Received: 29.11.2023
Revised: 12.12.2023
Accepted: 29.12.2023
Published: 9.1.2024

Slovak Journal of
Food Sciences

Potravinarstvo Slovak Journal of Food Sciences
vol. 18, 2024, p. 66-76
https://doi.org/10.5219/1941
ISSN: 1337-0960 online
www.potravinarstvo.com

Development of wheat composite bread using barley β-glucan rich flour

Boris Kovac, Andreja Bregar

ABSTRACT

The study highlights the potential for enhancing the nutritional value of wheat bread by incorporating β-glucan-enriched flour derived from barley. Initially, the study explores the air separation of barley flour into fractions rich in β-glucans. The procedure significantly increases the β-glucan concentration from 4.5% in barley to 8.0-10.5% in the separated coarse fractions, with yields varying from 33% to 77%. The optimal fraction of β-glucan-rich flour (β-GRF), balancing β-glucan concentration and yield, was chosen for subsequent evaluations. The impact of different levels of β-GRF supplementation (ranging from 0% to 30%) on the dough texture and sensory characteristics of the mixed wheat bread was tested. The addition of β-GRF has a noticeable influence on the rheological properties of the dough, resulting in longer development times and decreased stability compared to control samples. As the concentration of β-GRF increases to 10%, the specific volume generally rises, reaching 3.5 cm^3/g, compared to the control bread with a specific volume of 3.2 cm^3/g. However, beyond the 10% β-GRF level, the specific volume starts to decrease. Furthermore, β-GRF addition affects sensory and texture aspects, including bread volume, crumb, and crust characteristics. Despite these alterations, the bread remains within acceptable sensory parameters, and the final product, with 3g of β-glucan per 100 g of bread, meets the criteria for a health claim related to cholesterol reduction. This research underscores the potential to create healthier bread options by harnessing the nutritional benefits of dry concentrated β-glucans from barley, offering a promising avenue for improving the nutritional profile of bread products.

Keywords: β-glucan, barley, supplementation, mixed bread, health claim

INTRODUCTION

There is a growing interest in foods with added nutritional value that come with approved health claims. With its valuable nutrients, including β-glucans, barley has become an attractive option for functional food preparation due to its nutritional and technological benefits **[1]**. On the other hand, white wheat bread, consumed in large quantities in many European countries, is lacking in dietary fiber. Hence, public demand and research interest is in enriching bread with non-digestible prebiotic ingredients with health-promoting effects **[2]**. According to Hu et al. **[3]**, β-glucan reduces the glycemic index of bread and α-amylase activity by interacting with starch to form a more stable gel network structure, reducing the contact area between amylase and starch. The European Food Safety Authority (EFSA), through Commission Regulation **[4]** and the US Food and Drug Administration, has approved health claims for β-glucans. β-glucans are a key component for reducing the glycemic index of various processed foods **[5]**. EFSA's health claim depends on the source of β-glucans and the amount of β-glucan in one portion of the meal. Consumption of β-glucans from oats or barley as part of a meal reduces blood glucose levels after that meal. The claim may be used only for food that contains at least 4 g of β-glucans from oats or barley for every 30 g of available carbohydrates in a quantified portion as part of the meal. β-glucans contribute to maintaining normal blood cholesterol levels, and the claim may be used only for food containing at least 1 g of β-glucans from cereals or mixtures of these sources per quantified portion. The health claims related to β-glucans have led to increased interest in β-glucan-rich cultivars of barley and oats and concentrated β-glucan flours. Garcia-Gimenez et al. **[6]** reported that barley has a considerably high β-glucan concentration, ranging from 2.5% to 8% w/w, compared to other cereals such as wheat or rice, where the concentration does not exceed 1%. Various extraction and purification methods are available to produce cereal β-glucans **[7]**. The nature of the extraction procedure has a profound effect on the structure and molecular weight of β-glucan. Water and alkali extractions are preferred as the yield and recovery of extracted β-glucan concentration exceed 50% **[8]**. Pure β-glucan was commercially produced through high-temperature extraction with concentrated sodium hydroxide. However, using pure or highly concentrated β-glucan fractions to enrich cereal products is unsustainable and unnecessary. Practitioners in industrial-scale applications are constantly seeking the most cost-effective and environmentally sustainable procedures for obtaining highly concentrated β-glucan meal. Dry processing methods, such as grinding, screening, and/or air classification, use less energy and are environmentally friendly. The concentration of the final product can vary and is closely connected to the separation technique used and the initial content in the cereal. β-Glucans enriched flour by air classification could be a good ingredient for functional food preparation. However, the concentration of β-glucan will likely remain relatively low compared to wet extraction procedures. Ferrari et al. **[9]** concentrated β-glucan using a combination of micronization and air classification processes, resulting in a product ideal for integration into bread recipes. By combining grinding and screening **[10]**, fraction yields ranged from 30-70%, and the content of β-glucans ranged from 9.31% to 18.19%. While sieving allows the separation of fractions based solely on particle size and volume, the advantage of air classification is that fractions in the air stream are also separated based on specific gravity. The process of dry air separation is both environmentally and financially superior. Wheat flours are optimal for bread-making quality and can be blended with barley β-glucan. However, integrating purified β-glucan into a dough recipe can affect its rheological properties. Several authors **[11]** and **[12]** have reported that the overall effect may not necessarily be positive when integrating purified β-glucan into a dough recipe, affecting bread texture quality. The successful integration of β-glucan flours, rather than purified β-glucan, with minimal impact on the final bread quality, still presents a challenge.

