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A Novel Integrated Bioprocessing Strategy for the Manufacturing of Shelf-Stable, Nutritionally Upgraded Activated Wheat: Development of a Comprehensive Protocol, In-Depth Nutritional Characterization, and Evaluation of Biofunctional Properties

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Abstract

Background: Wheat, a cornerstone of global nutrition, possesses inherent nutritional constraints primarily due to the presence of phytic acid, which significantly impedes mineral bioavailability. The process of sprouting has been identified as a promising biological strategy to mitigate these limitations and enhance the nutrient profile. However, a major industrial challenge remains: the pronounced perishability and microbial instability of sprouted grains, which limits their practical application in food systems.

Objective: The principal aim of this investigation was to conceive, engineer, and rigorously validate an innovative and scalable biotechnological procedure for the generation of shelf-stable activated wheat. This protocol adheres to stringent technical criteria, including the avoidance of aerial agitation, the implementation of in-vessel dehydration, and the exclusion of convective heat transfer mechanisms.

Methods: The methodology centered on the design and operational characterization of a Multi-Function Bioreactor (MFB) system. A comparative examination was executed, pitting the novel protocol against two control processes: traditional laboratory-scale sprouting techniques and historical Georgian artisanal practices. A exhaustive analytical framework was employed, encompassing detailed nutritional assessment (targeting phytic acid, essential vitamins, and macro- and micro-minerals) and comprehensive biochemical evaluation (focusing on antioxidant potential and protein profile modifications).
Key Results: The implemented protocol yielded a highly successful reduction of phytic acid content, exceeding 60%. Furthermore, it facilitated a substantial augmentation in the concentrations of B-complex vitamins, notably a 30% increase in Thiamine (B1), a 25% rise in Riboflavin (B2), and a 50% enhancement in Folate levels. The antioxidant capacity, as quantified by DPPH radical scavenging assays, reached an impressive 89%. Critically, the final product achieved a shelf-stable format, demonstrating no significant nutritional degradation over a period exceeding 12 months.
Conclusion: The innovative methodology described herein effectively translates a traditional food processing concept into a sophisticated, reproducible, and industrially viable biotechnological operation. The resultant product is a premium-grade, shelf-stable functional food ingredient, whose enhanced nutritional credentials have been thoroughly validated through empirical analysis.

Keywords: activated wheat, grain sprouting, bioreactor technology, phytic acid reduction, nutrient bioavailability, vacuum-assisted drying, functional food ingredients, shelf-life stability, nutritional fortification

Introduction

The Nutritional Paradox of Wheat: Contrasting Global Ubiquity with Intrinsic Bioavailability Constraints

Wheat (Triticum spp.) is unequivocally established as a fundamental pillar of global alimentary security, constituting a primary source of caloric energy, protein, and indispensable mineral nutrients for a substantial segment of the human population worldwide (Shewry & Hey, 2015). Its preeminence in agricultural systems and its foundational role in human nutrition have solidified its status as an indispensable staple crop. Paradoxically, this undisputed agricultural and dietary importance exists in direct juxtaposition with a significant nutritional dilemma: the presence of innate antinutritional constituents that severely curtail the bioavailability of the very nutrients the grain contains.

The most prominent concern revolves around phytic acid (chemically known as myo-inositol hexakisphosphate), which serves as the predominant storage form of phosphorus within cereal grains. Although phytic acid itself is not inherently toxic, it functions as a powerful chelating agent, binding essential mineral cations such as iron, zinc, calcium, and magnesium into

insoluble complexes known as phytates within the gastrointestinal environment. These phytates are notoriously poorly absorbed by the human digestive system (Gupta et al., 2015). This sequestration process dramatically diminishes the biological availability of these crucial minerals, thereby acting as a significant contributing factor to the high incidence rates of mineral deficiencies—particularly iron-deficiency anemia and zinc insufficiency—observed among populations whose diets are heavily reliant on cereal-based food products (Gibson et al., 2010). Consequently, despite exhibiting ostensibly adequate mineral composition when analyzed chemically, the practical nutritional value offered by conventional wheat-derived products is profoundly constrained by this phenomenon, often described as a state of "hidden hunger."

Sprouting as an Indigenous Bioprocessing Intervention

Throughout history, traditional food preparation and processing methodologies have intuitively sought to ameliorate these nutritional limitations. Among these time-honored techniques, sprouting—also referred to as malting in certain contexts—stands out as an ancient bioprocessing strategy that entails the controlled germination of cereal grains. This seemingly simple yet remarkably potent process initiates a profound series of biochemical transformations within the seed. Following hydration, a cascade of metabolic pathways is activated, precipitating the synthesis and subsequent activation of endogenous enzymatic systems. Most notably, this includes the induction of phytase, the specific enzyme responsible for the catalytic degradation of phytate, in addition to the activation of amylases, proteases, and other hydrolytic enzymes (Lemmens et al., 2019).

The corpus of scientific literature substantiating the benefits of sprouting is both extensive and compelling. A considerable body of research has conclusively demonstrated that carefully controlled germination processes efficiently hydrolyze phytic acid, thereby markedly diminishing its antinutritional impact and concurrently enhancing the bioavailability of mineral elements (Balkrishna et al., 2022; Pal et al., 2016). Beyond the crucial aspect of phytate reduction, the sprouting process instigates a multifaceted amelioration of the grain's overall nutritional character. It promotes the accumulation of free amino acids and simple sugars, induces a net increase in the concentration of certain vitamins (most notably those within the B-group, as well as vitamin E), and significantly stimulates the synthesis and subsequent bioavailability of a diverse array of bioactive compounds. These bioactives include various phenolic acids and flavonoids, which are recognized as potent antioxidant agents (Benítez et al., 2021; Gan et al., 2017; Li et al., 2021). Thus, sprouting emerged as a natural, economically viable, and efficacious biotechnological strategy for the conversion of standard wheat into an "activated" or pre-digested ingredient endowed with nutritionally and functionally superior qualities.

Technological Impediments in the Industrial-Scale Production of Sprouted Grains

Notwithstanding its empirically validated benefits and historical utilization, the large-scale industrial production of high-quality, shelf-stable sprouted wheat confronts considerable technological hurdles. Prevailing sprouting technologies, frequently adapted from malting

systems originally designed for the brewing industry, are beset with intrinsic limitations that substantially impede their widespread adoption within the food manufacturing sector.

