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Comparative analysis of physicochemical and functional properties of germinated barley flour and beta glucan extract

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Abstract

Germination of cereal grains is an established method for enhancing their nutritional and functional qualities. This study systematically evaluated barley germination under various conditions to identify those that maximize nutritional content, bioactive compounds, and functional properties. The results indicated that a 48-hour germination period was the most effective, achieving a total malt recovery rate of 95.35% and a malting loss of only 4.65%, which reflect high process efficiency. Germination induced significant compositional changes, including increases in protein, dietary fiber (ranging from 13.03 to 14.54 g/100 g), and ash content, while fat content decreased concurrently. The levels of bioactive compounds were notably enhanced, with total phenolic content nearly doubling from 1.6 to 3.1 mg GAE/g, and radical scavenging activity increased markedly from 37.5% to 93%. Additionally, β-glucan extracted from germinated barley exhibited improved solubility, water-binding capacity, and a recovery rate of 80.28%. These findings suggest that germination induces structural modifications that enhance the functional utility of barley. The study also highlights that controlled germination is a cost-effective, natural, and scalable bioprocessing technique for adding value to barley, making it a promising approach for food and feed applications.

Keywords: Amylase assay, Antioxidant activity, Barley, Beta-glucan, Dietary fiber, Germination process.

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Contents

Contribution of this paper to the literature

This study statistically establishes that a 48-hour germination period is optimal for barley, revealing significant correlations ($p < 0.05$) among water absorption properties, malt loss, enzyme activity, and β-glucan yield. Germination induces compositional and structural changes, as evidenced by (FTIR), along with antioxidant enhancements. These modifications improve the techno-functional and nutritional qualities of barley, supporting the incorporation of β-glucan into functional food formulations. Such formulations can synergize effectively with dairy systems, offering potential health benefits and functional advantages.

1. Introduction

Barley (Hordeum vulgare L.) is a cereal grain that is highly nutritious. It belongs to the Poaceae family and ranks fourth among the most widely cultivated cereals globally, following wheat, rice, and corn. Its composition includes approximately 65-68% starch, 10-17% protein, 2-3% lipids, and 4-9% β-glucans. This nutritional profile provides significant health benefits for humans, supporting various bodily functions and contributing to overall well-being [1]. Barley not only consists of macronutrients but also includes vitamins, minerals, and various phytochemicals, which together promote heart health, control of blood sugar level, immune booster and overall metabolic well-being [2, 3]. Among these substances, β-glucans, a type of soluble dietary fiber found exclusively in barley and oats, are particularly well-known for their diverse physiological effects, which depend on their viscosity. These effects include a reduction in postprandial glucose response, a decrease in serum cholesterol levels, and an increase in hunger satisfaction [2]. These features make barley very attractive as a source of raw material for the production of functional foods and nutraceuticals.

Germination (malting) is a natural, low-cost bioprocess that enhances the quality of cereals on nutritional, biochemical, and functional levels. During germination, water uptake activates endogenous hydrolytic enzymes such as amylases, proteases, and β-glucanases. This process converts starch into sugars, makes proteins more accessible, and breaks down cell walls, facilitating the entry or synthesis of phenolic compounds [4]. Besides the increase in starch and protein, enzyme activity also results in the reduction of digestive resistance, soluble dietary fiber fractions, and antioxidant potential. The use of germinated barley in food products has not ceased; on the contrary, it is often reported that high levels of bioactive compounds and strong radical scavenging activity are the results of vigorous metabolic activity and phenolic liberation [5]. The use of germinated barley in such products is not an issue for processing due to the biochemical changes mentioned earlier. However, the quality attributes, such as water interaction properties, dispersibility, and stability of barley-based ingredients, will also be modified. This makes germinated barley a key ingredient for health-conscious and vulnerable groups targeted with specialized foods [6].

The functionality of β-glucan is significantly influenced by the germination process. During malting, structural rearrangements occur, and if the process is properly managed, the solubility, extractability, viscosity, and water-binding capacity of β-glucan can be enhanced. As a result, the physiological effectiveness and technological performance in food systems [4, 5] are greatly improved. These functional enhancements are crucial for producing stable beverages, reconstitutable powders, texture-modified foods, and other specialized formulations. Although germination presents a promising technology and nutritional source, research comparing the effects of germination duration on nutrient availability, antioxidant capacity, and β-glucan recovery remains limited. Additionally, understanding germination-induced changes in protein content and physicochemical properties is essential for optimizing barley's application as a high-performance functional ingredient.

Given these gaps, the present study aims to:

(a) Compare the nutritional composition, antioxidant activity, and bioactive profile of germinated versus non-germinated barley flour.

(b) Evaluate the extractability and functional properties of β-glucan isolated from both flours.

The study aims to establish an optimized germination protocol that maximizes the nutritional, functional, and bioactive attributes of barley, thereby enhancing its application potential in functional foods, nutraceuticals, and health-oriented formulations.

2. Materials and Methods

Barley sourced from the Mysuru market was stored at 4°C in a cold room, packed in airtight bags to prevent contamination. All chemicals used for proximate analysis were of analytical grade from Sigma, unless specified otherwise.