Scientific Hypothesis

There is growing interest in foods with added nutritional value and labeled with approved health claims. Preparing β-glucan-enriched functional products, such as mixed wheat bread, using pure or highly concentrated β-glucan fractions may pose technological and economic challenges. This concern has prompted our study, where we explored two hypotheses:

a. The β-glucan-rich fraction (β-GRF) obtained from barley through the dry separation method is sufficiently concentrated to support health claims related to lowering cholesterol levels.

b. Integrating β-GRF into mixed wheat bread yields acceptable dough characteristics and final bread quality.

These hypotheses aim to address the feasibility and effectiveness of incorporating β-glucan into mixed wheat bread, both in terms of health benefits and overall product quality.

MATERIAL AND METHODOLOGY

Samples

Commercially known as Beta Gerstel, Barley was used to produce 21 samples of β-GRF through the dry separation method. In the second level, dough samples were prepared by blending wheat flour with the β-GRF samples to determine the rheological characteristics of the dough. The third level involves preparing bread samples for final analyses. The same ratio of wheat flour to β-GRF, as used in the second phase, is employed, along with yeast and salt.

Chemicals

Kit for β-glucan determination: β-Glucan Assay Kit K-BGLU, Megazyme Ltd., Ireland. All chemicals were of analytical grade quality.

Animals, Plants, and Biological Materials

Wheat (*Triticum aestivum*) flour (12.5% protein, 0.50% ash, 1.3% crude fat, 14.7% moisture) produced by Mlinotest d.d., Slovenia.

Barley (*Hordeum vulgare*), Beta gerste, Germany (10.5% protein, 2.50% ash, 4,5% β-glucan,14.0% moist.

Instruments

Mill, hammermill, microniser: Sangatti, Italy.

Farinograph: Brabender, Germany.

Spiral mixer: IP10F Fimar, Italy.

Baking oven: Dibas, Germany.

Spectrophotometer: Mettler Toledo, USA.

Laboratory Methods

The amount of β-glucan was determined using the Megazym kit, following the AAAC Method 32-23 modified according to McCleary and Codd **[13]**. This method relies on the enzymatic degradation of polysaccharides by β-glucan hydrolases (specific for mixed-linkage [(1-3)(1-4)]-β-D-glucan), followed by spectrophotometric measurement of the generated D-glucose.

Dough rheology was determined following the AACC Farinograph Method 54-29 **[14]**. The farinograph is an instrument used to measure and record the rheological properties of dough during mixing. It is used to evaluate flour's water absorption and to determine dough development time, stability, and other dough mixing characteristics.

The seed displacement method determined the bread volume and specific volume according to AACC Method 10-05.01 **[15]**. It can be used to accurately measure the volume of oddly shaped objects by measuring the volume of displaced material when an object is submerged in a volumetric tube. This method relies on the principle that the bread volume displaces an equivalent volume of the rapeseed.