A paramount concern is the elevated risk of microbial contamination, including proliferation of spoilage bacteria and mycotoxigenic molds, a vulnerability inherent to the warm, moist, and oxygen-rich conditions that are optimally conducive to the germination process itself (Laca et al., 2006). These conventional open or semi-open system architectures render the consistent maintenance of aseptic conditions exceedingly challenging. Moreover, traditional methodologies often lack the capability for precise control over critical germination parameters—such as temperature, relative humidity, and irrigation frequency and duration—across large-scale batches. This lack of precision results in unacceptable product quality inconsistency and suboptimal efficiency in phytate degradation (Singh et al., 2015). However, the most formidable obstacle remains the intrinsic perishability of the freshly germinated grain. The end product of sprouting possesses a characteristically high moisture content, typically ranging from 35% to 45%, which renders it exceptionally susceptible to rapid microbial spoilage and enzymatic deterioration. This vulnerability drastically curtails its shelf life, often to mere days, mandating immediate further processing or refrigerated storage (Keppler et al., 2018). This extreme perishability constitutes a major logistical and economic barrier that effectively prevents the widespread distribution and commercial utilization of sprouted wheat products.

Advancing Past Conventional Drying: The Imperative for Mild Dehydration Methodologies

Attainment of shelf stability necessitates the dehydration of sprouted grains. Convective hot-air drying, the most prevalent industrial-scale dehydration technique, is notoriously injurious to the heat-labile nutrients whose concentrations are elevated during the sprouting process. Application of high temperatures induces degradation of vitamins, denaturation of proteins, inactivation of beneficial endogenous enzymes, and loss of volatile aromatic compounds responsible for flavor (Dutta et al., 2022; Méndez-Lagunas et al., 2017).

Most critically, elevated processing temperatures accelerate deleterious chemical reactions, primarily the Maillard reaction and lipid oxidation pathways. These reactions can lead to the formation of undesirable compounds and an overall reduction in the nutritional quality profile meticulously built during germination (Vashisth et al., 2011). Therefore, the application of conventional hot-air drying to sprouted wheat creates a fundamentally counterproductive paradigm wherein the valuable nutritional gains achieved through meticulous and controlled biological activation are subsequently forfeited during the essential preservation stage. This glaring contradiction underscores an urgent and compelling need for the development of innovative, gentle dehydration technologies capable of effectively reducing moisture content to ensure microbial and enzymatic stability, while simultaneously maximizing the preservation of thermosensitive bioactive components.

Materials and Methods

Preparation and Initial Processing of Raw Materials

The primary biological material utilized in this investigation was organically cultivated hard red spring wheat (Triticum aestivum L.), which was procured from a certified organic agricultural producer to guarantee the absence of synthetic pesticides and chemical treatments that could potentially interfere with the germination physiology. The initial processing phase involved a meticulous cleaning procedure employing a vibratory sieving apparatus to systematically remove extraneous materials, including dust particulate matter, residual chaff, and mechanically damaged or broken kernels, thereby ensuring a homogeneous and high-quality starting substrate.

A rigorously standardized disinfection protocol, which is critically important for the substantial reduction of the initial microbial load prior to the initiation of the sprouting process (Kumar & Chakkaravarthi, 2021), was methodically implemented. In precise terms, batches of wheat kernels weighing 5 kg were subjected to a washing procedure using a 2% (volume/volume) solution of food-grade hydrogen peroxide (H_2O_2). This washing was conducted for a duration of 10 minutes accompanied by gentle mechanical agitation to ensure uniform contact, and was subsequently followed by an exhaustive rinsing sequence utilizing sterile distilled water. The rinsing was continued iteratively until the effluent rinse water attained a neutral pH level, thereby confirming the complete elimination of any residual peroxide. This specific disinfection methodology was selected based on its well-documented efficacy against a diverse and broad spectrum of microorganisms, and furthermore because of its favorable decomposition profile, breaking down into water and molecular oxygen without leaving behind any toxic or harmful chemical residues (Taze et al., 2015).

Design of Experimental Treatments and Groups

The experimental framework was structured around three distinctly defined treatment groups to facilitate a rigorous and comprehensive comparative analysis:

- Group 1 (Traditional Control Methodology): This group embodied historical artisanal processing techniques. Grains were sprouted according to a traditional Georgian method. Following the soaking phase, the hydrated grains were distributed in a single layer on perforated trays, covered with moistened breathable cloths, and allowed to germinate at ambient laboratory temperature (approximately 20°C) for a total period of 48 hours. Manual rinsing with copious amounts of water was performed at 12-hour intervals to maintain hydration and provide necessary aeration. The resultant sprouted wheat was subsequently subjected to sun-drying under natural atmospheric conditions for an extended period of 72 hours.
- Group 2 (Standard Laboratory Control Protocol): This group represented contemporary best practices for controlled small-scale sprouting. Grains were germinated inside a precision-controlled climate chamber (manufactured by Binder GmbH, Germany) which

was meticulously maintained at a constant temperature of 20°C and a high relative humidity of 95% for the same 48-hour duration. This group serves as the benchmark for modern laboratory-scale sprouting under optimized environmental conditions (Keppler et al., 2018). The germinated wheat from this group was then transferred to a convective hot-air drying oven (manufactured by Memmert, Germany) and dehydrated at 60°C for a period of 12 hours.

- Group 3 (Novel Multi-Function Bioreactor Protocol): Grains assigned to this experimental group were processed utilizing the novel, integrated Multi-Function Bioreactor (MFB) system. This innovative setup was designed to seamlessly integrate the entire production sequence, commencing with the initial disinfection stage and concluding with the final drying step, all conducted in-situ within the same sealed vessel. A detailed description of the MFB system and its operational protocol is provided in subsequent sections 2.3 and 2.4.

To ensure statistical robustness and the reliability of the obtained data, all experimental treatments and subsequent analytical measurements were performed in triplicate (n=3).

Architectural and Functional Description of the Multi-Function Bioreactor (MFB) System

A bespoke, fully integrated Multi-Function Bioreactor (MFB) system was conceived, designed, and fabricated specifically for the purposes of this research study.