2.1. Sample Preparation

2.1.1. Preparation of the Native Flour

The grains were hulled and polished, then pulverized and sieved through a 60-mesh filter. For subsequent use, the resulting flour was stored in zip-lock bags to maintain quality and facilitate easy access.

2.1.2. Preparation of the Germinated Flour

The barley was soaked in water for 6 hours and germinated on muslin cloth for 24 to 120 hours. Kilning was performed at 70–80°C for 30 to 45 minutes to eliminate enzymes and reduce microbial activity. After hulling, the grains were pulverized into flour. The flour was sieved through a 60-mesh filter and stored in zip-lock bags for further use (Figure 1).

Figure 1. Different stages of germination.

2.2. Sample Extraction

To extract, 100 g of barley flour was refluxed with 80% ethanol for 6 hours at 70°C to remove soluble phenolic compounds. The residue was then mixed with water in a 1:10 ratio and stirred at 55°C for 90 minutes. The suspension obtained was then centrifuged at 4000 g for 20 minutes at 40°C, and the supernatant's pH was adjusted sequentially (8.5 and 4.0) using sodium bicarbonate. The supernatant was then subjected to repeated centrifugation (up to 21,000 g), after which it was mixed with ethanol, and the precipitate was freeze-dried for 24 hours [7].

2.3. Germination Properties

2.3.1. Malting Loss

According to Malleshi et al. [8], barley grains (100 g) were steeped in distilled water at room temperature (25 ± 3°C) for 12 ± 2 hours and germinated at 25°C in a BOD incubator for up to 120 hours. Samples were collected every 24 hours, dried at 55 ± 3°C, and weighed post-drying to calculate malting loss using the appropriate formula.

$$\text{Malting Loss (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

2.3.2. Amylase Assay

Amylase activity was measured by incubating the enzyme extract with 1% soluble starch at 37°C for 30 minutes. The reaction was stopped with DNS reagent, and the mixture was heated, cooled, diluted, and analyzed at 540 nm. Enzyme activity was calculated as the amount of maltose released per gram of malt over 30 minutes.

$$\textit{Amylase activity } (\mu mol/min) = \frac{\mu g \textit{ of maltose released}}{\textit{molecular weight}} \times 1000$$

2.4. Physical Properties of Flour

2.4.1. Bulk and Tapped Density

A 10 mL cylinder was filled with 2 g of flour, and the initial (bulk) and final (tapped) volumes were recorded after 50 taps. Bulk and tapped densities were calculated, and flow properties were assessed using Carr's Index (C) and Hausner Ratio (HR) [9].

2.5. Proximate Analysis

The flour samples underwent proximate composition analysis following AOAC methods [10]. Moisture and ash content were determined using the AOAC method. Fat content was extracted with a Soxhlet apparatus. Total nitrogen and crude protein contents were measured using the micro-Kjeldahl method. Crude fiber was determined through acid and alkali treatments. Dietary fiber content was determined using the Englyst and Hudson [11] while mineral content was analyzed via Plasma Atomic Absorption Spectroscopy after ash digestion with concentrated hydrochloric acid.

2.6. Color Measurement

A Colorimeter CM 5 was calibrated using a white standard plate, and samples were analyzed for L*, a*, and b* values to assess lightness, hue, and saturation.

2.7. Particle Size Analysis

Particle size distribution was determined using a Microtrac Analyzer, employing laser diffraction. Fluctuations in light intensity caused by Brownian motion were analyzed to assess the particle sizes of the sample.

2.8. Antioxidant Profile

2.8.1. Total Phenolic Content (TPC)

TPC was determined using the Folin-Ciocalteu method, measuring absorbance at 760 nm [12].

2.8.2. Radical Scavenging Activity (DPPH)

Radical scavenging activity was measured by mixing 0.5 mL of extract with 3 mL of 10^{-3} M DPPH solution, incubating for 30 minutes, and measuring the absorbance at 517 nm [13].

2.8.3. Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay measures the reduction of Fe^{3+} to Fe^{2+} at 700 nm after incubation with phosphate buffer, potassium ferricyanide, and $FeCl_3$ [14].

2.8.4. ABTS Assay

ABTS solution (7 mM), incubated with potassium persulfate, was diluted to an absorbance of 0.7 ± 0.02 at 734 nm and mixed with extracts. The reduction in absorbance was measured after 6 minutes [15].