A sensory profile that included the attributes related to texture was completed according to ISO 13299 **[16]**. A sensory panel of eight trained experts (five females, three males; age range, 28-58 years) was used to evaluate the bread samples. All of the experts had experience in descriptive evaluation of bread. The initial panel discussion and training session were compressed to two 2-h sessions. In the first phase of the test, the panel developed 9 descriptors for the sensory attributes of the control bread and the bread with β-GRF addition. The panellists created a 10-point unstructured line scale with descriptor labels at either end. Attribute ranges (weak = 0 vs. intense = 10): Crust thickness: thick = 0 vs. thin = 10, crumb colour: white = 0 vs. brown = 10, crumb pore homogeneity: inhomogeneous = 0 vs. homogeneous = 10, crumb pore size: small = 0 vs. big = 10. Texture cohesiveness: less cohesive = 0, very cohesive = 10, springiness: springless = 0 vs. springy = 10, toughness: weak = 0 vs. intense = 10, hardness: smooth = 0 vs. hard = 10, adhesivity: not stick = 0 vs. sticky = 10. The control bread was chosen as the reference sample, to reduce the variation among the panellists. The panellists defined the intensity value of the reference sample concerning each sensory attribute. Based on the questionnaires, the panelists rated the samples (one whole loaf of bread, two slices/per person) individually, using a balanced test design in which the serving order was randomized for each panellist.

The β-GRF was prepared following the procedure outlined in reference **[9]**. Barley underwent dehusking, with 12% of the peripheral parts being separated. The grains were then hammer-milled and subjected to two rounds of micronization using a high-speed rotor micronizer operating at 10,000 rotations per minute. Micronized fractions were separated in a cyclone under an airflow. An aspirating pump, positioned at the system's end, propelled the airflow, regulated by an inlet valve. Before the pump, a cyclone and a filter were placed to eliminate excessively fine powder. The apparatus sorted the flour into two portions: a coarse fraction and a fine fraction. Particle size control and separation were achieved by modulating the airflow using an inlet valve.

Description of the Experiment

Sample preparation: Preparation of β-GRF samples from barley:

Micronized flours were separated into coarse, medium, and fine fractions in cyclone by a controlled radial airflow. The yield and β-glucan concentration of fractions were determined. β-GRF coarse fraction was adjusted to 10% of β-glucan and used in dough preparation and baking trials.

Farinograph dough samples: The wheat flour was blended with β-GRF at 5%, 10%, 15%, 20%, 25%, and 30% (w/w). A control sample was prepared without β-GRF. The water addition was adjusted based on the farinograph result.

Bread samples: The dough was prepared from wheat flour blended with β-GRF at levels of 0%, 5%, 10%, 15%, 20%, 25%, and 30% (w/w), 2% yeast, 2% salt and 56.5% to 60% water (all w/w). The water addition was adjusted based on the previous farinograph results. Subsequently, the mixed dough was divided into 450 g pieces, shaped manually, and placed into baking pans. After fermenting at 30 °C and 85% relative humidity for 60 minutes, the loaves were baked in a convection oven at 230 °C for 30 minutes.

Number of samples analyzed: 21 samples of β-GRF, 7 samples of dough, 7 samples of bread.

Number of repeated analyses: 3.

Number of experiment replications: The sensory evaluation involved 16 replications, while other tests were conducted with 6 replications.

Design of the experiment:

1. Preparation of β-GRF from barley by milling, micronization, and dry separation; samples were used to determine β-glucan content and yield.

2. Dough preparation and determination of rheological characteristics by farinograph. Water absorption development time, stability, degree of softening, and quality number were determined.
3. Baking trials and final bread characteristics determination; moisture content, loaf height, volume, and specific volume were determined.
4. Sensory evaluation; crust thickness and colour, crumb homogeneity and size, texture cohesiveness, springiness, toughness, hardness, and adhesivity were determined.

In the final phase, the obtained results were processed, subjected to statistical analysis, and verified the validity of our hypotheses.

Statistical Analysis

The SPSS software version 26 (IBM, Armonk, NY, USA) was used to confirm the statistical significance of differences between each experimental set of wells. Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by *post-hoc* tests to determine any differences between group means. Differences with $p < 0.05$ were considered to be statistically significant. The data are presented as means ± standard deviation for 6 replicates, and 16 replicates were conducted for sensory evaluation.