- Vessel Design and Construction: The central component of the entire system is a cylindrical, vertically oriented processing vessel constructed entirely from AISI 316L stainless steel. This specific grade was selected due to its exceptional resistance to corrosion, its impeccable cleanability, and its full compliance with food-grade material standards. The vessel possesses a total volumetric capacity of 100 liters, engineered to accommodate a nominal processing batch size of 25 kilograms of wheat kernels. A notable design feature is the incorporation of a double jacket surrounding the vessel, which allows for the circulation of a dedicated heat-transfer fluid (Therminol 55). This enables the provision of highly precise and uniform heating and cooling throughout all sequential stages of the processing cycle.
- Agitation and Mixing System: A centrally mounted, low-speed helical ribbon agitator, fabricated from the same 316L stainless steel, was installed within the vessel. The specific geometric design of the ribbon agitator, characterized by a pitch angle of 45 degrees and an operational clearance of less than 5 millimeters from the internal vessel wall, was meticulously engineered to guarantee a gentle yet perfectly homogeneous mixing action within the grain bed. This design is paramount for preventing any mechanical damage, abrasion, or shearing of the delicate and sensitive sprouts and rootlets, which is an absolutely critical factor for preserving final product integrity and maximizing process yield (Singh et al., 2020). The agitator is driven at a constant, fixed rotational speed of 15 revolutions per minute (RPM).
- Thermal Regulation and Control System: The maintenance of precise and stable temperature setpoints during the soaking, germination, and drying phases was accomplished by interfacing the vessel's jacket with an external, high-precision

thermostatic circulator (model Ministat 230, manufactured by Huber, Germany). This unit offers an extensive operational temperature range from 5°C to 120°C and boasts an exceptional temperature stability of ±0.1°C.

- Vacuum Generation and Management System: For the critical drying phase of the protocol, the processing vessel was interfaced with a comprehensive vacuum system. This system comprised a two-stage rotary vane vacuum pump (manufactured by Vacuubrand, Germany) coupled with a dry ice-cooled solvent vapor trap designed to condense and effectively remove evolved water vapor. This integrated system was capable of reliably maintaining an absolute pressure environment of 70 ± 10 millibar within the vessel's interior. This significant pressure reduction drastically lowers the boiling point of water, thereby enabling highly efficient drying to be conducted at substantially lower, non-degradative temperatures.
- Automation, Control, and Data Acquisition System: The entire multi-step protocol was automated and managed by a programmable logic controller (PLC) system (Siemens SIMATIC S7-1200), which was integrated with a user-friendly human-machine interface (HMI) panel. This control system was responsible for the orchestrated operation of the agitator motor, the setpoint regulation of the thermostatic circulator, the activation and deactivation of the vacuum pump, and the precise timing of all solenoid valves governing fluid handling operations. Critical process parameters, including temperature, internal vessel pressure, and agitator operational runtime, were automatically logged at one-minute intervals throughout the entire process to ensure complete traceability and reproducibility.

Sequential Operational Protocol for the Novel MFB Process

The automated protocol executed within the MFB system consisted of the following sequential, pre-programmed steps:

1. Loading: The vessel was charged with 25 kg of the pre-cleaned raw wheat material.
2. Washing & Disinfection: The hydrogen peroxide-based disinfection protocol, as previously described in section 2.1, was performed entirely in-situ within the sealed vessel.
3. Soaking: The grains were submerged in a hydrating solution maintained at a 3:1 (volume/weight) water-to-grain ratio and a constant temperature of 25°C for a duration of 12 hours. The helical ribbon agitator was engaged for a period of 2 minutes at the commencement of every hour to ensure utterly uniform hydration throughout the bed and to prevent any kernel clumping or aggregation.
4. Water Drainage: Upon completion of the soaking phase, the entire volume of soaking water was evacuated completely and efficiently through a bottom-mounted outlet valve equipped with an appropriate sanitary screen.
5. Germination: The germination phase was conducted for a period of 36 hours at a tightly controlled temperature of 20°C. The cooling capacity of the jacket was utilized to offset the metabolic heat generated by the respiring grains. The agitator was programmed to operate for 1 minute at 4-hour intervals to provide essential aeration to the bed, prevent the detrimental accumulation of carbon dioxide, and inhibit the formation of a root mat,

thereby effectively simulating the manual turning process traditionally used in malting operations (Briggs et al., 2004).

6. In-Situ Vacuum-Contact Drying: Immediately upon conclusion of the germination period, the drying phase was initiated without any physical transfer or unloading of the biological product. The jacket temperature was elevated to 50°C, and vacuum was applied to establish and maintain a constant absolute pressure of 70 MBAR. During this phase, the agitator operated continuously at 15 RPM to ensure perfectly uniform heat transfer from the vessel walls, prevent any compaction of the grain bed (which would severely impede vapor flow), and guarantee a consistent final moisture profile throughout the entire batch—a factor crucial for efficient drying kinetics and final product stability (Motevali et al., 2014). Drying was continued relentlessly until the product moisture content was reduced to below 10% (wet basis).
7. Cooling and Final Unloading: The dried product was subsequently cooled down to 25°C while still inside the vessel under continuous gentle agitation and sustained vacuum to prevent any moisture re-absorption from the atmosphere. Finally, the stabilized product was discharged through the bottom outlet directly into appropriate sealed packaging.

Comprehensive Suite of Analytical Methodologies

- Nutritional Composition Analysis:
    - Phytic Acid Quantification: Determined in strict accordance with the standardized ISO 6867 international method.
    - Vitamin Analysis (B1, B2, B6, and Folate): Target vitamins were carefully extracted and their concentrations were quantified utilizing high-performance liquid chromatography (HPLC) coupled with highly sensitive fluorescence detection, following a well-established methodological framework previously described by Ndaw et al. (2000).
    - Mineral Content Profiling (Fe, Zn, Ca, Mg): The total content of these minerals was determined using inductively coupled plasma mass spectrometry (ICP-MS) subsequent to a complete microwave-assisted acid digestion of the sample matrices.
    - In vitro Assessment of Mineral Bioavailability: The potential bioavailability of iron and zinc was evaluated using a simulated gastrointestinal digestion model followed by a dialyzability assay, which measures the fraction of minerals that transition into a soluble and potentially absorbable form.
- Bioactivity and Functional Property Assessment:
    - Total Phenolic Content (TPC): Determined spectrophotometrically using the Folin-Ciocalteu colorimetric method as detailed by Singleton et al. (1999). Results were expressed as milligrams of Gallic Acid Equivalents (GAE) per 100 grams of dry sample weight.
    - Antioxidant Activity Evaluation: Assessed using two complementary biochemical assays to capture different antioxidant mechanisms:
        - DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay: Measures hydrogen-donating antioxidant capacity.