2.9. Beta-Glucan Analysis

2.9.1. Recovery and Quantification

Beta-glucan was extracted using lichenase digestion followed by β-glucosidase hydrolysis. The resulting glucose was measured with a GOPOD reagent at 510 nm [16].

$$\beta \text{ glucan (\%w/w)} = \Delta A \times F / W \times FV \times D \times 0.9$$

2.10. Functional Properties

2.10.1. Foaming Capacity

Foaming capacity (FC) of powders was determined using a method based on Narayana and Narasinga Rao [17], with slight modifications. One gram of flour sample was added to 50 mL of distilled water at 30 ± 2°C in a graduated cylinder. The suspension was thoroughly mixed and shaken for 5 minutes to induce foaming. The volume of foam at 30 seconds after whipping was measured and expressed as foam capacity using the appropriate formula.

$$\text{Foam capacity (\%)} = \frac{\textit{Volume of foam AW - Volume of foam BW}}{\textit{Volume of foam BW}} \times 100$$

Where, AW = after whipping, BW = before whipping

2.10.2. Water Binding Capacity (WBC)

The water binding capacity of powders was determined using the method described by Robertson et al. [18]. One gram of the sample was hydrated in 30 ml of distilled water at room temperature (~30°C). After reaching equilibrium (18 hours), the sample was centrifuged at 3000 RPM for 20 minutes, and the supernatant was decanted. The weight of the residue was recorded before and after drying at 105°C until a constant weight was achieved. Water binding capacity (WBC) was calculated as the amount of water retained by the sample per gram of dry weight.

$$\text{WBC (g/g)} = \frac{\textit{residue fresh weight} - \textit{residue dry weight}}{\textit{Residue dry weight}}$$

2.11. FTIR Analysis

Fourier Transform Infrared (FTIR) spectra were recorded using a Tensor II Bruker FTIR spectrometer over the range of 400–4000 cm^{-1}, utilizing TANGO software for regulatory compliance in accordance with 21 CFR Part 11.

2.12. Statistical Analysis

Statistical analysis was conducted to evaluate the significance of differences between the samples, specifically native barley (NB) and germinated barley (GB), for each parameter assessed. Data were analyzed using appropriate statistical tests, including one-way ANOVA for multiple comparisons. All results were expressed as mean ± standard deviation (SD). Statistical significance was set at a threshold of $p < 0.05$. The analysis was performed using JASP software. Each test was conducted in duplicate to ensure the reproducibility and robustness of the findings.

3. Results and Discussions

3.1. Germination Properties

3.1.1. Moisture Dynamics During Malting

Germination duration had a critical effect on barley hydration profile and thus malting quality. The moisture content (Table 2) increased during steeping from an initial 10-15% to about 40%, which is an indication of water absorption and activation of hydrolytic enzymes. This trend is in accordance with previously published studies claiming that water absorption to 38-42% is necessary for metabolic activity to begin during malting [19, 20].

Germination lasting up to 120 hours resulted in an increase in kernel moisture from 32.7% to 41.3%. This increase was due not only to tissue hydration but also to metabolic heat production and seedling development. Kilning reduced the grain moisture to approximately 5%, effectively terminating enzymatic activity and contributing to malt stability. These processes align with industrial malting standards, ensuring quality and consistency in the final product [21]. An additional increase in soaked and germinated weights is reflected in both water imbibition and biomass accumulation, as shown in Table 1. Such trends have also been observed in the literature on barley and sorghum malting, where moisture-driven enzymatic mobilization directly impacts the biochemical and physical properties of grains [22]. These results, taken together, indicate that regulated hydration and germination duration are key factors in maximizing enzymatic activation, substrate modification, and final malt quality.

Table 1. Weight of fractions at each step of the germination process.

Germination hours	Initial weight(g)	Soaked weight(g)	Germinated weight(g)	Kilned weight (g)	Weight of the vegetative grains(g)	Pulverized weight(g)
24	100.029 ± 0.50	155.825± 0.80	200.661±2.08	94.326±0.56	92.716±0.65	90.345±2.3
48	100.024 ± 0.51	155.658 ± 0.79	203.122±2.10	95.369±0.85	91.756±0.15	88.453±3.5
72	100.422 ± 0.55	154.936± 0.92	298.126±3.26	93.456±0.12	90.756±0.52	87.234±2.45
96	100.382 ± 0.52	154.381 ± 0.85	300.238±2.56	92.543±0.45	88.321±0.78	85.651±1.24
120	100.582 ± 0.60	155.758 ± 0.70	310.218±4.565	91.246±0.12	85.518±1.26	82.256±2.36

Table 2. Moisture estimation of grains in the malting process.

Germination hours	Initial Moisture	Soaked Moisture	Germinated Moisture	Kilned Moisture	Pulverized Moisture
24	10.7%	40.2%	32.7%	4.67%	7.47
48	10.7%	41.2%	33.8%	5.42%	8.40%
72	10.7%	40.2%	36.6%	4.74%	9.6%
96	10.7%	42.6%	39.6%	5.36%	9.31%
120	10.7%	40.7%	41.3%	5.5%	9.3%

3.1.2. Malt Loss

The duration of germination had a significant influence on the losses caused by vegetative and malting processes. When germination time was extended from 24 to 120 h, the vegetative loss escalated from 1.43% to 6.70%, whereas the malting loss increased from 5.70% to 9.28% (Refer to Table 3). The mentioned increases are due to the active metabolic processes, respiration, and the elimination of rootlets and shoots, which were also noted by Aubert et al. [23].

Table 3. Malting Loss and Amylase Activity.

