ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue V May 2026

# Germination as a Bioprocessing Strategy for Enhancing Functional Properties and Protein Structural Profiles of *Moringa Oleifera* Seed Protein Isolates: A Comparative Review With Soy Protein Isolates

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**DOI: https://doi.org/10.51244/IJRSI.2026.1305000163**

**Received: 08 May 2026; Accepted: 13 May 2026; Published: 05 June 2026**

## ABSTRACT

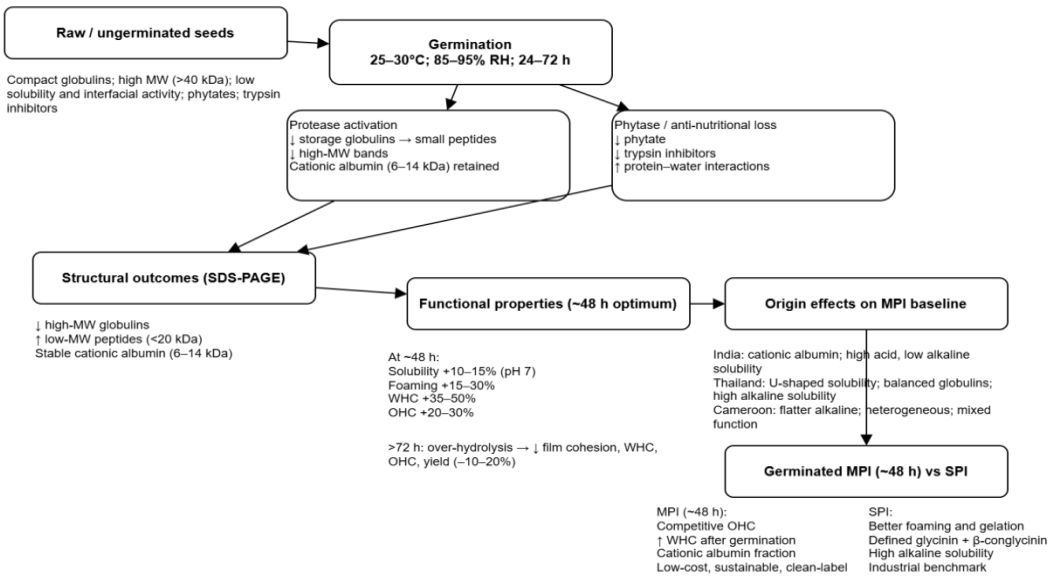
*Moringa oleifera* seed protein isolates (MPI) have gained increasing attention as sustainable plant protein alternatives; however, their functional properties remain highly variable and underutilized compared to soy protein isolate (SPI). This review critically evaluates germination as a bioprocessing strategy for enhancing MPI structural and functional properties, including SDS PAGE, solubility, foaming, water holding capacity (WHC), and oil holding capacity (OHC), while providing a comparative perspective with SPI.

Evidence indicates that germination improves MPI functionality primarily through enzymatic proteolysis of storage globulins, resulting in reduced molecular weight, increased surface polarity, and improved protein-water and protein-lipid interactions. Optimal germination (~48 h) enhances solubility by 10-15%, foaming capacity by 15-30%, WHC by up to 50%, and OHC by approximately 20-30%. However, SPI consistently outperforms MPI in foaming and gelation due to its structured glycinin and β-conglycinin fractions.

The review further highlights strong geographic origin effects on MPI functionality, driven by variations in protein composition, particularly the ratio of globulins to cationic albumins. Indian MPI shows acid-soluble albumin dominance, Thai MPI exhibits soy-like solubility behavior, and Cameroonian MPI displays mixed functional profiles. Despite these advances, key gaps remain in molecular characterization, standardization of extraction methods, and integration of omics-based approaches. Germination represents a low-cost, sustainable strategy to enhance MPI functionality, but full industrial competitiveness with SPI will require integrated bioprocessing and a better understanding of genotype-environment-protein interactions.

**Keywords:** *Moringa oleifera*; germination; *Protein Isolate*; Solubility; SDS-PAGE; foaming; water holding capacity; oil holding capacity; soy protein

### Graphical Abstract



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# INTRODUCTION

The global shift toward plant-based diets has accelerated interest in sustainable protein ingredients with acceptable nutritional quality and techno-functional performance (Mensink et al., 2016). Soy protein isolate (SPI) remains the most commercially established plant protein because of its excellent emulsification, gelation, foaming, and water-binding capacities (Nishinari et al., 2014). These properties are largely attributed to its major storage proteins, glycinin and β-conglycinin, which provide structural flexibility and network-forming ability in food systems.