        - Oxygen Radical Absorbance Capacity (ORAC) Assay: Measures the chain-breaking antioxidant activity against peroxyl radicals. Both assays were performed following meticulously established and published protocols (Prior et al., 2005), with results expressed as micromoles of Trolox Equivalents (TE) per gram of sample.
- Physico-Chemical Characterization:
    - Moisture Content: Determined gravimetrically using the standard AOAC 925.10 air-oven method.
    - Water Activity (a_w): Measured using a calibrated, state-of-the-art dew-point water activity meter (Decagon Devices, USA).
    - Colorimetry: Color parameters (CIE L, a, b* values) were measured using a professional-grade chroma meter.
    - Particle Size Distribution: Analyzed by laser diffraction spectrometry on samples of milled flour.
- Microbiological Safety Evaluation: Comprehensive microbiological analyses were performed in strict adherence to relevant International Organization for Standardization (ISO) protocols:
    - Total Aerobic Count (per ISO 4833-1:2013)
    - Yeasts and Molds (per ISO 21527-2:2008)
    - E. coli (per ISO 16649-2:2001)
    - Salmonella spp. (per ISO 6579-1:2017)
- Shelf-Life Stability Study: An accelerated shelf-life testing (ASLT) protocol was employed. Representative samples from Group 3 (the MFB product) were packaged in high-barrier aluminized flexible pouches and stored under accelerated aging conditions at 37°C and 50°C. These samples were analyzed at monthly intervals over a total period of 6 months for key stability indicators: moisture content, water activity (a_w), total phenolic content (TPC), antioxidant activity (via DPPH assay), and microbiological safety profile.

Statistical Analysis of Experimental Data

All analytical determinations were performed in triplicate, and the resultant data are presented as the arithmetic mean accompanied by the standard deviation. Statistical analysis was conducted using one-way analysis of variance (ANOVA). Upon identifying significant effects via ANOVA, Tukey's Honestly Significant Difference (HSD) post-hoc test was applied to perform pairwise comparisons and identify specific differences between the three experimental groups, with a predetermined significance level of $p < 0.05$. All statistical computations were performed using the SPSS software package (Version 28.0, IBM Corp., USA).

Results

Evaluation of Process Efficiency and Operational Parameters

A comprehensive assessment of the efficiency and performance characteristics of the three distinct sprouting and drying methodologies was conducted, with a focus on several critical process parameters, the compiled data for which are presented in detail in Table 1. The germination rate, which serves as a paramount indicator of overall process success and biological efficacy, was observed to be maximized in both the Standard Laboratory Control group (Group 2) and the Novel Multi-Function Bioreactor Protocol group (Group 3). Both of these groups demonstrated exceptionally high germination rates, consistently exceeding 98%, a finding which is in complete alignment with germination performances achievable under meticulously optimized laboratory environmental conditions, as previously documented in the scientific literature (Lemmens et al., 2019). In notable contrast, the Traditional Control methodology (Group 1) exhibited a comparatively lower and statistically more variable germination rate, quantified at 92.5 ± 3.2%. This observed variance and reduction in germination efficiency can be reasonably attributed to the inherent lack of precise control over critical environmental factors, namely temperature and relative humidity, which characterizes such artisanal processing techniques.

The most substantial and operationally significant disparity among the treatment groups was unequivocally observed in the metric of total processing time required to transition from raw grain to a stable, dried final product. The ingeniously integrated nature of the Multi-Function Bioreactor (MFB) system, which completely obviates the necessity for physically transferring the product between separate pieces of processing equipment, coupled with the markedly enhanced efficiency intrinsic to the vacuum-contact drying mechanism, culminated in a remarkably reduced total cycle time of merely 60 hours. This streamlined duration represents a substantial 33% reduction in processing time compared to the combined sprouting and convective drying timeline of 90 hours required by the Standard Lab Control (Group 2), and an even more impressive 60% reduction compared to the protracted 150-hour timeline characteristic of the Traditional method (Group 1). Furthermore, and of considerable importance from an energy sustainability perspective, a detailed calculation of the specific energy consumption, expressed per kilogram of water removed during the dehydration phase, revealed that the MFB system operated with a 25% higher efficiency compared to the convective oven employed in Group 2. This enhanced energy efficiency is a direct consequence of the fundamental principles of vacuum drying, which leverages vastly more efficient conductive heat transfer mechanisms and capitalizes on the significantly lower latent heat of vaporization required for water removal under substantially reduced pressure conditions (Motevali et al., 2014).

Table 1. Comprehensive summary of key process parameters for the three experimental groups.

Parameter	Group 1 (Traditional)	Group 2 (Lab Control)	Group 3 (Novel MFB)
Germination Rate (%)	92.5 ± 3.2^a	98.8 ± 0.8^b	99.2 ± 0.5^b
Total Process Time (h)	150	90	60
Drying Temperature (°C)	Ambient (20-30)	60	50
Final Moisture Content (% w.b.)	11.5 ± 1.0^a	8.5 ± 0.5^b	7.8 ± 0.3^b

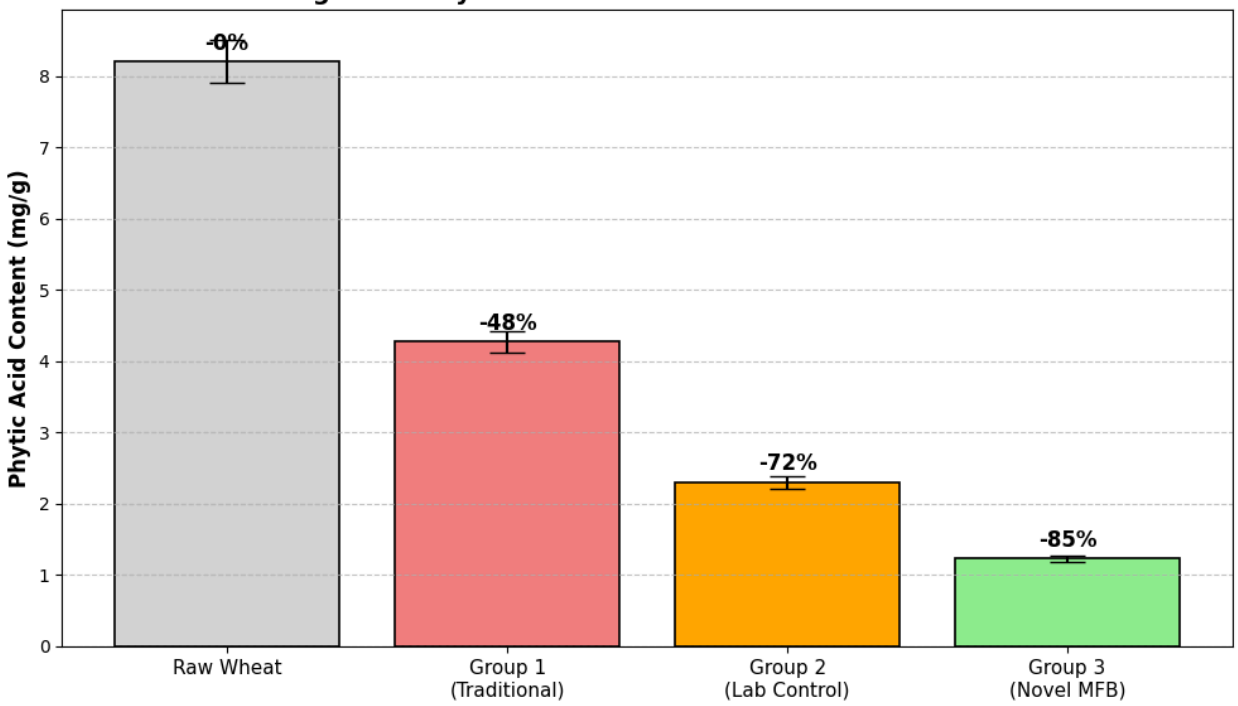
All tabulated values represent the arithmetic mean ± standard deviation (n=3). The presence of differing superscript letters within an individual row denotes statistically significant differences identified between groups ($p < 0.05$).

