Investigating the Potential of Proso Millet (*Panicum miliaceum* L.) as a Functional Food

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Abstract

Proso millet (*Panicum miliaceum* L.) is among the earliest domesticated millet species and serves as a vital staple food for populations living in arid and semi-arid regions worldwide. This review highlights its rich nutritional profile, including high-quality proteins rich in essential amino acids (such as isoleucine, methionine, and leucine), essential fatty acids (notably linoleic acid, an omega-6 fatty acid), and antioxidant compounds such as phenolic acids and glycosylated flavonoids. Compared to common cereals like rice and wheat, proso millet offers higher protein quality and a richer profile of bioactive compounds, highlighting its potential as a valuable functional food ingredient. Beyond its nutritional value, proso millet exhibits multiple health-promoting properties, including antioxidant, anti-diabetic, anti-cancer, antiproliferative, anti-liver injury, and anti-obesity effects. Its gluten-free nature also makes it suitable for individuals with celiac disease.

It may have significant benefits for maintaining the gastrointestinal, respiratory, and cardiovascular systems, as well as hair health. Furthermore, advanced processing techniques, including physiochemical and bioprocessing techniques (such as fermentation and germination), thermal processing, and non-thermal technologies, have shown promise in enhancing its nutritive and antioxidant compounds while reducing antinutritional factors. In addition, multidisciplinary approaches, including genomics and bioinformatics, play a crucial role in advancing millet improvement and achieving nutritional security. Despite its functional potential, proso millet remains underutilized commercially due to technical limitations, including the short shelf life of millet flour, the degradation of micronutrients, total proteins, and dietary fiber during dehulling, and a lack of studies focused on industrial scaling. Overall, this review highlights proso millet as a sustainable, health-promoting crop that aligns with global efforts to combat malnutrition and chronic diseases while enhancing food system resilience to climate change.

Keywords: Proso millet; Functional foods; Bioactive compounds; Food security; Sustainable nutrition; Bioinformatics

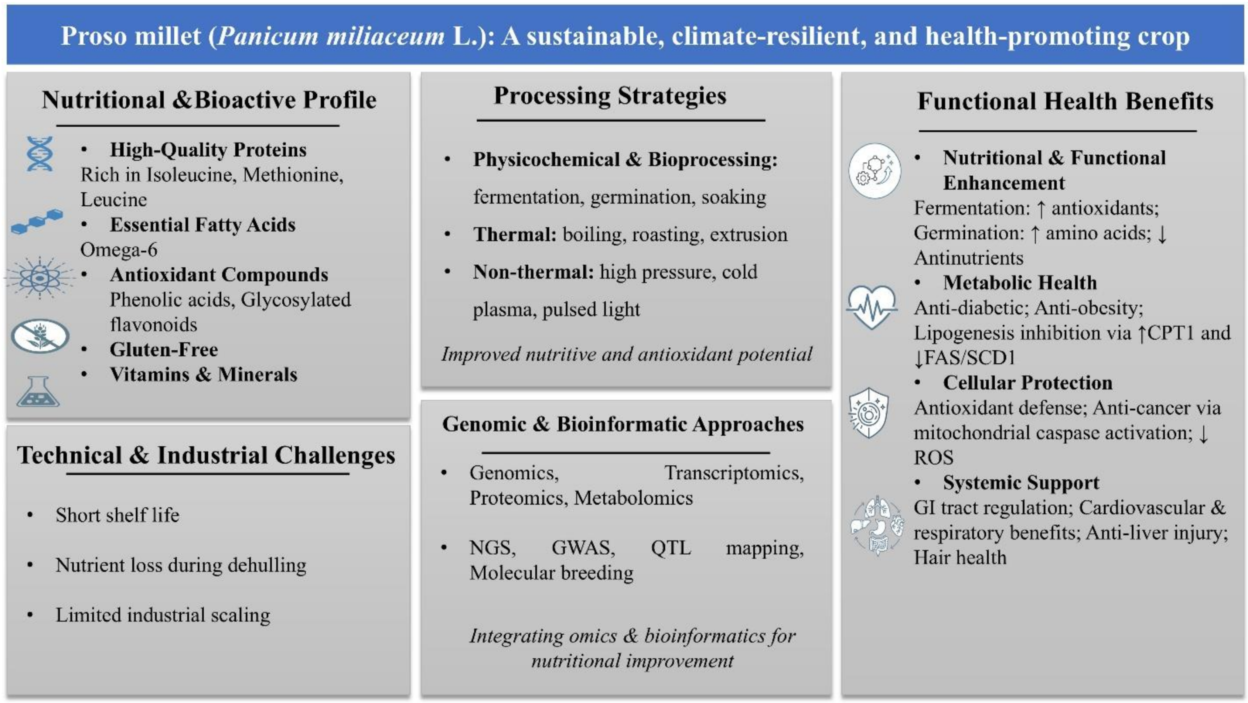

Figure 1. Graphical abstract summarizing the bioactive composition, processing strategies, and functional health mechanisms of Proso millet (*Panicum miliaceum* L.).