RESULTS AND DISCUSSION

Separation of barley flour into β-glucan enriched fractions

The primary aim was to separate micronized barley flour into fractions with as high a concentration of β-glucans as possible using an air cyclone. The cyclone could classify flour particles into coarse, medium, and fine fractions based on airflow settings. The results, presented in Table 1, show the yield and concentration of β-glucans in g/100g dry matter for all three fractions were obtained through classification at various airflow settings. As the intensity of air flow increases by airflow variation (valve settings from 1 to 7) the β-glucan content in the coarse fraction tends to concentrate. The β-glucan content in the medium fraction generally decreases with higher airflow intensities. As the airflow intensity increases, the yield of the coarse fraction decreases steadily. In contrast, the yield of the medium fraction increases as the airflow intensity increases. The separation phenomenon is consistent with the results of **[10]**. This suggests that β-glucan particles are redistributed from the medium to the coarse fraction at higher air flow intensities. The trends of the yield and β-glucan curves are similar to those described by Ferrari **[9]**. The yield of the fine fraction remains relatively low compared to the coarse and medium fractions across all airflow intensities. Overall, the results indicate that the choice of airflow intensity in cyclone separation can significantly impact both yield and β-glucan content in different fractions. The study found that at valve setting 6, the maximal β-glucan content in the coarse fraction was 2.1 times higher than that of the initial material. The initial material had a β-glucan content of 4.5 g/100 g dry matter, which is lower than that reported in barley tested by another source **[10]**. The efficiency of β-glucan concentration in the coarse fraction is comparable to the findings of Ferrari et al. **[9]**. They started with 8.7% β-glucan content in barley flour and, after two cycles of air classification, with a relatively low yield (29.8%), achieved 15.6%, β-glucan content respectively. The classic extraction of β-glucans from cereals typically encompasses a minimum of four comprehensive stages **[17]**: (a) wet milling of raw cereal and deactivation of β-glucanase to preserve the native high molecular weight of β-glucan, (b) extraction of β-glucans, (c) a purification and isolation step, and (d) drying of the final product.

Table 1 The yields and β-glucan content (g/100g) and SD in coarse, medium, and fine fractions as a function of airflow regulated by separator valve settings.

Airflow settings	**Fraction yield (yield ±SD)**			**β-glucan content (g.100g^{-1} ±SD)**		
	coarse fraction	**medium fraction**	**fine fraction**	**coarse**	**medium**	**fine fraction**
1	0.75 ± 0.0^{def}	0.22 ± 0.01^{a}	0.03 ± 0.01^{a}	8.01 ± 0.02^{a}	6.15 ± 0.02^{d}	5.91 ± 0.28^{f}
2	0.70 ± 0.07^{cf}	0.28 ± 0.02^{b}	0.03 ± 0.01^{a}	8.33 ± 0.02^{a}	5.23 ± 0.03^{c}	4.78 ± 0.20^{d}
3	0.66 ± 0.05^{be}	0.31 ± 0.01^{bc}	0.04 ± 0.01^{a}	8.69 ± 0.05^{ab}	5.25 ± 0.04^{c}	3.75n $\pm 0.12^{d}$
4	0.65 ± 0.03^{bd}	0.34 ± 0.02^{cd}	0.03 ± 0.01^{a}	9.13 ± 0.03^{b}	4.71 ± 0.04^{bc}	1.22 ± 0.10^{b}
5	0.61 ± 0.03^{ac}	0.35 ± 0.0^{d}	0.03 ± 0.01^{a}	10.18 ± 0.03^{c}	4.13 ± 0.03^{ab}	1.3 ± 0.13^{b}
6	0.59 ± 0.04^{ab}	0.38 ± 0.02^{d}	0.02 ± 0.01^{a}	10.51 ± 0.04^{c}	3.99 ± 0.03^{a}	1.84 ± 0.21^{c}
7	0.53 ± 0.07^{a}	0.45 ± 0.01^{e}	0.02 ± 0.01^{a}	10.01 ± 0.04^{c}	3.9 ± 0.02^{a}	0.28 ± 0.04^{a}

Note: Data are medians. Data with different superscript letters in the same column are significantly different ($p < 0.05$).

In contrast, our procedure involves only the milling and mechanical separation phase, making the process more sustainable and cost-effective compared to the traditional wet process with its multiple stages. Cajzek et al. **[18]** also asserted that a two-step process involving milling and sieving is effective in preparing flours rich in beta-glucan and bioactive compounds, offering potential enhancements in both functional and health-related properties for bakery products. However, it is important to note that our approach as well as the previously mentioned process **[18]** results in a lower final concentration of beta-glucan. This drawback could potentially be mitigated by selecting higher-quality raw materials. We can conclude that the dry process offers great potential for environmentally and economically friendly β-glucan concentration; however, we have to stress the importance of barley cultivars with higher initial β-glucan content for large-scale production. Industrial production would require scale-up with further process optimization. Based on the yield and β-glucan concentration, we identified coarse fractions that are suitable for our upcoming tests in dough preparation and baking trials. The β-GRF fraction was adjusted to contain 10% β-glucan.