Germination hours	Vegetative loss	Malting loss	Malt yield	Amylase activity (µmol/min)
24	1.429	5.701	94.299	0.79
48	2.68	4.654	95.346	2.21
72	4.094	6.937	93.063	3.65
96	5.412	7.809	92.191	4.03
120	6.698	9.282	90.718	4.05

The malt yield, however, was still quite high (90.72–95.35%) even with the above losses. The 48-hour period for germination was the one demonstrating the best performance, as it led to the highest malt yield (95.35%) and the lowest malting loss (4.65%). This behavior indicates that the germination process has to be controlled very precisely; otherwise, it will lead to over-modification and substrate degradation. This observation aligns with the works of Park et al. [24] and Cai et al. [25], who mentioned that prolonged germination has the contrary effect of enhancing the enzymatic activity whilst diminishing the extract potential and grain mass. Thus, the results confirm that 48 h is the time period in which the best compromise is reached between enzymatic conversion and mass preservation, thereby supporting both yield efficiency and commercial malting quality.

3.1.3. Amylase Activity

Amylase activity increased progressively with germination duration, rising from 0.79 µmol/min at 24 h to a peak of 4.05 µmol/min at 120 h (Table 3). This increase reflects the development of hydrolytic enzymes required for starch mobilization, a core objective of malting. The observed trend corroborates earlier findings that extended germination enhances α- and β-amylase synthesis due to intensified gibberellin signaling [26].

However, beyond 96 h, the benefits of increased amylase activity were offset by excessive dry matter losses. Similar trade-offs between enzymatic development and malt yield have been reported in commercial malting studies by Aubert et al. [23]. Thus, although longer germination boosts enzyme activity, 48 hours remains the optimal duration for balancing enzyme formation and product recovery.

3.2. Physical Properties

Germination brought about major transformations in the physical properties of the barley flour (Table 4). Germinated barley (GB) had a bulk density of 0.45 g/mL, which was significantly higher ($p < 0.05$) than that of the raw barley (0.38 g/mL). The higher density indicates that there is an improvement in the packing efficiency that is probably due to the loosening of the structure during germination and then the repacking during milling. This is in line with the findings of Abdullah and Geldart [27], who stated that the particles that are finer and more uniformly modified will have an increased bulk density. True density was also slightly increased for GB (1.43 g/cm^3), indicating that there were changes not only in the composition but also in the microstructure. The Hausner ratio and Carr index did not show any significant differences ($p > 0.05$), suggesting that flowability remained consistent between NB and GB. This consistency is crucial for processing, conveying, and packaging operations. Overall, germination

improved the packing behavior without affecting the flow properties, thereby supporting the use of GB in powder-based formulations.

Table 4. Physicochemical Composition of Native and Germinated Flour

Parameters	*NB*	*GB*
Physical Properties		
Bulk Density(g/mL)	0.38±0.028[a]	0.45±0.014[b]
Tapped Density(g/mL)	0.495±0.25[a]	0.550±0.56[b]
True density	1.39±0.13[a]	1.43±0.43[a]
Porosity	74.1±0.32[a]	69.2±0.06[a]
Haussner ratio	1.30±0.04[a]	1.29±0.03[a]
Carr index	23.40±0.02[a]	22.80±0.01[a]
Proximate composition (d.b)		
Moisture (%)	9.53±0.02[a]	9.16±0.00[a]
Protein (%)	12.74±0.20[a]	14.83±0.30[b]
Ash (%)	1.75±0.05[a]	2.03±0.02 [b]
Fat (%)	2.69±0.13 [a]	2.04 ± 0.10 [b]
Fiber (%) Insoluble Soluble Total	 10.21±0.08 [a] 2.82 ±0.12[a] 13.03±0.095 [a]	8.16±0.23 [b] 6.38±0.54 [b] 14.54±0.38 [b]
Crude Fiber	1.72±0.08 [a]	3.23±0.09 [b]
Mineral estimation (d.b)		
Calcium (mg/100g)	55.8±0.80 [a]	58.69±0.63[a]
Iron(mg/100g)	8.7±0.10 [a]	7.1±0.20[b]
Potassium (mg/100g)	150±0.63[a]	100±0.25 [b]
Magnesium (mg/100g)	75.8±1.25 [a]	78.2±0.69 [b]
Sodium (mg/100g)	20.6±0.84 [a]	18.21±0.78[b]
Particle size analysis		
Diameter (µm)	162.6 [a]	138.9 [b]
Volume (%)	55.1 [a]	71 [b]
Width((µm)	198.4 [a]	193 [a]
Colorimetric Analysis		
L*	74.99 [a]	74.5 [a]
a*	0.57 [a]	1.52[b]
b*	7.75 [a]	10.43 [b]
Antioxidant Analysis		
Total Phenolic Content (mgGAE/G)	120±0.59[a]	161±0.69[b]
Ferric Reducing Antioxidant Property (umol/ml Fe^{2+})	693.09±0.85[a]	1185.95±0.93[b]
ABTS Assay (%)	42.6±0.35[a]	95.5±0.54[b]
%Radical Scavenging Activity (%)	37.5±0.21[a]	93.0±0.74[b]

Note: Values are mean ± Standard deviation of 2 determinations (n = 2). Values with the different superscript within rows(b) are significantly different at $p < 0.05$ compared to the respective control(a).