1. Introduction

Millets are small-seeded cereal crops belonging to the Poaceae family (Nayak et al., 2024) and rank sixth among the world's most important cereals (Jevcsák and Sipos, 2016). These grains are highly resilient and can withstand diverse climatic conditions, making them both sustainable and drought-resistant. They are also energy-rich and nutrient-dense, playing a key role in addressing malnutrition globally. They serve as a valuable source of medicinal and nutraceutical properties. Over the past few decades, their production has grown to meet the dietary needs of the expanding world population. Due to their nutritional composition and bioactive components, millets are considered a long-term and sustainable solution for ensuring a steady supply of food and feed materials (C.A. et al., 2024). The incorporation of millet and its various components into everyday foods may help combat undernutrition and support overall health (Sabuz et al., 2023). Among the grasses classified as small millets, there are around 35 species across 20 genera. The most commonly cultivated types include finger millet, foxtail millet, proso millet, barnyard millet, kodo millet, and little millet (Singh et al., 2020). Proso millet (*Panicum miliaceum* L.), one of the minor millets, is among the earliest cultivated millet crops and serves as a staple food for most populations living in the arid and semi-arid tropics, including regions of Asia, Africa, and parts of Europe. This review provides a comprehensive overview of the nutritional profile and therapeutic properties of proso millet, explores advanced processing techniques to enhance its bioactive potential, and discusses key challenges hindering its commercial utilization (Shen et al., 2018).

2. Nutritional Composition and Bioactive Constituents of Proso Millet

Proso millet exhibits nutritional qualities comparable to or exceeding those of other cereals. Its nutrient composition closely aligns with the recommended ratios of protein, carbohydrates, and lipids (Ren et al., 2023). Proso millet shows a high nutritional value, providing significant amounts of nutrients, including carbohydrates, proteins, vitamins, and minerals, along with some other phytochemical components such as flavonoids, phenolic acid derivatives, alkaloids (Xiang et al., 2023), fat, and crude fiber (Shen et al., 2018). The seed coat of millet contains the highest concentration of bioactive compounds (Nayak et al., 2024). Its energy-rich composition makes proso millet effective in addressing malnutrition (C.A. et al., 2024).

- **Carbohydrates and starch:** Carbohydrates and starch: Starch, the most abundant nutrient in proso millet, accounts for nearly 70% (Shen et al., 2018).
- **High protein content:** Proso millet has a protein content of 9.5-17%, which is similar to or higher than that of wheat and other grains. Regarding wheat protein, proso millet protein is enriched in essential amino acids, including isoleucine, methionine, and leucine (Ren et al., 2023). Studies have also shown that protein accounts for 12% and the essential amino acid index (EAAI) of proso millet was higher (51%) compared to wheat (Shen et al., 2018). High levels of essential amino acids, particularly those containing sulfur (methionine and cysteine), are found in typical millet protein (Amadou et al., 2013).
- **Essential nutrients:** Besides, proso millet also contains minerals (iron, zinc, magnesium, calcium, and phosphorus) and vitamins (niacin, thiamine, riboflavin, folate, and vitamin E) (C.A. et al., 2024).
- **Fatty acids:** Proso millet is described as a good source of fatty acids, predominantly linoleic acid.
- **Antioxidant Compounds:** Proso millet is a rich source of antioxidant compounds, including phenolic acids and glycosylated flavonoids, which are recognized for their prebiotic properties (Lima et al., 2021).

3. Functional Properties and Therapeutic Potential

Proso millet offers significant health benefits and functional components, including antioxidant, anti-diabetic, anti-cancer, antiproliferative, and anti-liver injury effects. Table 1 summarizes the main health effects of proso millet, the experimental models used, and key findings.

A large number of studies have shown that the aqueous, ethanol, and methanol extracts of proso millet possess antioxidant properties (Shen et al., 2018). The high antioxidant activity of proso millet compared to some other grains is attributed to its phenolic compounds (Jevcsák and Sipos, 2016).

