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Review

A Comprehensive Review on the Extraction and Determination of Vitamins and Minerals in Kodo Millet (Paspalum scrobiculatum)

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Abstract

Kodo millet (*Paspalum scrobiculatum*) is increasingly recognized as a nutrient dense cereal with substantial potential for addressing micronutrient deficiencies. This review synthesizes current methodologies and emerging approaches in the extraction and determination of vitamins and minerals from kodo millet. Emphasis is placed on its B complex vitamins (thiamine, riboflavin, niacin) and essential minerals (iron, calcium, zinc, magnesium), which collectively offer diverse health benefits. Factors affecting nutrient retention such as antinutritional compounds (phytates, tannins), enzymatic activities, and environmental parameters are discussed in detail, underscoring the complexity of accurately quantifying micronutrients in this grain. The paper examines traditional and modern extraction techniques (solvent based, microwave assisted, enzymatic) as well as advanced analytical tools, including high performance liquid chromatography (HPLC), inductively coupled plasma mass spectrometry (ICP-MS), and other spectroscopic methods. Special attention is afforded to method standardization challenges, the importance of rigorous validation (including the use of certified reference materials), and potential pathways for biofortification and crop improvement. Moreover, the review highlights the cereal's resilience under marginal growing conditions, aligning its cultivation with the broader goals of sustainable agriculture. By identifying knowledge gaps and proposing harmonized protocols for nutrient analysis, this article seeks to catalyze further research, promote consumer acceptance, and encourage policy support for integrating kodo millet into mainstream diets. Ultimately, leveraging its robust nutritional profile could make kodo millet a vital contributor to addressing malnutrition and advancing global food security initiatives.

Keywords: Kodo millet, vitamins, minerals, extraction methods, analytical techniques, nutritional analysis, biofortification, food security

INTRODUCTION

Kodo millet (*Paspalum scrobiculatum*) is an ancient cereal cultivated predominantly in regions with harsh agronomic conditions, celebrated for its resilience and adaptability under marginal lands [1]. Despite its longstanding use in Asia and parts of Africa, global dietary reliance on major cereals like wheat, rice, and maize has often overshadowed the nutritional potential of kodo millet [2]. However, a paradigm shift toward diversifying staple diets to combat micronutrient malnutrition has thrust millets back into the spotlight [3]. Among these, kodo millet has garnered attention due to its robust macronutrient and micronutrient profile, as well as its moderate input requirements and relative drought tolerance [4].

Recent research underscores the significance of kodo millet in addressing global dietary deficiencies, particularly iron deficiency anemia and vitamin B complex shortfalls [5,6]. Accurate characterization of its micronutrient composition is imperative for developing evidence based consumption guidelines, breeding initiatives, and public health interventions [7]. Yet, the interplay between environmental factors (soil composition, rainfall patterns) and the inherent properties of the grain (fiber content, antinutritional compounds) introduces variability in reported nutrient values [8]. Consequently, establishing reliable extraction and analytical methods for vitamins and minerals is vital [9].

Traditional practices like milling, soaking, or fermentation influence the availability and stability of micronutrients [10]. Concurrently, advanced extraction and detection technologies ranging from solvent based methods to microwave assisted extraction and from atomic absorption spectroscopy to ICP-MS have facilitated more precise nutrient quantification [11,12]. Nonetheless, inconsistencies in protocols, methodological complexities, and limited access to state of the art instrumentation can lead to discrepancies in reported nutrient levels [13,14]. These variations pose challenges for industry players, researchers, and policy makers seeking to integrate kodo millet into mainstream nutritional programs and commercial products [15].

Beyond public health, kodo millet aligns with global calls for sustainable agriculture. As climate change intensifies, crops that thrive under minimal inputs and can withstand stressors are increasingly valuable [16]. Its promising capacity to grow in marginal soils, coupled with emerging biofortification approaches, situates kodo millet at the nexus of nutrition and resilience [17]. This convergence underscores the urgency of refining scientific understanding around its nutritional attributes.

This review aims to provide an exhaustive overview of kodo millet's nutritional composition, focusing on both vitamins and minerals. It delves into the intricacies of various extraction protocols, detailing their operational principles, advantages, and limitations. Subsequently, it explores analytical techniques for accurate micronutrient measurement, discussing validation requirements, challenges in data comparability, and future directions. By consolidating current knowledge and identifying methodological gaps, the paper seeks to inform best practices and catalyze further research, ultimately bolstering the cereal's role in global food systems [18–20].