3.3. Proximate Analysis

GB flour had less moisture (7.47–9.31%) than NB (10.7%). The reason is that the initial water uptake, which is necessary for the activation of the embryo, is soon followed by the consumption of water through metabolic activities. Therefore, the initial moisture is reduced after drying. Similar findings were observed with cereals, where drying post-germination led to lower residual moisture due to the accelerated metabolism [28]. The protein content in the flour significantly increased from 12.74% in NB to 14.83% in GB ($p < 0.05$). This increase is in line with the degradation of storage proteins into amino acids and peptides during germination. The proteolytic enzymes that are naturally present in the plant are activated [29]. Moreover, the relative increase in protein percentage might partly be due to the depletion of starch reserves; thus, the proportion of protein is rising. Ortiz et al. [30] also made similar comments when they reported that barley sprouting resulted in an increase of ~38% in protein content. But in the case of β-glucan extracts, the content of protein was reduced from 7.27% (Nβ) to 6.34% (Gβ). The reason for the reduction in protein content is twofold: Protein breakdown during germination, which leads to the reduction of the high-molecular-weight proteins that could otherwise be co-extracted along with β-glucan. The purification effects during extraction that are targeting polysaccharides for isolation while proteinaceous components are being removed [26, 30].

The data collected demonstrate that the germination process improves protein quality in whole flour, but at the same time decreases the amount of protein remaining in the β-glucan fractions isolated. This is an advantageous feature for the application of functional ingredients that require low-protein, high-purity fibers. The levels of fat decreased remarkably from 2.69% in NB to 2.04% in GB. The reduction has been attributed to the activation of lipases, which hydrolyze triglycerides into free fatty acids and glycerol, serving as energy substrates for the developmental growth during germination [31]. Comparable reductions in lipid content have been reported in various studies on sprouted cereals and legumes. The decrease in fat is considered beneficial from a nutritional standpoint, particularly in terms of prolonging the shelf life and oxidative stability of processed foods. The germination process also resulted in a significant increase in dietary fiber components. Total dietary fiber content increased from 13.03 g/100 g (NB) to 14.54 g/100 g (GB). This enhancement aligns with previous research, which indicated reorganization of cell walls and increased synthesis or release of polysaccharides during germination [28]. There was also an increase in insoluble fiber, while crude fiber showed a remarkable increase from 1.72% to 3.23%. This indicates that the process of structural polysaccharide deposition is advanced as the germinating seedlings grow. However, a reverse trend was observed in the β-glucan extracts: there was a decrease in both insoluble fiber (5.21%

to 4.53%) and soluble fiber (87.90% to 79.03%). This suggests that a small portion of β-glucan is enzymatically broken down during germination, resulting in a lower molecular weight and reduced solubility. The activity of β-glucanase, which typically increases during germination, is likely responsible for these changes. Although this reduction affects extraction yield, it enhances the functional properties of the product, particularly solubility and viscosity. These improvements significantly impact the product's applications in beverages and nutritional supplements, where enhanced solubility and viscosity are desirable for better functionality and consumer acceptance [2]. The ash content under study (1.75%) was higher in the non-germinated sample (NB) compared to its germinated counterpart (GB) (2.03%), thereby showing a more pronounced disintegration of the mineral–phytate complex, indicating enhanced mineral availability. It has also been reported that the same trend of increments was seen in the case of sprouted lentils, chickpeas, and soybeans [32]. Mineral profiling disclosed a rise in magnesium (75.8 → 78.2 mg/100 g), calcium (55.8 → 58.69 mg/100 g), and a decrease in potassium. These alterations signify the disintegration of mineral–phytate complexes and increased bioavailability through the activation of endogenous phytase during germination [33]. Potassium reduction may result from leaching during soaking or redistribution of minerals within the germinated seed.

3.4. Antioxidant Properties

Germination has indeed an impressive effect on antioxidant potential (see Table 4). The total phenolic content (TPC) was almost twofold higher, moving from 1.6 mg GAE/g in NB to 3.1 mg GAE/g in GB ($p < 0.05$). The reason for this increase is the liberation of phenolic compounds from bound forms and the activation of phenylpropanoid pathways that occur during germination [28]. Besides that, the FRAP activity also showed a significant increase (693 → 1185 μmol Fe^{2+}/g), which indicates an overall improvement in reducing power. The scavenging activity of the ABTS radical also increased from 37.5% to 93%, demonstrating a substantial enhancement in radical neutralization capacity. These results support the notion that germination transforms barley into a more potent source of antioxidants, aligning with similar studies conducted on sprouted cereals and legumes.