Proso millet protein concentrate may have therapeutic potential for the treatment of type 2 diabetes (Shen et al., 2018). Additionally, proso millet protein is gluten-free (Ren et al., 2023); therefore, persons with celiac disease could consume it (Jevcsák and Sipos, 2016). Proso millet is regarded as a potential preventive food for liver injury, as studies have examined the effects of dietary proso millet protein on D-galactosamine-induced liver damage in rats (Shen et al., 2018). The antiproliferative activity of proso millet was also examined in vitro against MDA human breast cancer and HepG2 liver cancer cells. The results showed differential and potentially selective inhibitory effects. Such findings could help guide the consumption of proso millet to enhance its health benefits (Zhang et al., 2014).

Proso millet has demonstrated significant effects on metabolic health. Among extracts from nine different cereal grains tested, proso millet extract showed the strongest anti-lipogenic activity in 3T3-L1 cells. Furthermore, feeding its extract at 1% (w/w) for 4 weeks to ob/ob mice (an obesity model) effectively reduced body weight and liver weight, as well as blood triglyceride and total cholesterol levels, compared to obese ob/ob mice on a standard diet. Also, the extract decreased hepatic lipogenic-related gene (PPARα, L-FABP, FAS, and SCD1) expression, whereas it increased lipolysis-related gene (CPT1) expression. Consumption of the extract also led to a significant reduction in long-chain fatty acid levels and in the C18:1/C18:0 fatty acid ratio in the adipose tissue of the animals. Additionally, serum inflammatory mediators decreased significantly. These results suggest that proso millet extract is useful in the chemoprevention or treatment of obesity and obesity-related disorders. Through these mechanisms, proso millet demonstrates a promising role in supporting metabolic health (Park et al., 2011).

Proso millet might have significant potential as a functional food for maintaining hair health. In an in vitro study using human immortalized dermal papilla cells (iDPCs), a complex of millet and wheat extracts (MWC) was found to increase the mRNA levels of antioxidant enzymes (catalase and SOD1), growth factors (IGF-1, VEGF, and FGF7), and hair growth-related factors (wnt10b, β-catenin), while reducing the mRNA expression of inflammatory cytokines (IL-6 and TNFα). In an anagen-synchronized mouse model (in vivo), it was demonstrated that the mRNA levels of hair follicles (HFs) in the dorsal skin of the mouse model (in vivo) in the early and late telogen phases were also measured. It was demonstrated that the mRNA levels showed trends similar to those observed in the in vitro study during both the early and late telogen phases. In this model, MWC treatment prolonged the anagen phase of the hair cycle. These results suggest that MWC can reduce oxidative stress and inflammation, potentially extending the anagen phase by upregulating growth factors involved in the Wnt10b/β-catenin signaling pathway. (Choi et al., 2023).

A study explaining the effectiveness of proso millet in gastrointestinal tract, respiratory, and cardiovascular system ailments. It was shown that proso millet has antidiarrheal, spasmolytic, bronchodilator, and vasorelaxant activities, likely due to activation of ATP-dependent K^+ channels (Saqib et al., 2022).

Table 1. Summary of *In Vitro*, *In Vivo*, and Clinical Studies on the Health-Promoting Effects of Proso Millet.

Health Effect	Type of Study	Cell Line / Animal Model	Extract / Active Component	Key Findings	Reference
Anticancer	In vitro	HepG2 (human liver cancer)	Aqueous extract of proso millet	Reduced cell proliferation, antioxidant activity, selective antiproliferative effects	Zhang et al., 2014
Anticancer	In vitro	MDA-MB-231 (human breast cancer)	Butanol (BuOH) fraction of proso millet	Induced apoptosis via mitochondrial pathway, BAK/BAX activation, caspase-8/-9/-3 activation, PARP cleavage	Jun et al., 2023
Anti-obesity and metabolic improvement	In vivo	Type 2 diabetic mice (HFD + STZ)	Whole proso millet (WPM)	Reduced fasting blood glucose & serum lipids, improved glucose tolerance, alleviated liver/kidney injury, inhibited gluconeogenesis genes, activated PI3K/AKT pathway	Deng et al., 2023
Anti-obesity and lipid-lowering	In vivo	Type 2 diabetic mice	Proso millet protein (PMP)	Increased HDL & adiponectin, reduced glucose & insulin, upregulated adiponectin, downregulated TNF-α	Park et al., 2008