Despite its industrial importance, soy production faces environmental and geographical limitations, including deforestation concerns and restricted adaptability to semi-arid climates (Aiking & de Boer, 2020). Consequently, alternative protein sources adapted to harsh environments are increasingly being explored. Moringa oleifera is a drought-tolerant multipurpose tree widely cultivated in Africa and Asia. Its seeds contain approximately 30-40% protein on a dry matter basis and possess a balanced amino acid composition rich in arginine and glutamic acid (Moyo et al., 2011). Recent studies have shown that alkaline extraction followed by isoelectric precipitation can produce moringa protein isolates with protein purity exceeding 80%. However, inconsistencies in functional performance between studies suggest that protein composition and behavior are strongly influenced by seed origin, extraction conditions, and pretreatment methods.

Among the available pretreatments, germination has attracted significant attention because it naturally activates endogenous enzymes that hydrolyze storage macromolecules without the use of harsh chemicals (Jakubczyk et al., 2020). Controlled germination modifies protein structure, improves digestibility, reduces anti-nutritional compounds, and enhances functional performance. Nevertheless, the mechanisms through which germination affects MPI functionality remain fragmented across the literature.

This review therefore aims to:

i. Evaluate the effects of germination on the structural characteristics of MPI using SDS-PAGE evidence.

ii. Compare the impact of germination on solubility, foaming, WHC, and OHC of MPI relative to SPI.

iii. Discuss the influence of geographic origin on MPI functionality and identify major research gaps.

# METHODOLOGY

This review was prepared through a comprehensive evaluation of published studies focusing on the effect of germination on the structural and functional properties of *Moringa oleifera* seed proteins. Relevant literature mainly published between 2005 and 2026 was retrieved from ScienceDirect, Scopus, Web of Science, and Google Scholar using combinations of keywords including "*Moringa oleifera*", "germination", "protein isolate", "functional properties", "SDS-PAGE", "protein solubility", "foaming capacity", "water holding capacity", and "soy protein isolate". Priority was given to peer-reviewed articles reporting germination-induced changes in protein structure, extraction behavior, and techno-functional properties of moringa seed proteins. Comparative studies involving soy and other leguminous protein systems were also included to provide mechanistic and functional context. Studies focusing exclusively on medicinal, pharmacological, or non-food applications were excluded unless directly related to protein characterization or functionality.

Data extracted from the selected studies included germination conditions, extraction methods, SDS-PAGE profiles, pH-solubility behavior, foaming properties, water holding capacity (WHC), and oil holding capacity (OHC). The collected information was critically compared and synthesized to identify consistent trends, mechanistic relationships, and major research gaps related to the application of germination as a bioprocessing strategy for moringa protein isolates.

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## Germination-Induced Structural and Functional Modification of Moringa Seed Proteins

### Biochemical Changes During Germination

Germination initiates a series of biochemical and structural changes that significantly influence the functionality of *Moringa oleifera* seed proteins. During this process, endogenous enzymes such as proteases, phytases, and amylases become activated to mobilize storage reserves required for seedling development (Bewley et al., 2013; Nkhata et al., 2018). Among these enzymes, proteases play the most important role in modifying protein functionality by partially hydrolyzing compact storage globulins into smaller peptides and amino acids. This controlled proteolysis reduces molecular size, increases structural flexibility, and exposes previously buried hydrophilic and hydrophobic residues. As a result, germinated proteins generally show improved extractability, solubility, and interaction with water and oil systems compared with ungerminated proteins.

In addition to protein hydrolysis, germination contributes to the degradation of anti-nutritional compounds such as phytates and trypsin inhibitors, which are known to interfere with protein digestibility and functionality (Aviles-Gaxiola et al., 2018). Reduction of phytate-protein interactions improve mineral availability and enhances protein-water interactions within the isolate matrix. Similar biochemical effects have also been reported in soy, chickpea, mung bean, lentil, and kidney bean proteins, indicating that moderate germination-induced hydrolysis is a conserved mechanism underlying functional improvement in leguminous proteins.

### SDS-PAGE and Structural Characterization

Structural modifications occurring during germination are commonly evaluated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), which should is regarded as a molecular characterization technique rather than a functional property. Electrophoretic profiles in *Moringa Oleifera* seed products (cake and protein isolate) consistently demonstrate progressive disappearance of high-molecular-weight storage protein bands alongside the accumulation of low-molecular-weight peptide fragments as germination advances (Mune Mune et al., 2016). These changes provide direct evidence of proteolytic hydrolysis and structural remodeling within the protein matrix.

Compared with soy protein isolate (SPI), moringa proteins display distinctive electrophoretic characteristics due to the persistence of low-molecular-weight cationic albumin bands within the 8.5 to 32.5 kDa range (Illingworth et al., 2022). These albumins remain detectable even after germination and partly explain the unusual alkaline insolubility observed in some moringa isolates (Jain et al., 2019). In contrast, soy proteins are dominated by glycinin and β-conglycinin fractions, whose controlled hydrolysis during germination is closely associated with improvements in interfacial and hydration properties.