NUTRITIONAL COMPOSITION OF KODO MILLET

Kodo millet's appeal lies in its balance of carbohydrates, proteins, and dietary fiber, alongside a diverse micronutrient profile [21]. Carbohydrates in kodo millet are often rich in non starchy polysaccharides that slow glucose release, aiding glycemic control in populations prone to metabolic syndrome [22]. Its protein content, while moderate, can complement other dietary staples when combined in a balanced diet [23]. A key highlight is the grain's significant dietary fiber content, which supports digestive health and contributes to satiety [24].

In terms of vitamins, kodo millet provides notable concentrations of B-complex vitamins, specifically thiamine (B1), riboflavin (B2), and niacin (B3) [25]. These cofactors play central roles in energy metabolism, DNA repair, and antioxidant mechanisms, making their deficiency a major public health concern worldwide [26]. Although present in lesser amounts, vitamin E in kodo millet adds antioxidant value, potentially mitigating oxidative stress and chronic disease risks [27]. However, the actual retention of these vitamins can vary with processing and storage conditions, prompting a need for standardized analytical methods to capture the true nutritional potential [28].

Minerals such as iron, calcium, magnesium, and zinc are also abundant in kodo millet, each fulfilling critical physiological functions [29]. Iron is essential for hemoglobin synthesis and oxygen transport, while calcium supports skeletal health [30]. Magnesium aids in hundreds of enzymatic reactions, and zinc underpins immune defenses and cellular repair mechanisms [31]. The presence of antinutritional factors (phytates, tannins) can influence the bioavailability of these minerals, leading researchers to explore processing interventions like germination and fermentation [32]. The variability introduced by environmental conditions, genotypic differences, and agricultural practices further complicates efforts to establish precise nutrient baselines [33].

Traditional culinary methods such as soaking, malting, or fermenting kodo millet can mitigate phytates and enhance mineral absorption [34]. Conversely, excessive refinement or prolonged cooking can degrade heat labile vitamins [35]. Thus, recommended practices often include minimal milling and controlled heating to maximize nutrient retention [36]. Additionally, blending kodo millet with legumes or complementary cereals may bolster protein quality and ensure a more complete amino acid profile [37]. From a public health perspective, leveraging the cereal's inherent nutrition for widespread consumption demands verifiable data on its vitamin and mineral content hence the importance of rigorous, standardized extraction and analytical techniques [38].

SIGNIFICANCE OF VITAMINS IN KODO MILLET

Vitamins are organic compounds required in trace amounts yet essential for sustaining normal metabolism. In kodo millet, the emphasis frequently rests on the B-complex group thiamine (B1), riboflavin (B2), niacin (B3) given their involvement in critical metabolic pathways [39]. Thiamine facilitates the conversion of nutrients into ATP, crucial for neuronal and cardiac function. Riboflavin serves as a cofactor in redox reactions, supporting antioxidant defenses by regenerating glutathione. Niacin is indispensable for synthesizing NAD and NADP, vital cofactors in countless biochemical reactions [40]. Even minor vitamin E content contributes to protecting cell membranes from oxidative stress [41].

These vitamins gain prominence in regions plagued by dietary insufficiencies, where reliance on refined grains lacking these micronutrients aggravates the risk of deficiency [42]. Kodo millet, if properly processed, can bridge some of these nutritional gaps. However, factors such as storage temperature, moisture, and exposure to light can degrade thermolabile vitamins [43]. Postharvest handling protocols like low temperature storage and reduced exposure to oxygen help preserve vitamin integrity but demand additional resources and infrastructure. Cooking methods also matter: prolonged boiling or high heat roasting can cause substantial vitamin losses [44]. Innovations like microwave cooking or mild steaming have shown promise in retaining water soluble vitamins. Moreover, the synergy among B complex vitamins is noteworthy riboflavin, for instance, helps activate pyridoxine (vitamin B6) and folate (vitamin B9), underscoring the holistic value of maintaining the entire vitamin spectrum [45]. Despite these advantages, consumer familiarity with kodo millet and consistent access to high quality grain remain limiting factors in maximizing its nutritional impact.

SIGNIFICANCE OF MINERALS IN KODO MILLET

Minerals are inorganic, serving structural and regulatory roles in the human body. Kodo millet is particularly lauded for iron, calcium, magnesium, and zinc, aligning it with global efforts to combat anemia, osteoporosis, and immune deficiencies [46]. Iron deficiency afflicts large swaths of the global population, especially women and children in low income settings, underscoring the potential of iron rich cereals like kodo millet [47]. Calcium supports bone mineral density, while magnesium is a cofactor for enzymes involved in protein synthesis and glucose metabolism [48]. Zinc is indispensable for DNA synthesis and immune function, further bolstering the cereal's nutritional cachet [49].