Hair health promotion	In vitro / In vivo	Human immortalized dermal papilla cells (iDPCs) / anagen-synchronized mouse model	Complex of proso millet and wheat extract (MWC)	Increased growth factors (IGF-1, VEGF, FGF7), hair growth genes (Wnt10b, β-catenin), antioxidant and anti-inflammatory effects	Choi et al., 2023
Hair growth stimulation	In vivo	C57BL/6 mice with testosterone-induced hair inhibition	Millet seed oil (MSO)	Promoted HFDPC proliferation, activated β-catenin pathway, stimulated hair growth	Lee et al., 2023
Anti-inflammatory	In vitro / In vivo	HT-29 cells / Nude mice	Bound polyphenols from foxtail millet bran (BPIS)	Inhibited IL-1β, IL-6, IL-8; increased IL-10; suppressed NF-κB-p65; reduced inflammation in vivo	Shi et al., 2017
Anti-inflammatory	In vitro	Human immortalized dermal papilla cells (iDPCs)	Complex of proso millet and wheat extract (MWC)	Increased antioxidant enzymes (catalase, SOD1), growth factors, hair growth genes; decreased IL-6 & TNFα	Choi et al., 2023
Hepatoprotective	In vivo	Rats (D-galactosamine-induced liver injury)	Proso millet protein	Reduced AST, ALT, LDH, indicating liver protection	Shen et al., 2018
Hepatoprotective	In vivo	Type 2 diabetic mice	Whole grain proso millet	Improved glucose tolerance, alleviated liver injury, reduced insulin resistance	Alzahrani et al., 2022
Hypoglycemic	In vivo	Type 2 diabetic mice	Whole grain proso millet (WPM)	Reduced fasting glucose, improved glucose tolerance, inhibited gluconeogenesis genes, activated PI3K/AKT pathway	Deng et al., 2023
Hypoglycemic	Humans (clinical trial)	Type 2 diabetic patients	Whole grain proso millet (WPM)	Reduced fasting & postprandial glucose; effects attributed to fiber, protein, resistant starch	Anitha et al., 2024

Cardioprotective	In vivo	Rat model	Aqueous extract	Reduced cardiac injury	Saqib et al., 2022
Blood pressure & myocardial protection	In vivo	Rat model	Aqueous extract	Decreased blood pressure and myocardial injury caused by high sodium intake	Wei et al., 2018

4. Genomic and Bioinformatic Approaches for Nutritional Improvement

With the growing global population, many people continue to experience food scarcity. Millets, although cultivated to a limited extent in regions facing food insecurity, hold significant potential to address nutritional deficiencies. This potential can be realized by expanding their cultivation area and implementing target crop improvement strategies. Genetic and genomic resources are essential for such improvement efforts (Sekhar et al., 2021).

The use of bioinformatics tools has transformed millet research by providing advanced computational approaches to analyze and interpret the large volumes of data generated by genomic studies. A variety of biotechnological and genomic approaches have been employed to further enhance millet crops. These include the development of genetic maps, next-generation sequencing (NGS), genotyping-by-sequencing (GBS), genome-wide association studies (GWAS), synteny studies, gene expression profiling, fine mapping of quantitative trait loci (QTL), candidate gene identification, genetic engineering, gene pyramiding, and systems biology. Additionally, integrating multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, provides a comprehensive understanding that supports the advancements and biofortification of this nutrient-rich cereal (Satyavathi et al., 2021). Functional genomics techniques, supported by bioinformatics tools, play a crucial role in the isolation and characterization of genes with nutritional importance. High-throughput 'omics' technologies provide useful insights and opportunities for the development of third-generation molecular markers, as well as the identification of candidate genes for engineering transgenic crops aimed at improving nutritional security. High-throughput DNA sequencing technologies, commonly referred to as next-generation sequencing (NGS), represent one of the key foundations of genomic research. These methods readily provide information on Single Nucleotide Polymorphism (SNP) in nutrient-rich genes (Sharma et al., 2017). Comparative synteny studies, enabled by bioinformatics, allow researchers to examine the genomic relationships between millets and non-millet cereals, helping to identify common genes associated with nutrition biosynthesis pathways. Once relevant genes, alleles, and QTLs are discovered, they can then be incorporated into elite lines through molecular marker-assisted selection or transgenic approaches. Synteny analyses may also aid in transferring beneficial traits from major cereals into millets (Manwaring et al., 2016). Further, proteomics has enhanced our understanding of how proteins influence mineral homeostasis in plants (Sharma et al., 2017). Genomic resources, including whole-genome sequencing (WGS) data for various millet species, are available in public databases such as NCBI, Phytozome, Gramene, and EnsemblPlants. These resources have facilitated the development of large-scale, genome-wide molecular markers, the construction of high-density genetic maps, and the identification of genomic regions associated with key traits, including nutritional qualities for use in breeding programs (Sekhar et al., 2021). The completion of several WGS and transcriptome profiling projects in small millets has been enabled by advances in NGS and bioinformatics platforms. These advancements have led to the discovery of numerous non-coding RNAs (ncRNAs) and have enhanced our understanding of their expression patterns and regulatory mechanisms. Millet genome sequences can be effectively used to identify candidate genes involved in abiotic stress tolerance and to advance breeding strategies such as genomic selection (El-Hashash et al., 2023). Overall, bioinformatics approaches, including the detailed analysis of millet genome sequences, have provided valuable insights into the genetic architecture of millets and the evolutionary patterns of key genes linked to agronomic and nutritional traits (Chellappan and Peramaiyan, 2024).