### Effect of Germination on Protein Solubility

Protein solubility is one of the most important functional properties influencing the application of plant proteins in food systems because it affects emulsification, foaming, gelation, and digestibility. Compared with SPI, which exhibits a symmetrical U-shaped pH-solubility profile with minimum solubility near pH 4-5, moringa protein isolates (MPIs) display considerable variation depending on seed origin and protein composition. Germination improves solubility by reducing molecular size and increasing exposure of ionizable amino acid residues capable of interacting with water molecules (Damodaran, 2008). Most studies consistently report maximum improvement after approximately 48 h germination, beyond which excessive hydrolysis may destabilize the protein matrix and reduce isolate recovery. This non-linear response highlights the importance of maintaining a balance between productive partial hydrolysis and excessive degradation during germination.

### Effect of Germination on Foaming Properties

Foaming properties are important in food systems where proteins are required to adsorb rapidly at the air-water interface and form stable interfacial films. Compared with SPI, moringa proteins generally show lower foaming capacity and stability. The lower foaming capacity of Moringa oleifera seed protein isolate compared

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with Soy protein isolate may partly be attributed to differences in globulin composition, particularly the higher proportion of β-conglycinin (7S globulin) in soy proteins, which possesses superior molecular flexibility, interfacial adsorption, and viscoelastic film-forming properties that enhance foam formation and stability (Wang et al., 2021; Cheng et al., 2021). Nevertheless, controlled germination improves foaming behavior by producing smaller and more surface-active peptide fragments capable of faster interfacial adsorption.

Studies consistently report moderate improvements in foaming capacity after approximately 48 h germination, although excessive hydrolysis beyond this stage may weaken film cohesion and reduce foam stability (Bera et al., 2023; Ben-Othman et al., 2020). Similar trends have been reported in soy, chickpea, mung bean, and lentil proteins, further supporting the role of moderate proteolysis in enhancing interfacial functionality across leguminous protein systems

**Effect of Germination on Water and Oil Holding Capacities**

Water holding capacity (WHC) and oil holding capacity (OHC) are strongly influenced by the structural modifications induced during germination. Improvement in WHC is mainly associated with exposure of polar amino acid residues and increased matrix porosity following partial protein unfolding. Ungerminated Moringa oleifera seed protein isolate generally shows lower WHC than Soy protein isolate because of its compact globular structure, but germination substantially improves water retention properties by increasing protein–water interactions. In contrast, OHC in MPI is relatively comparable to SPI even before germination, largely because moringa proteins contain appreciable amounts of hydrophobic amino acids such as leucine, phenylalanine, and proline (Alghooneh et al., 2023; Mune Mune et al., 2016). Germination further enhances OHC through exposure of additional hydrophobic regions within the protein matrix. However, prolonged germination beyond the optimum period may lead to excessive fragmentation and gradual loss of structural integrity.

# DISCUSSION

**Effect of Germination of Structural Characterization**

Taken together, SDS-PAGE evidence positions germination as a structurally transformative process that converts MPI from a rigid, globulin-rich system with limited functional versatility into a more flexible, peptide-enriched matrix capable of broader interactions with food system components. In ungerminated seeds, the electrophoretic profile is dominated by high-molecular-weight storage globulins whose compact quaternary structure limits extractability and interfacial activity, explaining why raw MPI consistently underperforms SPI in most functional assessments. As germination progresses, endogenous proteases become activated and systematically cleave these globulins, producing a reproducible shift toward low-molecular-weight fragments below 20 kDa, a pattern broadly conserved across leguminous protein systems including soy, chickpea, lentil, and mung bean (Nkhata et al., 2018; Ghumman et al., 2016).

The practical consequences are multi-directional: smaller peptides improve solubility, foaming, and hydration properties, but excessive proteolysis beyond approximately 72 h produces fragments too short to maintain stable interfacial films, accounting for the non-monotonic functional response consistently documented in germinated MPI studies (Mune Mune et al., 2016; Khattab & Arntfield, 2009). Throughout this transformation, the stable cationic albumin fraction (6-14 kDa, pI ~911) remains largely intact, a feature with no equivalent in soy protein systems and one that directly explains MPI's distinctive acid-solubility behavior and its divergence from SPI functionality (Illingworth et al., 2022; Jain et al., 2019). The proportion of this albumin fraction relative to globulins also varies across geographic seed sources, making the SDS-PAGE profile a practical fingerprint for both quality control and origin authentication of germinated MPI preparations.

Future proteomic and molecular mapping work should therefore specifically target this albumin fraction, as its stability and composition are likely key determinants of germination-responsive functionality across different ecotypes.