Dough characteristics

Table 2 shows notable effects on all determined Farinograph parameters when β-GRF is added to wheat flour dough. β-glucan supplementation leads to an increase in dough hydration. This observation is consistent with findings from a study by Zeng et al. **[19]**, which reported that hydrophilic colloids, like β-glucan, commonly increase dough viscosity, requiring slightly more water for dough preparation. β-glucan impacts hydration, development times, dough stability, and quality. In general, the trend is that β-glucan enhances these aspects, but the specific effects depend on its concentration. Up to 2% of β-glucan supplements contribute to the formation and development of gluten networks, promoting the formation of harder, stronger, and more stable gluten networks **[20]**. However, as the concentration of β-glucan continues to rise, the gluten structure weakens to a greater extent, as indicated by the work of Lii et al. **[21]**, which is not preferred in regard to dough stability **[22]**. Previous research **[12]** demonstrated that substituting hulless barley flour for wheat flour adversely affected gluten microstructure and dough mixing behavior. This was attributed to the partial breaking of cross-links in the gluten network, resulting in shortened dough development and stability times. The current study's results align with the mentioned findings, showing a significant increase in development time when 5% β-GRF is added to the dough. This suggests that β-glucan doughs require more time for proper gluten development during mixing than control dough, potentially leading to better stability during processing and handling. The current study indicates that β-GRF supplementation higher than 10% (equivalent to 1% β-glucan) negatively correlates with development time.

Table 2 Farinograph parameters of wheat flour dough as a control (0) and fortified dough with β-GRF at a range to 30% (β-GRF).

B-GRF (%)	**Farinograph dough parameters**				
	Water absorption (% ±SD)	**Development time (min ±SD)**	**Stability (min ±SD)**	**Degree of softening (BU* ±SD)**	**Quality number (number ±SD)**
0	56.5 ±3.0^{a}	7.0 ±0.8^{ab}	12.14 ±1.63^{ab}	40 ±1.63^{a}	160 ±14^{bcd}
5	57.5 ±0.6^{a}	9.1 ±0.3^{df}	15.3 ±0.9^{c}	48.0 ±1.8^{bc}	160 ±15^{cd}
10	57.5 ±0.7^{a}	8.35 ±0.3^{cd}	13.40 ±1.2^{bc}	56 ±1.5^{cd}	144 ±6^{ac}
15	58.0 ±0.8^{a}	8.15 ±0.4^{cf}	10.1 ±0.5^{a}	61 ±1.8^{d}	134 ±6^{ab}
20	58.5 ±0.2^{a}	7.5 ±0.6^{bc}	10.2 ±1.2^{a}	51 ±2.9^{bc}	146 ±5^{ad}
25	59.5 ±2.4^{a}	6.1 ±0.2^{a}	10.1 ±0.8^{a}	50.0 ±4.1^{b}	129 ±10^{a}
30	60.0 ±1.8^{a}	6.15 ±0.3^{a}	10.0 ±0.8^{a}	49 ±1.5^{b}	124 ±6^{a}

Note: *BU Brabender farinograph units. Data are medians. Data with different superscript letters in the same column are significantly different (p <0.05).

This aligns with the observations of a previous study **[23]**, which noted that at concentrations over 1%, β-glucan molecules organize into a dense, pseudoplastic complex. The trend in dough stability mirrors that of development time, increasing at low β-GRF additions but decreasing when more than 10% β-GRF is added. The current study's findings are consistent with previous research **[11]**, **[24]**, where increased water absorption and β-glucan concentration resulted in delayed dough development time. However, it contrasts with the results of Mohebbi et al. **[24]**, who found a negative effect on stability and development time even with a 1% β-glucan addition.

Bread moisture content, volume, and specific volume

The moisture content of the final bread samples presented in Table 3 appears to remain relatively stable across different levels of β-glucan content, with slight variations ranging from 43.7% to 46%. This suggests that changes in β-glucan content significantly impact the moisture content of the bread. Increased water absorption and, consequently, higher bread moisture content have been reported in other studies where β-glucan from different sources was used to fortify wheat flour **[11]**. The literature describes a statistically significant correlation between dough characteristics and bread quality. That means that the results of the farinograph dough testing reliably predict the final characteristics of the enriched bread **[25]**.

Table 3 Bread moisture content, volume, and specific volume and loaf height for control (0) and fortified dough with β-GRF at range to 30%.