5. Processing Strategies to Enhance Nutritional Quality

Millets contain a wide range of nutrients, antinutrients, and antioxidants, highlighting the importance of understanding how different processing methods affect these components. Antinutritional factors can impair the digestibility of macronutrients as well as the bioavailability and bioaccessibility of minerals (Balyatanda et al., 2024). On the other hand, the bioactive compounds, nutrients, and functional properties of cereal grains can be influenced by processing techniques such as decortication, soaking, malting, milling, and fermentation (Sabuz et al., 2023). These necessitate methods to reduce or eliminate antinutrients while improving nutritive and antioxidant value in food (Balyatanda et al., 2024). Since antinutrients are biologically active secondary metabolites with both detrimental effects and pharmacological significance, it is essential to thoroughly understand processing techniques before selecting the most appropriate method. Understanding how processing techniques affect the antioxidant and antinutritional properties of phytochemicals in millets is essential for optimizing the nutritional quality and health benefits of millet-based foods. Employing appropriate processing techniques can preserve and enhance the antioxidant content, maximizing their protective effects against oxidative stress and chronic diseases. Furthermore, effective processing methods should substantially reduce antinutritional factors, thereby enhancing nutrient bioavailability and minimizing potential adverse effects. Based on the method of processing, the processing techniques can be grouped as follows: (1) physicochemical and bioprocessing techniques, such as germination, fermentation, enzymatic decortication, dehulling, milling, soaking, and malting (Balyatanda et al., 2024).

Most millets exhibit improvements in their nutritional mineral, fiber, and vitamin contents after germination and fermentation. Basic processing methods such as soaking, germination, and fermentation can enhance the absorption of minerals and improve protein digestibility, addressing issues related to protein-energy deficiency (Ravi and Rana, 2024).

(2) Thermal processing, including steaming, boiling, parboiling, roasting, flaking, extrusion, cooking, microwaving, etc.; (3) non-thermal technologies, such as irradiation, pulsed light, UV, ozone, cold plasma, high-pressure processing, pulsed electric fields, and ultrasound; and (4) combination techniques that synergistically reduce antinutritional factors (ANFs) (Balyatanda et al., 2024). Thermal and nonthermal processing inhibit lipase activity, ensuring microbial stability and improved storage durability. These methods show promise in enhancing millet properties while minimizing nutrient loss and energy consumption during processing (Ravi and Rana, 2024).

6. Technical and Industrial Challenges in Millet Utilization

Despite growing interest in millets for their health-promoting properties, several technical and industrial limitations continue to impede their full-scale utilization. The following challenges must be addressed to unlock their potential in functional food development and commercial applications.

1) To improve nutrients and produce novel functional food items, it is necessary to decrease antinutrients, increase nutrient bioavailability, and extend shelf life, as millet flour's low shelf life is due to increased lipids and enzyme activities; 2) Milling, dehulling, and decortication processes can reduce the levels of micronutrients, total proteins, and dietary fiber. Excessive dehulling can diminish fiber content and result in the loss of micronutrients by removing the nutrient-rich bran and germ section. Therefore, caution should be exercised when decorticating millets; 3) Additionally, while research on millets is extensive, few studies focus on large-scale production potential, creating a knowledge gap between laboratory findings and industrial applications (Ravi and Rana, 2024).

7. Conclusion

The growing global interest in sustainable and nutritious food sources positions millets favorably in the market. Proso millet emerges as a promising candidate in the global quest for sustainable, nutrient-dense, and climate-resilient crops. Its ability to address various health conditions, including undernutrition and chronic diseases, creates opportunities to develop targeted functional foods and nutraceuticals. Given its resilience and rich nutritional profile, proso millet holds immense promise as a strategic crop for enhancing global food security and supporting sustainable agriculture. However, its commercial potential remains largely untapped due to limitations in large-scale processing, low shelf life, and nutrient degradation during dehulling. To fully harness the benefits of proso millet, further multidisciplinary research is essential, particularly in advanced food processing technologies and industrial applications.

Authors statement of contribution

We wish to acknowledge that **Tahere Razzaghi** and **Fatemeh Naderi** contributed equally to this work and should be considered co-first authors.

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