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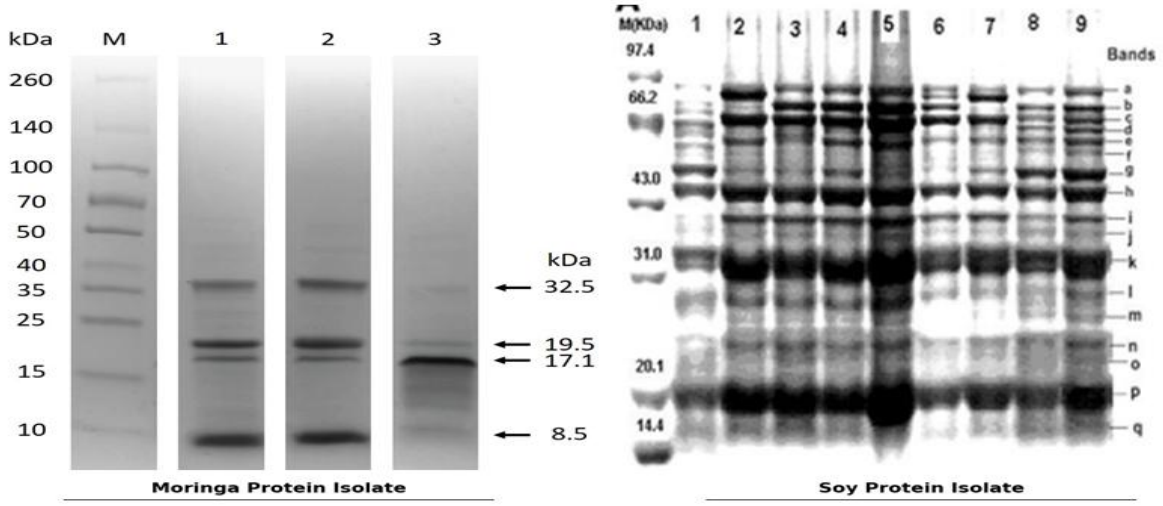
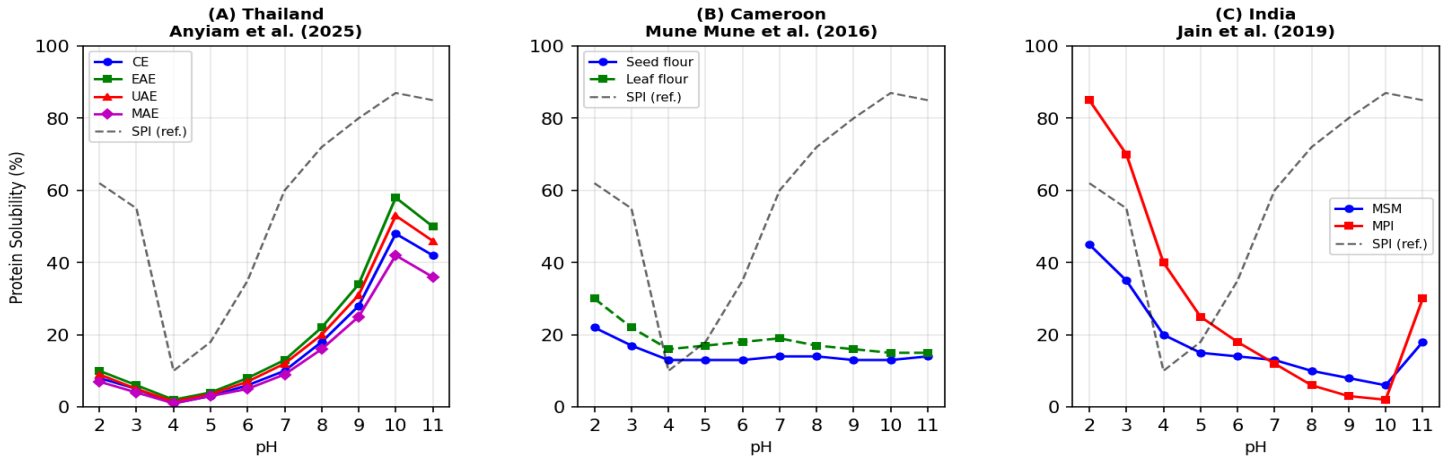
Figure 1. SDS-PAGE Protein Band Patterns for Moringa and Soy Protein Isolates (Illingworth et al., 2022; Liu et al., 2007).