Nonetheless, the bioavailability of these minerals can be hindered by the grain's antinutritional composition namely phytates that chelate divalent cations [50]. Processing interventions such as soaking, germination, and fermentation degrade phytates, freeing up minerals for easier absorption. Selecting appropriate processing and culinary methods is thus pivotal to unlocking kodo millet's full mineral potential. Furthermore, environmental conditions, such as soil pH and mineral composition influence the final mineral content of harvested grains, adding an agronomic layer to nutrient optimization. Addressing these variables effectively can position kodo millet as a potent tool against mineral deficiencies.

EXTRACTION METHODS FOR VITAMINS IN KODO MILLET

Extraction of vitamins from kodo millet involves liberating these compounds from the cereal matrix no simple feat due to the presence of fiber, protein complexes, and potential antinutritional factors [9,11]. Traditional solvent based methods employ polar solvents like water, methanol, or ethanol. Process parameters such as extraction time, temperature, and pH must be carefully controlled to avoid degradation of heat or pH sensitive vitamins [13]. In contrast, microwave assisted extraction (MAE) uses microwave energy to break cell walls more rapidly, often yielding higher vitamin recovery in reduced extraction times [14].

Enzyme assisted extraction is another promising approach, leveraging specific enzymes (e.g., cellulases, proteases) to dismantle the cereal's cell wall architecture. While it offers gentle extraction conditions, enzyme costs and reaction specificity can pose challenges [7]. Pressurized liquid extraction (PLE), also known as accelerated solvent extraction, harnesses elevated pressure and moderate temperature to enhance solvent penetration while minimizing thermal degradation [9]. Supercritical fluid extraction (SFE), particularly with supercritical CO2_22, is effective for fat soluble vitamins but less so for water soluble B complex vitamins [16]. Post extraction cleanup through filtration, centrifugation, or solid phase extraction is crucial for eliminating extraneous compounds that interfere with subsequent detection. Emerging green extraction technologies focus on reducing harmful solvents and lowering energy consumption, aligning with sustainability goals. Across all methods, validation typically involves spiking known vitamin standards into the cereal matrix and verifying extraction efficiency. The high variability in matrix composition necessitates careful optimization for each vitamin, underlining the complexity of consistently extracting the full vitamin spectrum from kodo millet.

EXTRACTION METHODS FOR MINERALS IN KODO MILLET

Mineral extraction from kodo millet typically demands breaking down its organic matrix to release tightly bound inorganic elements. Acid digestion often utilizing nitric acid (HNO3_33), hydrochloric acid (HCl), or mixtures thereof stands as a classical approach [8]. Open vessel acid digestion can be performed under heat, but microwave digestion, which uses sealed Teflon vessels, provides uniform heating and reduces oxidation time [11]. Dry ashing is another strategy, wherein organic matter is combusted at high temperatures (500–600°C), leaving an ash residue of minerals. However, volatile elements can be lost if not carefully regulated.

Wet ashing employs oxidizing agents at lower temperatures, potentially conserving volatile elements more effectively. Instrumental contamination and reagent purity are pivotal concerns; hence, high-purity acids and rigorously cleaned containers are mandated to prevent sample contamination [10]. Occasionally, chelating agents like EDTA are added to stabilize specific minerals in solution. Environmental considerations around chemical waste highlight a need for greener extraction protocols that reduce acid volumes and toxic byproducts.

Matrix complexity adds another layer of challenge phytates, fiber, and tannins can interfere with mineral solubilization. Pretreatments such as soaking, fermentation, or germination can lower phytate content before the digestion step [12]. Once extracted, the sample often undergoes filtration or centrifugation to yield a clear solution amenable to spectroscopic techniques. Consistency in digestion parameters (temperature, time, acid concentration) is vital for reproducibility, especially for multi sample analyses. As with vitamin extraction, validated protocols including checks with certified reference materials are essential to ensure data reliability.

ANALYTICAL TECHNIQUES FOR VITAMIN DETERMINATION

Accurate quantification of vitamins in kodo millet commonly involves chromatographic and spectroscopic methods. High performance liquid chromatography (HPLC) is a mainstay due to its separation efficiency and adaptability to various detectors (UV, fluorescence, electrochemical) [12]. Derivatization steps (e.g., forming thiochrome from thiamine) can enhance detectability, while reversed phase columns facilitate the analysis of water soluble vitamins [9]. For heightened specificity and lower detection limits, liquid chromatography–mass spectrometry (LC–MS) and tandem mass spectrometry (LC–MS/MS) have gained traction [14]. These systems confirm molecular weight and structure, reducing the likelihood of coeluting interferences.