β-GRF (%)	**Moisture content (% ±SD)**	**Loaf hight (cm ±SD)**	**Volume (cm^3 ±SD)**	**Specific volume (cm^3.g^{-1} ±SD)**
0	38.7 ±0.8^{a}	8.1 ±0.1^{b}	1200 ±8.1^{c}	3.2 ±0.1^{bc}
5	39.2 ±1.6^{a}	8.2 ±0.1^{b}	1240 ±8.7^{d}	3.3 ±0.1^{c}
10	40.0 ±1.6^{ab}	8.3 ±0.1^{b}	1250 ±8.7^{d}	3.5 ±0.2^{c}
15	40.5 ±1.3^{ab}	7.9 ±0.2^{ab}	1015 ±6.4^{b}	3.1 ±0.1^{b}
20	41.5 ±0.3^{ab}	7.9 ±0.2^{ab}	990 ±9.1^{a}	2.9 ±0.1^{ab}
25	42.2 ±0.8^{ab}	7.8 ±0,1^{a}	980 ±9.1^{a}	2.8 ±0.1^{a}
30	42.4 ±1.6^{b}	7.7 ±0.3^{a}	977 ±9.2^{a}	2.8 ±0.1^{a}

Note: Data are medians. Data with different superscript letters in the same column are significantly different (p <0.05).

The data indicates a significant impact of β-GRF addition on bread properties, particularly a noticeable decrease in loaf height when the β-glucan content exceeds 10%. The decrease in loaf height (Figure 1) is attributed to the higher levels of β-glucan, which intensively affect the bread's volume, leading to denser or less risen bread. Škrbic et al. **[26]** noted a similar effect when barley flour was added to wheat flour, causing a decrease in bread volume due to gluten dilution and less retention of CO_2 gas. The study observes that supplemented bread volume generally increases up to 10% β-GRF, after which it starts to decrease. Xu et al. **[3]** also reported a similar trend when adding different doses of oat β-glucan. Specific volume, which measures the volume per gram of bread, follows the same trend as bread volume, becoming denser and less voluminous as more β-glucan is added. The findings align with a previous report **[12]** for added barley β-glucan, and similar studies **[27]** describe a significant reduction in specific bread volume and an increase in firmness when enriched with fiber-rich substrates. Depending on the desired characteristics of the bread, such as loaf height and specific volume, one can use this data to determine the optimal level of β-glucan content. For instance, if a lighter and more voluminous bread, popular in some European regions, is preferred, a lower β-glucan supplement may be the choice.

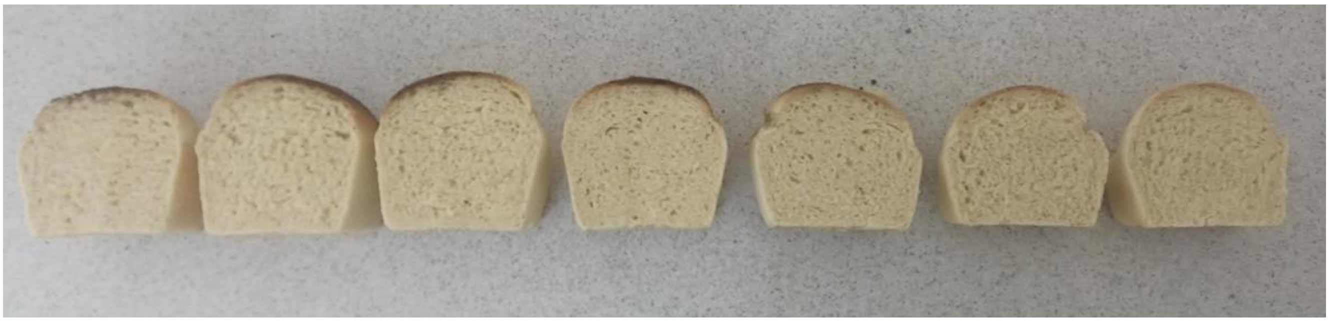

Figure 1 Influence of β-glucan on the appearance of the baked product. The concentration increases from left to right. Far left is the control with 0%, followed by samples with 5, 10, 15, 20, 25, and 30% β-glucan.