## Solubility Behavior

Germination consistently enhances the solubility of *Moringa oleifera* protein isolates (MPI), particularly around neutral pH, through a combination of controlled proteolysis, unfolding of compact protein structures, and increased exposure of ionizable amino acid residues. During germination, endogenous proteases partially hydrolyze storage globulins, reducing molecular size and increasing the number of free amino and carboxyl groups available for protein-water interactions. This improves hydration and dispersion behavior at physiological pH (Damodaran, 2008; Foegeding & Davis, 2011; Nkhata et al., 2018). SPI exhibits a more classical and symmetrical U-shaped solubility curve, with minimum solubility near its isoelectric point (~pH 4.5) and high solubility under alkaline conditions due to glycinin and β-conglycinin dissociation (Barac et al., 2017). Germination-induced improvements in MPI partially reduce this functional gap by shifting protein structure toward smaller, more hydrophilic peptides, but MPI still shows strong genotype- and origin-dependent variability. For instance, Indian MPI tends to show extremely high solubility at acidic pH due to cationic albumin dominance, whereas Thai and Cameroonian variants exhibit more globulin-like behavior (Jain et al., 2019; Anyiam et al., 2025). This comparative solubility behavior is further visualized in Figure 2, which shows how germination shifts MPI profiles upward at neutral pH while still maintaining structural divergence from SPI.

Figure 2. pH-Solubility Profiles of Moringa Protein Products from Three Geographic Source




(A) Thailand - soy-like U-shape, minimum at pH 4, maximum at pH 10 (Anyiam et al., 2025). CE=Conventional, EAE=Enzyme-Assisted, UAE=Ultrasound-Assisted, MAE=Microwave-Assisted Extraction. (B) Cameroon-acid-dominant, flat alkaline profile (Mune Mune et al., 2016). Seed flour (dashed)

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vs leaf flour (solid). (C) India - strongly inverted; MSM=Moringa Seed Meal; MPI=Moringa Protein Isolate (Jain et al., 2019). Grey dashed line = SPI reference (Kinsella, 1979). Values digitized from published figures.

**Foaming Properties**

Foaming behavior in protein systems is governed by a balance between rapid adsorption at the air-water interface and the ability to form a stable, viscoelastic film. Germination enhances MPI foaming capacity by generating smaller peptide fragments that diffuse more rapidly to the interface and partially unfold, thereby improving surface activity (Foegeding & Davis, 2011; Wilde, 2000). Across germination studies (Figure 3), a 15-30% increase in foaming capacity has been reported at around 48 h, indicating an optimal proteolysis window where functional improvement is maximized before excessive breakdown occurs (Mune Mune et al., 2016; Nkhata et al., 2018). However, even with germination, MPI foaming performance remains lower than SPI. This is primarily due to the absence of soy β-conglycinin-like flexible subunits, which provide superior interfacial film formation and elasticity (Renkema & van Vliet, 2002; Lam et al., 2018). SPI typically achieves foaming capacities above 120%, while MPI remains significantly lower. Enzymatic or physical pretreatments can narrow this gap (Anyiam et al., 2025). Beyond 48-72 h germination, over-hydrolysis reduces peptide size below the threshold required for stable film formation, leading to foam collapse (Khattab & Arntfield, 2009).

**Water Holding Capacity (WHC)**

Water holding capacity is strongly influenced by protein unfolding, surface polarity, and matrix porosity. Germination enhances WHC in MPI by exposing hydrophilic amino acid residues and increasing the number of available hydrogen bonding sites. Proteolytic cleavage during germination disrupts compact globulin structures, allowing water molecules to penetrate and become physically entrapped within a more open protein matrix (Damodaran, 2008). Experimental evidence (Figure 3) shows that WHC in germinated Moringa protein systems can increase by approximately 35-50% after 48 h, bringing values closer to the lower range of SPI functionality (Mune Mune et al., 2016). SPI still maintains superior WHC (3.5-5.0 g/g protein) due to its well-developed gel network-forming glycinin fraction, which creates stable water-retaining structures upon hydration and mild heating (Foegeding & Davis, 2011; Renkema & van Vliet, 2002). Nonetheless, germination clearly improves MPI suitability for hydrated food systems such as bakery and meat analogues.

**Oil Holding Capacity (OHC)**

Oil holding capacity in protein systems is primarily governed by surface hydrophobicity and the ability of proteins to physically entrap lipid molecules within their matrix. Unlike solubility and WHC, OHC is less sensitive to pH but strongly influenced by exposure of non-polar residues such as leucine, isoleucine, and phenylalanine during structural modification (Moure et al., 2006). Germination enhances MPI OHC moderately (approximately 20-30%) by increasing surface hydrophobic exposure through partial proteolysis, allowing improved lipid binding and retention. However, MPI and SPI remain functionally comparable in OHC (1.5-2.5 g/g protein range), suggesting that lipid-binding capacity is a conserved property among legume proteins (Mune Mune et al., 2016; Lam et al., 2018). SPI's similar OHC is attributed to its balanced distribution of hydrophobic residues across glycinin and β-conglycinin fractions (Wolf, 1970; Boye et al., 2010). Overextended germination (>72 h) reduces OHC due to excessive hydrolysis (Figure 3), which breaks proteins into peptides too small to physically entrap oil droplets, highlighting the importance of controlled germination duration (Nkhata et al., 2018).