3.5. Beta-Glucan Recovery

The β-glucan contents were nearly identical for both NB (5.94 g/100 g) and GB (5.70 g/100 g), however, the recovery efficiency was 80.28% for GB and 83.82% for NB (Table 5), so the recovery was somewhat lower in GB. The reduction in yield could be ascribed to the loosening of the structure and modification of the β-glucan during the germination phase. Nevertheless, on the strength of the slight improvement in effectiveness, the functional changes in the GB β-glucan have made it more appropriate for use in food applications, which agrees with Din et al. [34] findings.

Table 5. Beta-glucan recovery of flour samples.

Sample	Beta-glucan (g/100g)	% β- glucan recovery
GB flour	5.7038	80.28%
GB β-glucan extract	4.5793	
NB flour	5.9426	
NB β-glucan extract	4.9813	83.82%

Table 6. Physicochemical Composition of Beta-Glucan in Native and Germinated Flour

	Nβ	Gβ
Proximate Analysis		
Moisture (%)	10.10±0.36 [a]	9.98±0.25 [a]
Protein (%)	7.27±0.58 [a]	6.34±0.71 [b]
Fiber (%)		
Insoluble	5.21±0.84 [a]	4.53±0.35 [b]
Soluble	87.90±0.52 [a]	79.03±0.27 [b]
Total	93.11±0.46 [a]	83.56±0.167 [b]
Functional Properties of Betaglucan		
Water binding capacity (%)	3.98±0.85 [a]	4.21±0.59 [a]
Foaming capacity (%)	154.9±1.25 [a]	150.98±0.1.89 [a]
Colorimetric Analysis		
L*	78.38 [a]	76.28 [a]
a*	1.71 [a]	1.29 [b]
b*	7.93 [a]	11.20 [b]

Note: Values are mean ± Standard deviation of 2 determinations (n = 2). Values with the different superscript within rows(b) are significantly different at $p < 0.05$ compared to the respective control(a).

3.6. Functional Properties

The increase in the water-binding capacity (WBC) of β-glucan due to germination was very pronounced (4.21% in Gβ vs. 3.98% in Nβ), and it was attributed to the structural changes and the improvement of the molecular interaction and hydration [4]. It contributes to the improvement of texture, viscosity, and moisture retention in prepared foods. There was a small reduction in the foaming capacity in Gβ (150.98%) compared to Nβ (154.9%), which was probably due to the weakening of the protein-polysaccharide interactions that are vital for bubble stabilization [5, 35]. Though this reduction is very small, it indicates that germination may have a slight impact on the functionalities related to aeration.

3.7. Particle Size Analysis

Particle size analysis revealed a significant difference between the particle sizes of NB (162.6 μm) and GB (138.9 μm), with GB having the smaller particles. Taniguchi et al. [36] mentioned that smaller particle sizes improve the rheological properties of barley-based products, making their yield stress and dynamic viscoelasticity higher, which is an advantage in batter formulations.

3.8. Color Analysis

Color analysis showed that the samples of germinated barley (GB and Gβ) had much less lightness (L*) but a great deal more redness (a*) and yellowness (b*) when compared to the non-germinated samples (NB and Nβ) as per (Table 4 and Table 6). The changes are attributed to the formation of melanoidins and other color-active compounds that are produced during germination by the enzymatic process of browning and the degradation of starches and proteins.

3.9. FTIR Analysis

The FTIR analysis separated the germinated barley (GB) samples by their specific vibrational signatures and showed the molecular transformations that took place during germination (Figure 2-5). One of the most important signatures was that of the methyl mercapto (methyl thiol) functional groups, which is indicated by the presence of vibrational modes corresponding to the carbon-hydrogen (C-H) bending motion within the methyl group. The C-H bending vibrations were observed in two ranges: 1340-1300 cm^{-1} and 1455-1405 cm^{-1}, which are typical for the structures of the methyl (CH_3) group. These changes may indicate transformations in the structure and composition of biomolecules caused by fermentative processes during germination. Other key observables were: • C-O Stretch Vibrations: The presence of such bonds is revealed as alcohols. • O-H Vibrations: The presence of hydroxyl (-OH) groups is indicated, which are the chief markers of hydrophilic compounds. • C-H Bend (CH_2/CH_3): The bending motion of the carbon-hydrogen bonds in methyl (CH_3) or methylene (CH_2) groups is suggested, which leads to further identification of the alcohol compounds.

The sulfur-containing compounds, particularly aliphatic thioethers and thio compounds, were identified within the range of 2500-2600 cm^{-1}, corresponding to the stretching vibrations of sulfur-hydrogen (S-H) bonds. These compounds are considered the primary contributors to the aroma and flavor profile of germinated barley [37]. Besides those, other molecular signatures that were seen in the FTIR spectrum are: • Alkenes (C=C bonds): Absorptions in the range of 1620-1680 cm^{-1} indicate the presence of unsaturated hydrocarbons. Alkynes, characterized by C≡C bonds, are observed within the range of 2100-2260 cm^{-1}. Additionally, aliphatic alkoxy and linear bromo compounds appear in the range of 500-1300 cm^{-1}, suggesting that the organic compounds produced during germination are highly complex. FTIR analysis has also shown the formation of tertiary alcohols, CF_3 groups, and saturated hydrocarbons, the combined presence of which contributes to the unique flavor profiles, aroma characteristics, and nutritional properties of germinated barley. The detection of these functional groups is a clear indicator of the biochemical processes occurring during germination, including oxidation, halogenation, and various enzyme-mediated reactions. The identification of sulfur-containing compounds, such as aliphatic thioethers, along with other complex organic transformations, suggests they are major contributors to the flavor and aroma of germinated barley. These compounds also influence the texture and nutritional qualities of the sample. In summary, FTIR analysis reveals the molecular complexity and biochemical transformations involved in germination, leading to improved nutritional and sensory properties. Further research into the functional significance of these compounds in food applications could provide valuable insights into their potential benefits and uses.