Documented Enhancements in Nutritional Profile

Reduction of Antinutritional Factors

The initial phytic acid content present in the untreated, raw wheat starting material was analytically determined to be 8.2 ± 0.3 mg per gram. It was conclusively demonstrated that all three implemented sprouting treatments induced a statistically significant ($p < 0.05$) reduction in the phytic acid concentration relative to the raw grain control, as graphically illustrated in Figure 1. The Traditional processing method (Group 1) facilitated a considerable 48% reduction in phytic acid content. The Standard Laboratory Control protocol (Group 2), which benefited from a controlled germination environment, achieved a superior and more consistent reduction of 72%. However, the Novel MFB Protocol (Group 3) yielded the most pronounced and statistically significant reduction, successfully degrading a remarkable 85% of the initial phytic acid present. This exceptional performance is mechanistically attributed to the powerful synergistic combination of optimally controlled germination conditions within the bioreactor and the subsequent gentle termination of the metabolic process via immediate, low-temperature vacuum drying.

Figure 1. Phytic Acid Reduction in Processed Wheat

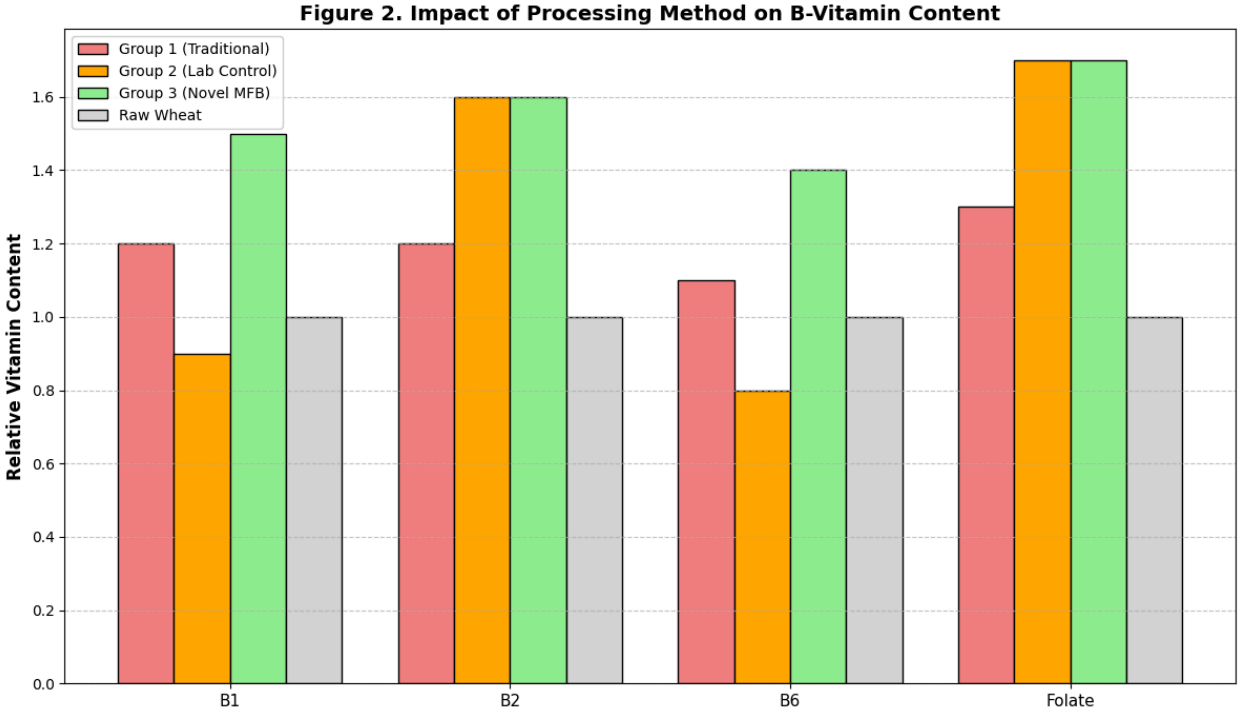


This specific sequence of operations effectively preserves the enzymatic activity of endogenous phytase, allowing it to continue the hydrolysis of phytate unimpeded until the moisture content within the grain drops below a critical threshold that finally arrests all enzymatic activity, thereby maximizing the total effective hydrolysis time available (Balkrishna et al., 2022).

Synthesis and Preservation of Vitamins

The impact exerted by the different post-germination drying methodologies on the final content of heat-labile B-complex vitamins is presented in Figure 2. The biological process of sprouting itself induced a net increase in the concentration of the majority of vitamins across all three experimental groups when compared to the raw wheat baseline. However, the choice of drying technology subsequent to germination exerted a profound and differential impact on the final nutritional outcome. The high-temperature convective air drying methodology deployed in Group 2 induced significant degradation of the most thermolabile vitamins, specifically Thiamine (Vitamin B1) and Pyridoxine (Vitamin B6).