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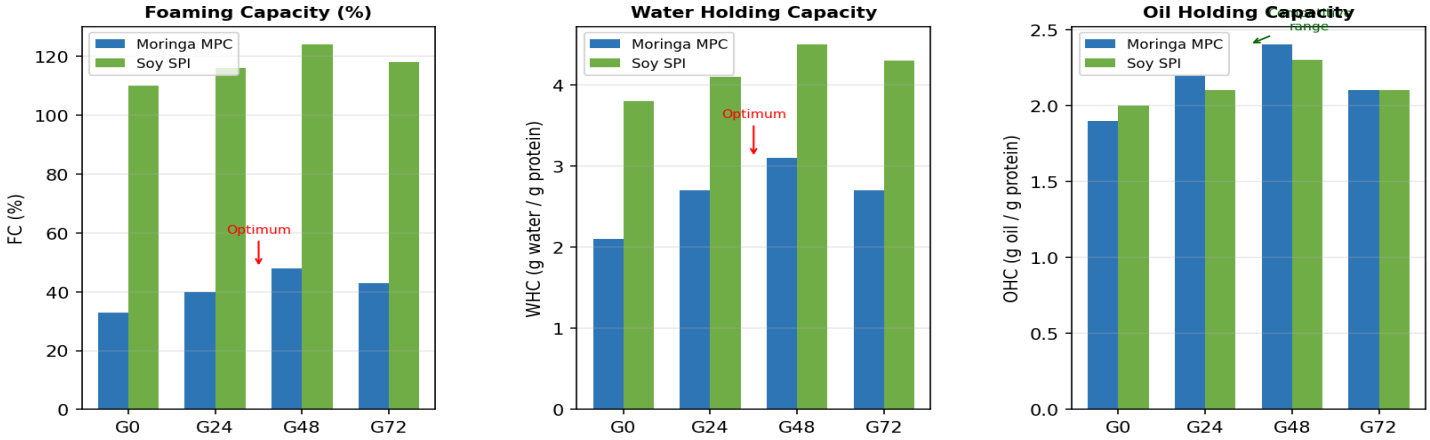
Figure 3. Effect of Germination Duration on Foaming Capacity (FC), Water Holding Capacity (WHC), and Oil Holding Capacity (OHC) of Moringa Protein Concentrates and Soy Protein Isolate




Data compiled from multiple independent sources: Moringa values from Mune Mune et al. (2016) with G24 interpolated from Mubarak (2005) and El-Adawy (2002); soy values from Sangronis & Machado (2007) and Kinsella (1979). These data are not from a single experiment and should be interpreted as indicative trends rather than directly comparable measurements. Non-monotonic response (improvement to G48, decline at G72) in all three properties reflects the balance between productive partial proteolysis (improving surface exposure) and matrix fragmentation (reducing film coherence and pore structure). Inter-study methodological differences, including variations in extraction pH, drying conditions, and defatting procedures, may influence reported values.

## Trade-offs Between Functionality Gains and Protein Yield or Sensory Quality

While germination consistently enhances the functional properties of MPI, it is important to acknowledge that these gains do not come without trade-offs. One of the most significant concerns is the reduction in protein yield associated with prolonged germination. As endogenous proteases hydrolyze storage proteins into smaller peptides and free amino acids, a portion of the soluble nitrogen is lost during isoelectric precipitation, leading to a measurable decrease in isolate recovery. Studies on germinated legume proteins suggest that protein yield can drop by 10-20% under extended germination conditions (beyond 48-72 h), partly offsetting the functional improvements gained (Nkhata et al., 2018; Sangronis & Machado, 2007). This highlights the need to carefully optimize germination duration relative to the intended application and acceptable yield thresholds.

Sensory quality is another underexplored dimension of germination-induced changes in MPI. Partial proteolysis during germination generates short-chain peptides and free amino acids, some of which contribute to increased bitterness or off-flavors in the resulting isolate. Bitter taste is typically associated with hydrophobic peptide fragments produced from the cleavage of storage globulins, and the extent of bitterness is generally proportional to the degree of hydrolysis (Damodaran, 2008). In addition, the inherent beany or grassy notes of raw Moringa seed material may be intensified or altered by germination-related enzymatic activity, particularly lipoxygenase-driven oxidation of unsaturated fatty acids during the early stages of germination. These sensory concerns are rarely addressed in protein functionality studies but represent a practically important barrier to consumer acceptance and commercial adoption, particularly for beverage and dairy analogue applications. Future studies should include sensory evaluation and bitterness profiling alongside functional measurements to provide a more complete picture of germination's suitability as an industrial pretreatment.