Crust and crumb attributes and texture profile of the bread

There is a clear trend of decreasing crust thickness as the percentage of β-GRF increases, indicating that higher β-GRF content leads to thinner crusts. The crust colour darkens with higher levels of β-GRF supplementation, likely due to the Maillard reaction during baking, resulting in crust browning. The data indicates a decrease in crumb pore homogeneity with β-GRF supplementation, suggesting that β-GRF may lead to an inconsistent and non-uniform crumb structure throughout the bread. Crumb pore size increases with β-GRF supplementation up to 15%, associated with a more open and airy texture, preferred for certain types of bread, such as artisanal loaves. Beyond 15% supplementation, crumb size reduces, leading to a decrease in bread volume. Similar behaviour was observed in a study **[28]** where wheat flour was partially substituted with an oat flour fraction rich in β-glucans. Springiness represents the ability of bread to return to its original shape after compression. Current work indicates that the springiness of the bread samples increases with higher β-GRF supplementation levels, suggesting improved elasticity. This aligns with findings from Kurek et al. **[29]**, who observed increased springiness with low levels of β-glucan supplementation. The toughness and hardness of bread samples in current research decrease as β-GRF supplementation increases. This is consistent with the study by Skendi et al. **[11]**, where an increasing level of pure β-glucan supplementation resulted in a coarser structure and decreased breadcrumb firmness. The cohesiveness of tested bread samples increases, indicating a decreasing tendency of the bread to fracture and crumble with higher β-GRF levels. This contrasts with the findings of **[28]**, where oat beta-glucans led to decreasing cohesiveness, although not significantly different between samples. Adhesivity of bread samples increases with higher β-GRF supplementation, in line with other works showing that bread containing β-glucans exhibits lower moisture loss during storage **[11]**. Abdel-Gawad **[30]** reported that β-glucan enrichment could prolong bread shelf life, and the reduction in moisture loss is evident with an increasing concentration of β-glucans. The results confirm that β-glucan can impact bread quality parameters, potentially causing some damage, as observed by other authors **[31]**. The results conclude that further research and testing may be necessary to optimize β-GRF levels for achieving the desired bread texture and optimal nutritional properties. In summary, the results provide comprehensive insights into how β-glucan supplementation influences various textural properties of bread. The observed effects may have implications for product quality and nutritional considerations, highlighting the need for careful optimization based on intended use and consumer preferences.

Table 4 Crust and crumb attributes, texture profile of the bread prepared from the wheat flour dough for control (0) and fortified dough with β-GRF at range to 30%.

β-GRF (%)		**0**	**5**	**10**	**15**	**20**	**25**	**30**
Crust	**Thickness (score ±SD)**	6.3 $\pm1.0^{de}$	6.1 $\pm0.9^{bcde}$	6.0 $\pm0.5^{ae}$	5.5 $\pm0.7^{ad}$	5.3 $\pm0.7^{ac}$	5.0 $\pm0.8^{ab}$	5.0 $\pm0.9^{a}$
	Colour (score ±SD)	2.0 $\pm0.8^{ae}$	2.5 $\pm0.5^{abf}$	3.0 $\pm0.8^{ac}$	3.0 $\pm0.7^{ad}$	3.5 $\pm1.0^{bcde}$	4.0 $\pm0.8^{cdf}$	4.0 $\pm1.0^{cd}$
Crum pore	**Homogeneity (score ±SD)**	8.0 $\pm1.1^{e}$	7.0 $\pm0.7^{bcde}$	6.3 $\pm0.7^{ad}$	6.3 $\pm0.7^{ac}$	6.0 $\pm0.8^{ab}$	5.8 $\pm0.8^{a}$	5.8 $\pm0.8^{a}$
	Size (score ±SD)	5.0 $\pm0.8^{a}$	5.3 $\pm0.9^{a}$	6.0 $\pm0.7^{a}$	6.0 $\pm0.7^{a}$	5.5 $\pm0.9^{a}$	5.0 $\pm0.8^{a}$	4.9 $\pm0.8^{a}$
Texture	**Cohesiveness (score ±SD)**	5.9 $\pm0.1^{a}$	6.0 $\pm0.5^{a}$	6.1 $\pm0.7^{a}$	6.0 $\pm0.7^{a}$	6.0 $\pm0.7^{a}$	7.0 $\pm0.7^{b}$	7.0 $\pm0.8^{b}$
	Springiness (score ±SD)	5.6 $\pm0.8^{ac}$	6.0 $\pm0.8^{ab}$	6.3 $\pm0.5^{bc}$	7.8 $\pm0.9^{d}$	8.0 $\pm0.7^{d}$	8.0 $\pm0.7^{d}$	9.6 $\pm0.5^{e}$
	Toughness (score ±SD)	8.0 $\pm0.6^{cde}$	7.0 $\pm0.9^{be}$	7.0 $\pm0.9^{bd}$	7.0 $\pm0.9^{bc}$	6.3 $\pm0.7^{ab}$	6.0 $\pm0.8^{ab}$	5.8 $\pm1.0^{a}$
	Hardness (score ±SD)	6.0 $\pm1.6^{cdef}$	5.3 $\pm0.6^{bcde}$	5.0 $\pm0.6^{ae}$	5.1 $\pm0.9^{ad}$	5.0 $\pm0.7^{ac}$	4.8 $\pm0.6^{abf}$	4.0 $\pm0.7^{a}$
	Adhesivity (score ±SD)	3.0 $\pm0.5^{a}$	4.0 $\pm0.7^{a}$	3.9 $\pm0.7^{a}$	5.5 $\pm0.6^{b}$	7.3 $\pm0.6^{c}$	8.0 $\pm1.1^{c}$	8.1 $\pm1.1^{c}$