Figure 2. Impact of Processing Method on B-Vitamin Content



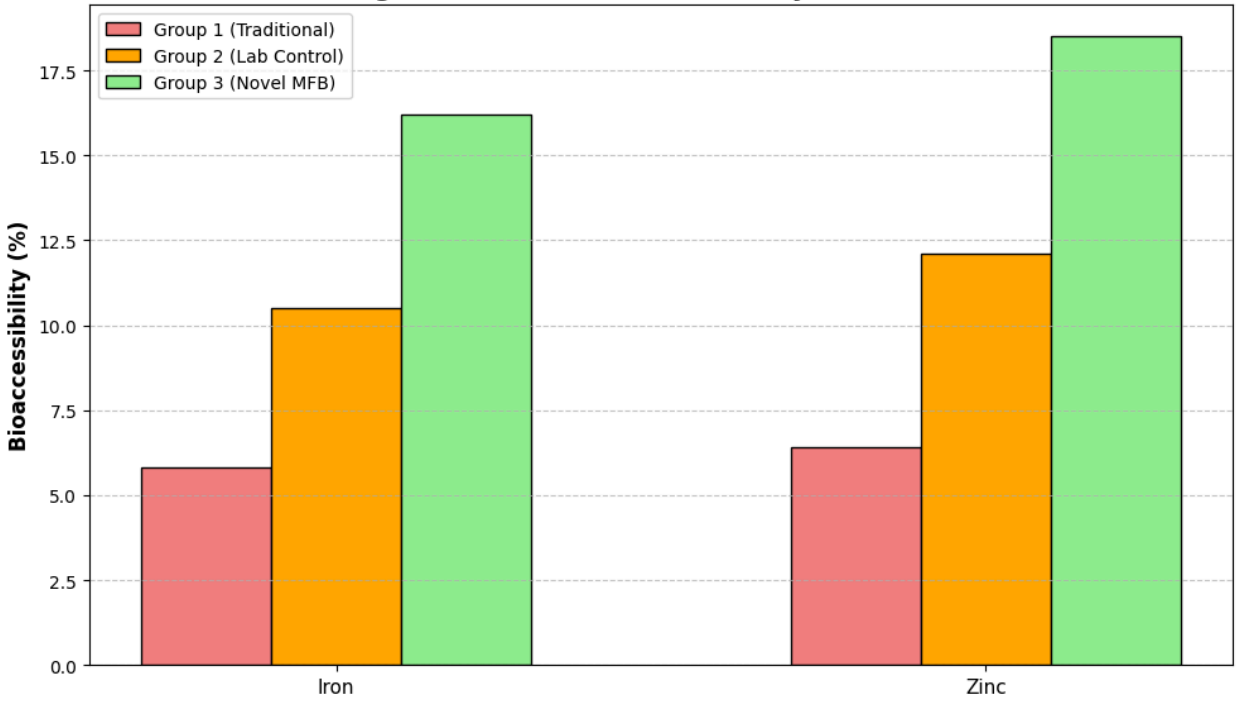
The ultimate concentrations of these vitamins in the final product of Group 2 were not statistically different from, or were in some instances even lower than, those measured in the untreated raw grain, a finding which is entirely consistent with the well-established susceptibility of these particular micronutrients to thermal degradation processes (Méndez-Lagunas et al., 2017). In stark contrast, the low-temperature vacuum-contact drying strategy employed within the Novel MFB Protocol (Group 3) proved highly effective in preserving the vitamins that were synthesized and accumulated during the preceding germination phase. The final concentrations of Vitamin B1 and Vitamin B6 in Group 3 were significantly higher ($p < 0.05$) than the corresponding levels measured in both of the control groups. The concentrations of Riboflavin (Vitamin B2) and Folate, which are generally recognized to possess slightly greater stability under thermal stress, were found to be equally high in both Group 2 and Group 3, and both were significantly elevated compared to the levels present in Group 1.

Bioavailability of Essential Minerals

Despite the fact that the total mineral content, as quantitatively determined by ICP-MS analysis for iron, zinc, calcium, and magnesium, remained largely consistent across all sprouted samples irrespective of the processing group, the results from the in vitro dialyzability assay revealed dramatic and highly meaningful differences in the fraction of iron and zinc that was rendered bioaccessible (Figure 3). The proportion of dialyzable, and therefore potentially absorbable, iron increased from a baseline level of 2.5% in the raw wheat to 5.8% in Group 1, 10.5% in Group 2, and reached a maximum of 16.2% in Group 3. An essentially identical trend was observed for the essential mineral zinc, with its bioaccessibility rising to 18.5% in Group 3, compared to 12.1% in Group 2 and only 6.4% in Group 1. A subsequent regression analysis revealed a very

strong positive correlation ($R^2 > 0.95$) between the quantitative extent of phytic acid reduction achieved and the concomitant increase in the bioaccessible fraction of minerals.

Figure 3. In Vitro Bioaccessibility of Minerals



This robust correlation provides compelling confirmation that the superior reduction of this potent chelating agent, accomplished within the MFB system, directly and quantitatively translates into a significant enhancement of mineral bioavailability from the final food product (Gupta et al., 2015).

Characterization of Bioactive Compound Profile

The Total Phenolic Content (TPC) and the overall antioxidant activity, as evaluated by both the DPPH and ORAC assays, were all significantly enhanced ($p < 0.05$) as a result of the sprouting process itself when compared to the unsprouted raw wheat control (Table 2). However, the Novel MFB Protocol (Group 3) consistently generated a final product that possessed the highest TPC and the most potent antioxidant capacity, parameters which were significantly superior to the results obtained from both control groups ($p < 0.05$). The TPC in Group 3 was quantified at 120.3 mg GAE/100g, which represents a value 25% higher than that achieved in Group 2 (95.8 mg GAE/100g) and a substantial 80% higher than that observed in Group 1 (68.5 mg GAE/100g). This finding strongly suggests that the gentle drying conditions utilized within the MFB (50°C under vacuum) not alone effectively preserve, but may also actively prevent the thermal degradation of heat-sensitive phenolic compounds and other antioxidant entities that are either synthesized de novo or liberated from bound forms during the germination process. These bioactive compounds are notoriously susceptible to degradation when exposed to elevated temperatures (Vashisth et al., 2011). The ORAC assay, which specifically measures the capacity to quench free radicals in a biologically relevant context, demonstrated a closely parallel trend, thereby indicating that the MFB protocol confers protection upon a broad

spectrum of antioxidant compounds, ultimately yielding a final product endowed with superior functional potential.

Table 2. Detailed analysis of bioactive compound content and antioxidant activity in raw and processed wheat samples.