## Germination in the Context of Alternative Bioprocessing Strategies

Germination, enzymatic hydrolysis, and fermentation are the three main bioprocessing strategies for improving plant protein functionality, and understanding how they compare helps contextualize the practical value of germination for MPI production. Enzymatic hydrolysis using exogenous proteases such as alcalase, papain, or flavourzyme offers a more controlled and targeted approach, allowing precise manipulation of the degree of

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hydrolysis and producing protein hydrolysates with predictable functional profiles. For MPI specifically, enzymatic hydrolysis has been shown to substantially improve solubility and emulsification, sometimes exceeding germination-induced gains (Anyiam et al., 2025), but it incurs higher processing costs, requires enzyme inactivation steps, and raises clean-label concerns that may limit consumer acceptance. Fermentation, particularly with lactic acid bacteria or filamentous fungi, improves digestibility, reduces anti-nutritional factors, and can enhance flavor profiles in legume-based systems (Nkhata et al., 2018), but introduces additional complexity around microbial strain selection, safety validation, and processing time. Germination, by contrast, is low-cost, chemical-free, and relies entirely on the seed's own enzymatic machinery without requiring specialized equipment or additives, making it the most practical and scalable pre-extraction treatment for improving baseline protein quality. From an industrial standpoint, germination is best positioned not as a replacement for more targeted post-extraction methods, but as a complementary first step. Combining it with mild enzymatic post-treatment or selective fractionation could maximize functional performance while minimizing the trade-offs inherent to any single approach.

**Recommendations for Industrial Application**

Translating germination-based improvements into commercial MPI production requires matching germination parameters to the functional demands of the intended end-use application. For meat analogues and restructured food products, where WHC and OHC are the primary requirements, germination for approximately 48 h at 25-30°C with controlled humidity of 85-95% RH is recommended. Under these conditions, partial protein unfolding and hydrophobic exposure are maximized while sufficient structural integrity is retained to support network formation during processing. For plant-based beverages, where broad pH solubility is critical, a germination period of 36-48 h at moderate temperatures combined with alkaline extraction at pH 9-10 is advisable, as this improves the nitrogen solubility index while limiting the formation of bitter-tasting peptides. For bakery applications, where water absorption, dough elasticity, and emulsification are most important, a shorter germination window of 24-36 h is more suitable, improving WHC and surface polarity without the excessive protein fragmentation that could compromise dough structure. Across all applications, aeration during germination is strongly recommended to ensure uniform enzyme activation and to prevent anaerobic conditions that generate undesirable volatile compounds. Post-germination drying should be conducted below 60°C to preserve functional gains and minimize Maillard browning and thermal denaturation.

**Influence of Geographic Origin on MPI Functionality**

The functional behavior of MPI is strongly shaped by geographic origin, reflecting underlying differences in agroecological conditions, soil chemistry, and genotype-environment interactions that directly influence protein composition. Across studies, MPI from India, Thailand, and Cameroon consistently exhibit distinct protein profiles, particularly in the ratio of globulins to cationic albumins, which governs solubility, interfacial activity, and hydration properties. Indian MPI is characterized by a high proportion of cationic albumins with elevated isoelectric points (pI ~9-11), resulting in unusually high solubility at acidic pH but reduced alkaline solubility. Thai MPI shows a more balanced, globulin-dominated profile with soy-like U-shaped solubility behavior, while Cameroonian MPI displays flatter, broader solubility curves reflecting a more heterogeneous protein composition (Jain et al., 2019; Mune Mune et al., 2016; Anyiam et al., 2025). These compositional differences extend beyond solubility to foaming, WHC, and OHC, with MPI from alkaline soil regions showing weaker interfacial stability and MPI from humid tropical soils exhibiting more balanced but generally lower overall functional performance compared to SPI. SPI continues to outperform all MPI variants in foaming and gelation due to its well-defined glycinin and β-conglycinin fractions, which remain largely absent or structurally inconsistent in MPI (Barac et al., 2017; Lam et al., 2018). Despite these insights, critical gaps remain, including limited proteomic mapping of Moringa ecotypes, the absence of standardized extraction protocols across regions, insufficient research on how climate change affects long-term protein expression, and incomplete industrial-scale comparisons with SPI. Addressing these gaps is essential for positioning MPI as a reliable and globally competitive plant protein ingredient.

