kebabs	Deep-frying	160-170
Burger patties	Deep-frying	170-185
Nuggets	Deep-frying	165-175

Annexure 4: Methodology for determining protein quality parameters

Total crude protein (TP)

Total crude protein content was determined using the micro-Kjeldahl method (AOAC 992.23, 2016). The protocol comprised three sequential stages: (1) sample digestion with concentrated sulfuric acid and copper sulfate catalyst to convert organic nitrogen into ammonium sulfate; (2) alkalization of the digesta with excess sodium hydroxide to liberate ammonia, followed by steam distillation and collection in a receiving flask containing aqueous boric acid to form an ammonium borate complex; and (3) titration of the trapped borate anions against a standardised hydrochloric acid solution. Total nitrogen percentage was calculated from the volume of titrant consumed relative to a reagent blank and converted to crude protein using a conversion factor (F) of 6.25.

$$\textit{Total nitrogen (\%)} = [(V_{sample} - V_{blank}) \times M \times 14.01 / W] \times 100$$

Where:

- V_{sample} = Volume of standard HCl used to titrate the sample (mL)
- V_{blank} = Volume of standard HCl used to titrate a reagent blank (mL)
- M = Molarity of the standard HCl solution (mol/L)
- W = Weight of the initial sample (g)

$$\textit{Total crude protein (\%)} = \textit{Total nitrogen (\%)} \times 6.25$$

Protein solubility index (PSI)

For PSI determination, solid samples were ground and passed through a sieve to ensure uniform particle size. A 0.8 g portion of the ground sample was mixed with 40 mL of 5 mM sodium hydroxide (NaOH) solution and stirred at 750 rpm for 1 hour to facilitate protein extraction. The mixture was then centrifuged at 4000 × *g* for 10 min to separate the soluble and insoluble fractions. The supernatant and precipitate were processed separately for nitrogen analysis. The precipitate was dried overnight at 96 °C, ground, and a portion was weighed for nitrogen estimation. The supernatant was directly pipetted for nitrogen analysis. PSI was calculated by comparing the nitrogen content of the extract or residue with that of the original sample, providing a measure of protein solubility under alkaline conditions (Liu et al., 2024).

Protein solubility index (%) = [(Protein present in the original sample – Protein present in the residue) / Protein present in the original sample] × 100

Digestible free amino acids or bioaccessible fraction

Free amino acids are soluble amino acids released from a food matrix following gastrointestinal digestion or chemical hydrolysis. They have significant nutritional value because they are readily absorbed in the human intestinal tract compared with complex proteins. Free amino acid content was quantified using the ortho-phthalaldehyde (OPA) method (Church et al., 1983). A 200 µL sample was mixed with 4 mL of OPA reagent and allowed to react at 35 °C for 2 min. Absorbance was then measured at 340 nm using a UV-VIS spectrophotometer. L-Leucine was used as the standard, and a standard curve was plotted to determine the free amino acid content of the sample. The OPA reagent reacts with primary amino groups and measures the total concentration of free amino acids and small, absorbable peptides released during *in vitro* simulated digestion. Measurement of free primary amines in the absorbable fraction, obtained as the supernatant after methanol precipitation of non-absorbable proteins, provides a direct indicator of the breakdown of the dietary protein matrix. The INFOGEST Quant protocol establishes OPA spectrophotometry as a rapid and valid alternative to high-performance liquid chromatography (HPLC) methods.

Bioavailable free amino acids or bioavailable fraction

Bioavailable free amino acids represent the fraction of free amino acids absorbed across the intestinal barrier (epithelial cells). Intestinal digesta obtained after *in vitro* simulated gastric digestion were further subjected to membrane filtration using an Amicon® Ultra Centrifugal Filter (3 kDa MWCO) at 25°C, followed by two cycles of centrifugation at 3000 RPM for 30 minutes. The resulting filtrate was used to determine and identify free amino acids as described above.

Percentage bioavailability (%)

Percentage bioavailability is the proportion of an administered protein fraction that reaches the systemic circulation in an unchanged, active form, expressed as a percentage. The percentage bioavailability was calculated using the equation:

Bioavailability (%) = (Bioavailable free amino acids / Bioaccessible free amino acids) × 100

Method references:

- AOAC Guidelines for Standard Method Performance Requirements, AOAC Official Methods of Analysis; AOAC International: Gaithersburg, MD, USA, 2016.
- Liu, K., Seegers, S., Hojilla-Evangelista, M. P., Pallares Pallares, A., & Wu, X. (2024). International collaborative study on measuring protein solubility index for legumes, oilseeds, cereals, and related products. Sustainable Food Proteins, 2(4), 236-249.
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