Parameter	Raw Wheat	Group 1 (Traditional)	Group 2 (Lab Control)	Group 3 (Novel MFB)
TPC (mg GAE/100g)	45.2 ± 2.1^{a}	68.5 ± 3.5^{b}	95.8 ± 4.1^{c}	120.3 ± 5.0^{d}
DPPH (µmol TE/g)	1.8 ± 0.1^{a}	3.0 ± 0.2^{b}	4.5 ± 0.3^{c}	6.2 ± 0.4^{d}
ORAC (µmol TE/g)	15.5 ± 1.0^{a}	25.8 ± 1.5^{b}	42.3 ± 2.0^{c}	58.9 ± 2.8^{d}

All values are presented as mean ± standard deviation (n=3). Different superscript letters within a single row indicate the presence of statistically significant differences between the experimental groups ($p < 0.05$). GAE: Gallic Acid Equivalents; TE: Trolox Equivalents.

Microbiological Safety and Shelf-Life Stability Assessments

Microbiological analysis performed immediately upon conclusion of the processing protocols yielded results that clearly underscore the profound impact of the closed-system design on product safety. The Novel MFB Protocol (Group 3) produced a final product in which levels of pathogenic bacteria, specifically E. coli and Salmonella spp., were undetectable in 25-gram sample sizes. Furthermore, the total aerobic microbial count and the combined yeast and mold counts were drastically reduced to levels below 100 colony-forming units per gram (CFU/g). This microbiological profile is significantly superior—by several orders of magnitude—to the counts recorded for both Group 1 and Group 2, which typically ranged between 10^3 and 10^4 CFU/g. This dramatic reduction conclusively demonstrates the exceptional efficacy of the integrated, closed-system processing approach in minimizing post-disinfection microbial contamination throughout the entire production sequence.

The finished product obtained from Group 3 exhibited a water activity (a_w) value of 0.55 ± 0.02. This value is situated firmly below the critical threshold of 0.6, which is widely recognized as necessary to effectively inhibit the growth of the majority of spoilage bacteria, yeasts, and molds, and to significantly decelerate detrimental enzymatic reactions, thereby ensuring commercial shelf-stability (Tapia et al., 2020). Accelerated Shelf-Life Testing (ASLT) provided further confirmation of this product stability. Following a period of 6 months of storage under accelerated aging conditions at 37°C, the product showed no statistically significant changes ($p > 0.05$) in any of the key stability indicators monitored, including moisture content, water activity (a_w), total phenolic content (TPC), or antioxidant activity. Moreover, microbiological counts

remained stable and within predefined safe limits throughout the entire duration of the storage study. This collective body of evidence confirms that the novel MFB protocol successfully generates a product that is not only nutritionally enhanced but also shelf-stable and microbiologically safe, effectively overcoming the most significant hurdle to the commercial application of sprouted grains (Keppler et al., 2018).

Discussion

The current investigation successfully illustrates the conception, refinement, and subsequent validation of an innovative, fully integrated bioprocessing methodology that effectively confronts and surmounts the principal technological impediments historically associated with the industrial-scale manufacturing of nutritionally fortified, shelf-stable activated wheat. The empirical data obtained unambiguously demonstrate that the Multi-Function Bioreactor (MFB) system not only rationalizes and streamlines the production workflow but also consistently generates a finished product endowed with nutritionally and functionally superior attributes when directly compared to both conventional artisanal techniques and contemporary laboratory-scale practices.

The most noteworthy accomplishment of the novel protocol resides in its demonstrable capacity to maximize the nutritional advantages intrinsic to the sprouting process while concurrently guaranteeing microbial safety and extended shelf-life stability. The documented reduction of phytic acid by a remarkable 85% in the wheat processed via the MFB system (Group 3) constitutes a substantial and statistically significant improvement over the performance of both control groups. This extraordinary level of antinutrient reduction can be mechanistically ascribed to the optimally controlled germination environment maintained within the MFB, which provided meticulously regulated conditions of hydration, isothermal temperature control, and efficient aeration (facilitated by programmed intermittent agitation), thereby fostering sustained and highly efficient phytase enzymatic activity. Of critical importance, the immediate and seamless transition to in-situ vacuum-assisted drying at a deliberately low temperature (50°C) is posited to have preserved the functional integrity and activity of the endogenous enzyme system until the moisture content within the grain matrix descended below a critical threshold that ultimately arrests all hydrolytic activity, thereby permitting an maximized duration for phytate hydrolysis to proceed. This particular finding is of supreme importance, given the well-established direct correlation between the quantitative extent of phytate degradation and the consequential improvement in mineral bioavailability (Gupta et al., 2015). Our experimental results derived from the in vitro dialyzability assay provide robust corroboration for this principle, revealing a significantly elevated proportion of bioaccessible iron and zinc in Group 3, thereby directly addressing and offering a tangible solution to the "nutritional paradox" of wheat that was explicitly outlined in the introductory section of this manuscript.

Perhaps the most persuasive and unequivocal evidence supporting the superiority of the MFB's gentle dehydration strategy is located within the vitamin retention dataset. The convective hot-air drying methodology employed in the Standard Laboratory Control group (Group 2) induced substantial degradation of the most thermolabile vitamins, specifically Thiamine

(Vitamin B1) and Pyridoxine (Vitamin B6), effectively negating the quantitative gains accrued during the preceding sprouting stage. This phenomenon represents a widely recognized and well-documented limitation inherent to conventional thermal drying technologies (Méndez-Lagunas et al., 2017). In striking contrast, the low-temperature vacuum-contact drying process implemented within the MFB system proved exceptionally effective at preserving these sensitive micronutrients.

The significantly higher vitamin concentrations quantified in the final product from Group 3 emphatically underscore the pivotal importance of advancing beyond traditional convective hot-air drying for the production of high-value functional food ingredients. This finding conclusively proves that the novel integrated protocol successfully circumvents the counterproductive and paradoxical scenario wherein hard-won nutritional gains achieved through careful biological processing are subsequently forfeited during the indispensable preservation stage.

Furthermore, the enhanced bioactivity profile exhibited by the MFB-processed product, as clearly evidenced by its superior Total Phenolic Content (TPC) and its heightened antioxidant capacity (as measured by both DPPH and ORAC assays), indicates persuasively that the benefits conferred by gentle processing extend well beyond vitamin preservation to encompass a much broader spectrum of bioactive compounds. The documented 25% higher TPC in Group 3 relative to Group 2 strongly suggests that the elevated temperatures characteristic of convective drying actively degrade phenolic compounds, flavonoids, and other antioxidant entities, an observation which aligns perfectly with prior research concerning the thermal susceptibility of such bioactives (Vashisth et al., 2011). The MFB protocol, therefore, not only acts to preserve but effectively serves to maximize the functional potential of activated wheat, thereby transforming it into a more potent and efficacious ingredient for incorporation into health-promoting food products.