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**Limitations of the Reviewed Literature**

The conclusions drawn in this review should be interpreted with an awareness of several important limitations in the underlying literature. First, the body of evidence for germinated MPI remains relatively small, with a disproportionate reliance on a limited number of primary studies, notably Mune Mune et al. (2016), Anyiam et al. (2025), and Jain et al. (2019). This narrow evidence base limits the statistical robustness of observed trends and makes it difficult to draw universally applicable conclusions across different production contexts. Second, the reviewed studies differ considerably in their germination conditions, including temperature, humidity, aeration, and seed lot origin, as well as in their protein extraction methods, such as extraction pH, defatting procedure, precipitation pH, and drying conditions. These methodological differences make direct comparisons between studies unreliable and may account for some of the variability in reported functional values. Third, several data points used for comparative analysis are drawn from protein concentrates rather than isolates, or from related species within the Moringaceae family, which may not be fully representative of commercially relevant MPI preparations. Together, these limitations suggest that the functional trends described in this review should be understood as indicative rather than definitive, and that future studies employing harmonized protocols across multiple Moringa ecotypes are needed to build a stronger evidence base.

Table 1. Summary of Key Published Studies on Germinated Moringa Protein Systems

| Study | Origin / Material | Germination Conditions | Extraction Method | Protein Purity (%) | Solubility (%) | WHC (g/g) | OHC (g/g) |
|---|---|---|---|---|---|---|---|
| Mune Mune et al. (2016) | Cameroon; seed protein concentrate | 0, 24, 48, 72 h; ~25°C; humidity NR | Alkaline extraction, isoelectric precipitation | ~72-78% | Improved at 48 h; flat alkaline profile | ~2.0-2.8 g/g | ~1.8-2.4 g/g |
| Anyiam et al. (2025) | Thailand; seed protein isolate | NR (conventional and assisted extraction methods compared) | CE, EAE, UAE, MAE; isoelectric precipitation | >80% | U-shaped; high alkaline solubility; method-dependent | NR | NR |
| Jain et al. (2019) | India; seed protein isolate | NR (post-harvest processing focus) | Alkaline extraction pH 9; isoelectric precipitation pH 4 | >80% | High acid solubility; low alkaline solubility (cationic albumin effect) | NR | NR |
| Nkhata et al. (2018) | Review; cereals and legumes (broader context) | Variable; 24-96 h reported across species | Various; review-based synthesis | Variable | Generally improved post-germination | Variable | Variable |
| Sangronis & Machado (2007) | Venezuela; soy and legumes (reference baseline) | 0, 24, 48, 72 h; 25°C; dark | Alkaline extraction; isoelectric precipitation | >90% (SPI) | U-shaped; high in alkaline range | ~3.5-5.0 g/g | ~1.5-2.5 g/g |

NR = not reported. CE = conventional extraction; EAE = enzyme-assisted extraction; UAE = ultrasound-assisted extraction; MAE = microwave-assisted extraction. WHC = water holding capacity; OHC = oil holding capacity. Values are representative ranges drawn from the cited studies.

## CONCLUSION

Germination emerges as a promising bioprocessing strategy for improving the functional properties and structural characteristics of *Moringa oleifera* seed protein isolates (MPI). Across multiple studies, germination consistently enhances solubility, foaming capacity, water holding capacity (WHC), and oil holding capacity (OHC) through controlled proteolysis and structural unfolding of storage proteins. However, the extent of improvement is highly dependent on germination duration, with approximately 48 hours at 25-30°C under controlled humidity identified as the optimal balance between beneficial hydrolysis and over-degradation. When compared to soy protein isolate (SPI), MPI demonstrates competitive functionality in certain properties, particularly OHC, but remains inferior in foaming and gelation due to the absence of structurally flexible β-conglycinin-like subunits. Nevertheless, germination narrows this functional gap by generating smaller peptides and improving surface activity, although it does not fully replicate SPI performance. Importantly, MPI retains unique advantages, such as stable cationic albumin fractions, which confer distinctive solubility behavior not observed in soy systems. The functional potential of MPI is not only dependent on processing (such as germination) but is also strongly influenced by geographic origin, which determines baseline protein composition. Trade-offs associated with germination, including potential reductions in protein yield and the development of bitter or off-flavors from extensive proteolysis, must be carefully managed through application-specific optimization of germination conditions. For beverage applications, germination of 36-48 h combined with alkaline extraction is recommended, while meat analogue and bakery applications benefit from 48 h or 24-36 h germination, respectively, with aeration and controlled post-germination drying below 60°C. Future optimization strategies combining germination with targeted fractionation or enzymatic post-treatment may further enhance MPI functionality and industrial applicability. A graphical abstract summarizing the mechanisms of germination-induced functional enhancement is provided as supplementary material.

### Declaration of Competing Interest

The author declares no known competing financial interests or personal relationships that could have influenced the work reported in this review.

### Funding

This review received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XIII Issue V May 2026


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