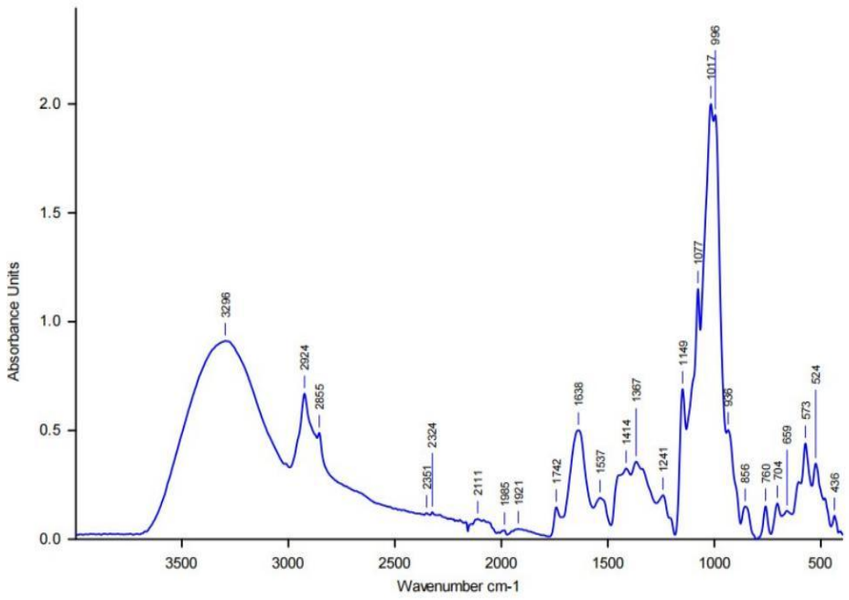

Figure 2. FTIR Spectrum of 24-Hour Germinated Barley.

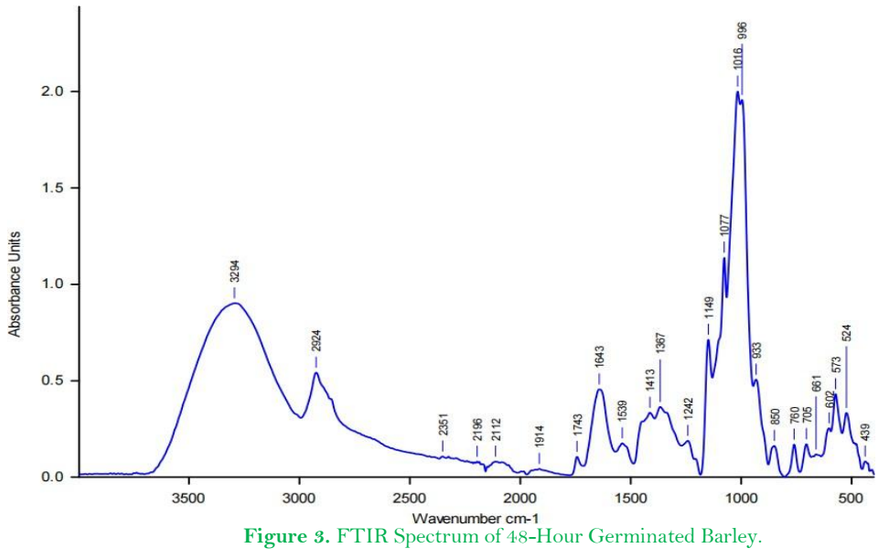

Figure 3. FTIR Spectrum of 48-Hour Germinated Barley.