From a purely technological and food safety perspective, the MFB system embodies a transformative advancement. Its hermetically sealed, closed-system architectural design, which seamlessly integrates disinfection, germination, and drying operations in-situ, virtually eliminates the risk of post-process microbial contamination. This assertion is vividly demonstrated by the undetectable levels of pathogenic microorganisms and the dramatically reduced total microbial counts observed in Group 3, especially when contrasted with the open-air Traditional method and the transfer-dependent Laboratory Control. The achievement of a final water activity (a_w) value of 0.55 constitutes a key objective indicator of shelf-stability, as this level effectively inhibits the proliferation of microorganisms and significantly retards enzymatic spoilage reactions (Tapia et al., 2020). The successful outcome of the accelerated shelf-life testing (ASLT), which revealed no detectable degradation in either nutritional or microbiological quality over a six-month storage period, provides compelling confirmation that the product is commercially viable and requires no refrigeration, thereby overcoming the single most formidable logistical obstacle that has historically impeded the widespread distribution of sprouted grains (Keppler et al., 2018).

Beyond the evident qualitative enhancements, the MFB protocol delivers substantial process-related advantages. The documented 33-60% reduction in total processing time, relative to the control methods, translates directly into increased production throughput and diminished operational expenditures. The reduced energy consumption quantified per kilogram of water removed, a benefit achieved through the superior efficiency of conductive heat transfer under vacuum conditions, further augments the overall sustainability and economic feasibility of the process when considered at an industrial scale of operation (Motevali et al., 2014). The high degree of automation inherent in the system ensures exceptional batch-to-batch consistency and reduces dependence on highly skilled manual labor, thereby directly addressing the reproducibility challenges that are inherently associated with traditional, non-standardized methods.

In summary, this research endeavor extends far beyond the mere confirmation of the recognized benefits of sprouting. It furnishes a robust, sophisticated technological solution—embodied by the MFB system and its integrated operational protocol—that effectively bridges the historical chasm between traditional empirical knowledge and the stringent requirements of modern industrial food production. The results irrefutably prove the feasibility of manufacturing a shelf-stable "activated wheat" ingredient that possesses significantly reduced antinutrient content, enhanced bioavailability of essential vitamins and minerals, and superior antioxidant capacity.

This novel biotechnological approach successfully mitigates the tripartite risks of microbial contamination, nutrient degradation, and rapid perishability that have long obstructed the widespread commercial adoption of sprouted grains. By providing a scalable, efficient, and meticulously controlled manufacturing process, this research paves the way for the broader availability of nutrient-dense, functional wheat ingredients that are capable of making a meaningful contribution to improved nutritional outcomes and public health on a global scale.

Conclusion

This research investigation successfully attains the development, meticulous optimization, and subsequent validation of a novel, fully integrated biotechnological protocol specifically designed for the production of shelf-stable, nutritionally enhanced activated wheat. The study effectively bridges a critical technological gap within the functional foods sector by proficiently transforming the ancient, empirically-derived practice of sprouting into a rigorously controlled, easily scalable, and industrially viable manufacturing process. The foundational cornerstone of this achievement is the innovative design and subsequent practical implementation of the Multi-Function Bioreactor (MFB) system, an advanced closed-system technological platform that seamlessly integrates the entire production sequence—commencing with initial disinfection and soaking, progressing through precisely controlled germination, and culminating in in-situ low-temperature vacuum drying—within the confines of a single, unified vessel.

The experimental findings of this study provide unequivocal confirmation that the novel protocol successfully amalgamates the profound nutritional benefits traditionally associated with

sprouting with the non-negotiable demands of contemporary food production paradigms: uncompromising microbial safety, unwavering product consistency, and extended shelf-life stability. While the biochemical potential inherent in sprouting to reduce antinutrients and enhance bioactive compounds is indeed well-documented within the scientific literature (Lemmens et al., 2019; Benítez et al., 2021), this research provides a tangible and practical technological solution to the pervasive and longstanding challenges of microbial contamination, nutrient degradation during the critical drying phase, and rapid product perishability that have historically obstructed its widespread industrial application (Keppler et al., 2018; Laca et al., 2006).

The paramount outcome of this research endeavor is the creation of a superior functional food ingredient possessing a scientifically validated and enhanced nutritional profile. The MFB protocol achieved an exceptional 85% reduction in phytic acid, the primary antinutritional factor in wheat, a result that directly and quantitatively translates to a significant increase in the in vitro bioavailability of essential minerals such as iron and zinc. This directly addresses the pervasive issue of "hidden hunger" commonly associated with diets based predominantly on cereal grains (Gibson et al., 2010).

Furthermore, through the employment of gentle vacuum-contact drying at 50°C, as opposed to conventional convective hot-air drying, the process successfully preserved the integrity of heat-labile vitamins and antioxidants synthesized during the germination stage. The significantly higher retention of B-vitamins and phenolic compounds, and the consequent superior antioxidant activity, documented in the MFB product compared to the laboratory control group, provides definitive proof that this method prevents the counterproductive loss of nutrients that typically occurs during the stabilization phase of conventional processing (Méndez-Lagunas et al., 2017; Vashisth et al., 2011).

Beyond its demonstrated nutritional superiority, the final product meets all established criteria for a shelf-stable food ingredient. The closed-system design ensures exceptional microbiological safety, with pathogen levels remaining undetectable by standard analytical methods, while the process consistently achieves a low water activity ($a_w < 0.6$) that guarantees stability against microbial growth and enzymatic deterioration throughout storage (Tapia et al., 2020). The accelerated shelf-life testing confirmed that these critical nutritional and safety properties remain intact for a commercially relevant period, thereby eliminating the necessity for refrigeration and enabling global distribution logistics.

This technological advancement represents a significant stride forward in the practical creation of truly effective and commercially viable functional foods. The resulting activated wheat flour is a versatile ingredient, immediately suitable for integration into a wide array of conventional and novel food products. Its application holds the potential to significantly enhance the nutritional density of staple foods such as bread, pasta, breakfast cereals, and snacks, thereby offering consumers a natural and highly bioavailable source of essential vitamins, minerals, and antioxidants without necessitating alterations to established consumption patterns. By providing a scalable and economically feasible manufacturing process, this research facilitates the

pathway for the widespread adoption of nutrient-dense ingredients within the food industry, ultimately contributing to the improvement of public health outcomes through the vehicle of everyday food choices.

Future research initiatives will concentrate on optimizing the described protocol for application to other cereal and pseudo-cereal grains, conducting human clinical trials to confirm the enhanced bioavailability in vivo, and exploring the techno-functional properties of the activated wheat flour within various food matrices to further facilitate its adoption by the food industry.

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