Simpler approaches like UV visible spectrophotometry or thin layer chromatography (TLC) may suffice for preliminary screening, yet they often lack the selectivity demanded by the complexity of kodo millet [3]. Sample cleanup via solid phase extraction or liquid liquid partitioning is typically integrated to remove proteins, fibers, and other interfering compounds. Calibration involves serial dilutions of vitamin standards, verified against reference materials when available. Instrument validation includes parameters such as linear range, limits of detection/quantification, accuracy, and reproducibility. Maintaining appropriate storage conditions for extracts is also critical, given the sensitivity of certain vitamins to light, temperature, and oxidation [13].

ANALYTICAL TECHNIQUES FOR MINERAL DETERMINATION

On the mineral front, atomic absorption spectroscopy (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) are routinely employed [2]. AAS (flame or graphite furnace) measures how free atoms absorb specific wavelengths, suitable for elements like iron, calcium, or magnesium. ICP-OES excites atoms in a plasma; their characteristic emission spectra are correlated to elemental concentrations [4]. ICP-MS extends sensitivity down to parts per billion or parts per trillion, making it invaluable for trace minerals [12]. Each technique mandates well digested, particle free samples to avoid instrumental blockages or signal drift.

Calibration relies on multi element standard solutions, facilitating the simultaneous detection of multiple minerals. Matrix matching or internal standards help offset ionization interferences, common when analyzing cereals with high alkali metal content. For accuracy checks, certified reference materials mimicking cereal matrices are crucial [5]. Though advanced, these instruments may be cost and resource intensive, limiting their use in smaller laboratories. Innovations like portable X-ray fluorescence (XRF) devices provide faster, though often semi quantitative, field based measurements. Ultimately, data robustness hinges on method validation, consistent sample preparation, and thorough matrix effect management.

CHALLENGES AND FUTURE DIRECTIONS

Despite the tangible promise of kodo millet in improving micronutrient intake, multiple obstacles persist. Environmental variables soil pH, rainfall, crop genetics induce fluctuations in vitamin and mineral content that complicate establishing standardized nutrient databases [6]. Antinutritional factors like phytates demand careful processing protocols, adding cost and labor that may deter widespread adoption [7]. Furthermore, global differences in extraction and analytical procedures result in inconsistent data, weakening cross study comparisons and stalling robust policy directives [8]. Many laboratories also face resource limitations, lacking advanced instrumentation like ICP-MS or HPLC-MS/MS, which restricts the scope of nutrient profiling [10].

Addressing these concerns calls for continued innovation in green extraction technologies, ensuring minimal environmental impact while maintaining high extraction efficiency [11]. Nanotechnology based sensors and adsorbents could advance detection sensitivity, though commercial scale feasibility remains to be demonstrated. Standardizing protocols, especially through interlaboratory validation and the use of certified reference materials, is critical for data harmonization and comparability. On a broader scale, biofortification and selective breeding can amplify the inherent nutrient density of kodo millet, provided reliable nutrient measurement underpins breeding decisions [17,18]. Partnerships among researchers, policy makers, and community stakeholders will be essential to translate these scientific advances into practice particularly in regions where micronutrient deficiencies remain pervasive. The intersection of nutrition science, sustainable agriculture, and consumer education underscores the urgency and complexity of tapping kodo millet's full potential.

CONCLUSION

Kodo millet stands as a compelling cereal candidate for mitigating micronutrient deficiencies while promoting climate resilient agriculture. Its array of vitamins particularly B-complex vitamins and minerals like iron, calcium, magnesium, and zinc highlight its significance in supporting human health. However, the accuracy of reported nutrient levels depends on rigorous extraction and analytical techniques. Conventional and modern methodologies ranging from solvent based extractions and acid digestions to sophisticated instrumentation like HPLC and ICP-MS offer valuable insights but also reveal methodological gaps. Antinutritional factors, environmental variability, and the lack of standardized protocols complicate efforts to fully harness kodo millet's nutritional promise.

Future directions revolve around harmonizing extraction and detection strategies, advancing "green" and nanotechnology enhanced methods, and facilitating interdisciplinary collaboration. Efforts to biofortify kodo millet or optimize cultivation and processing can further augment its nutritional profile. By integrating scientific innovation with community level engagement, kodo millet can transition from a largely underutilized cereal to a central component of sustainable diets and public health strategies. Ultimately, systematic research, robust validation, and policy backing are indispensable for elevating kodo millet's status and delivering tangible benefits to populations in need of affordable, nutrient rich food options.

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