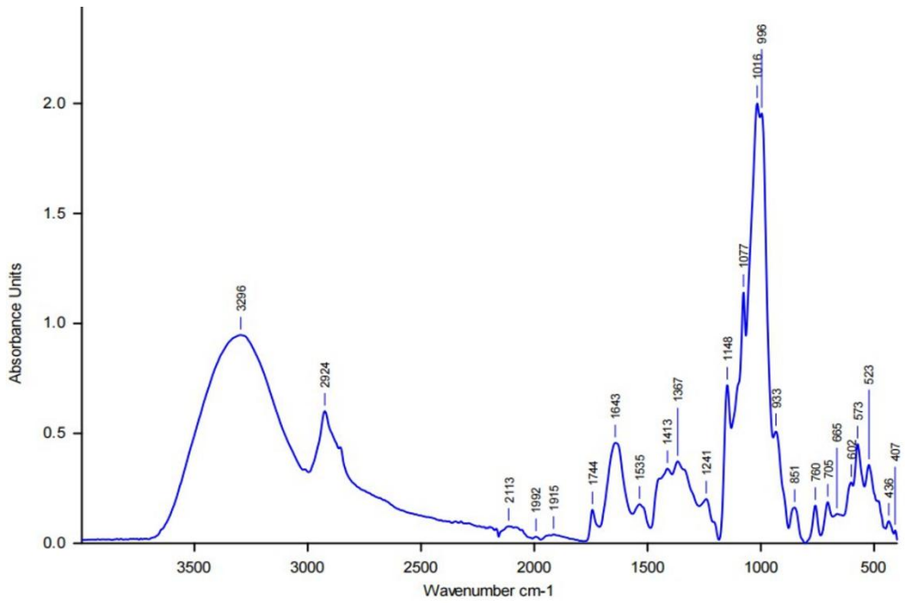

Figure 4. FTIR Spectrum of 72-Hour Germinated Barley.

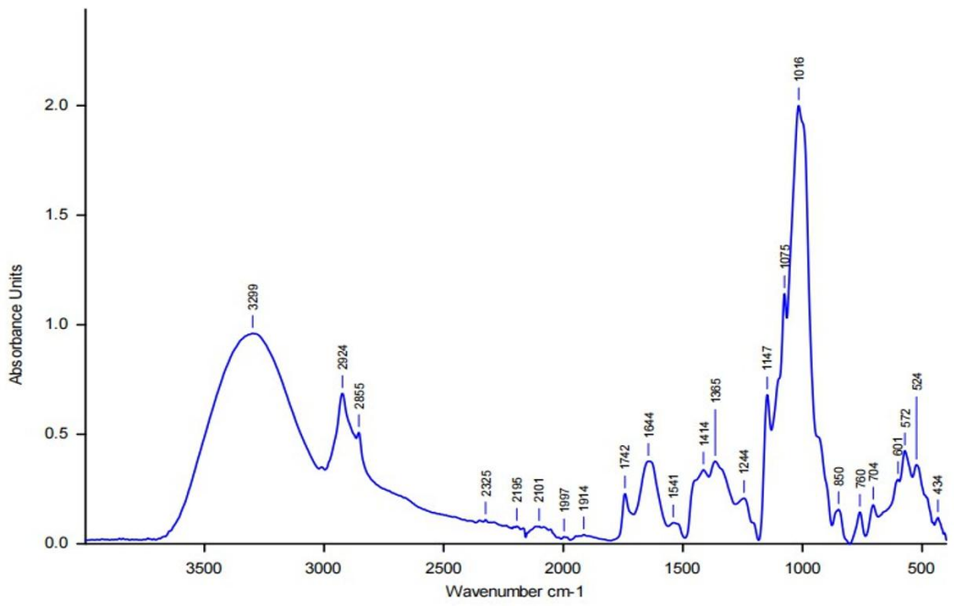

Figure 5. FTIR Spectrum of 96-Hour Germinated Barley.

4. Conclusion

The research reveals that the 48-hour germination of barley, which is very well controlled, results in an increase in yield (95.35%) for malting. Simultaneously, the germinated flour demonstrates enhancements in nutritional aspects, including protein content (14.83%), dietary fiber (14.54 g/100g), minerals such as calcium (Ca) and magnesium (Mg), and antioxidant capacity (Total Phenolic Content (TPC) 3.1 mg GAE/g, Ferric Reducing Antioxidant Power (FRAP) 1185.95 µmol Fe^{2+}/g, DPPH 93%). Additionally, there is an improved β-glucan water-binding capacity (4.21 g/g), although there are subtle reductions in β-glucan recovery (80.28%) and foaming properties. These changes are attributed to the presence of enzymes and their activities, which are supported by

(FTIR) results indicating that ground barley flour germinated together with its β-glucan extract are optimal ingredients for moisture-retaining foods rich in antioxidants. Such foods are targeted towards areas such as cardiometabolic health, glycemic control, and nutritional enhancement in vulnerable populations. Future studies should focus on optimizing germination parameters such as temperature (20–25°C), humidity (85–95%), and pH (5.5–6.0) for specific highly β-glucan barley cultivars to maximize extract retention and solubility, utilizing RSM for precise process modeling. In vitro digestion models combined with Caco-2 cell assays could elucidate the fermentation profiles of β-glucan, the production of short-chain fatty acids, and cholesterol-binding efficacy under gastrointestinal conditions. Comparative structural analysis using HPAEC-PAD and NMR should classify the degree of polymerization (DP3:DP4 ratios) and molar mass shifts between germinated and native β-glucan, correlating physicochemical changes with viscosity and health functionalities. Pilot extraction trials involving pH cycling (4.0–8.5), ethanol precipitation conditions, and lichenase digestion times could achieve recovery rates exceeding 85%, while maintaining molecular integrity suitable for nutraceutical applications.

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