Note: Attribute ranges; crust thickness: thick = 0 vs. thin = 10, crumb colour: white = 0 vs. brown = 10, crumb pore homogeneity: inhomogeneous = 0 vs. homogeneous = 10, crumb pore size: small = 0 vs. big = 10. Texture cohesiveness: less cohesive = 0, very cohesive = 10, springiness: springless = 0 vs. springy = 10, toughness: weak = 0 vs. intense = 10, hardness: smooth = 0 vs. hard = 10, adhesivity: not stick = 0 vs. sticky = 10. Data are medians. Data with different superscript letters in the same column differ significantly (p <0.05).

Nutritional value concerning health claims

The data in Table 5 reveals that β-GRF supplementation significantly impacts the nutritional composition of bread, particularly concerning β-glucan content. The results of many previous studies confirmed that β-glucans contribute to maintaining normal blood cholesterol levels, mainly when the food portion contains at least 1 g of β-glucans from sources like oats, oat bran, barley, barley bran, or mixtures of these per quantified portion **[32]**. To claim that the food has a beneficial effect on maintaining normal blood cholesterol levels, the consumer should be informed that this effect is obtained with a daily intake of 3 g of β-glucans from oats, oat bran, barley, barley bran, or mixtures of these β-glucans **[4]**.

At 5% β-GRF supplementation, the β-glucan content is low, requiring a larger amount of bread (600 g) to meet the daily recommended β-glucans for a health claim related to cholesterol reduction. However, at 30% β-GRF supplementation, only 100 g of bread is needed to meet the daily recommended β-glucans, making it more feasible without significant changes to bread consumption habits. The consumption of β-glucans helps reduce the post-meal rise in blood glucose **[4]**, a capability not shared by other polysaccharides present in bread. A health claim related to cholesterol reduction can be used for food containing at least 4 g of β-glucans from oats or barley for every 30 g of available carbohydrates in a quantified portion as part of the meal. The best ratio of 1.91 g β-glucan per 30 g carbohydrates was obtained at 30% β-GRF supplementation. However, it falls short of reaching the minimal criteria for the health claim related to blood glucose reduction. While our study demonstrates promising results in enhancing the nutritional value of wheat bread by incorporating β-glucan-enriched flour from barley, it's essential to acknowledge potential limitations. The study identifies a lower final concentration of beta-glucan compared to classic extraction methods. Further optimization of the milling and mechanical separation phase might be necessary to enhance the final concentration without compromising other desirable characteristics. While the bread remains within acceptable sensory parameters, individual preferences can vary. Conducting broader sensory studies with diverse consumer groups might provide more comprehensive insights into the acceptability of the β-GRF-enriched bread across different populations. Although the bread meets the criteria for a health claim related to cholesterol reduction, a broader nutritional assessment, considering factors such as overall caloric content, and other nutrients, would provide a more comprehensive understanding of the product's nutritional profile.

Table 5 Quantity of β-glucan-enriched bread per day, determined through calculation to meet the recommended amount for the health claim regarding lowering.

% of β-GRF supplement	**β-glucan/100g bread ($g.100g^{-1}$)**	**Quantity of bread (g)**
0	0	-
5	0.5	600
10	1	300
15	1.5	200
20	2	150
25	2.5	120
30	3	100

CONCLUSION

The procedure of air separation significantly increases β-glucan from 4.5% in barley to 8.0-10.5% in the separated coarse fractions, with yields varying from 33% to 77%. Because our process involves only milling and mechanical sieving, we can infer that it is more sustainable and cost-effective compared to the wet process, which typically includes at least four stages. The supplementation of wheat flour by β-GRF fraction, containing 10% β-glucan has a noticeable influence on the rheological properties of the dough, resulting in longer development times and decreased stability when compared to control samples. The specific volume generally increases up to a 10% β-GRF supplement, reaching 3.5 cm^3/g, in comparison to the control bread with a specific volume of 3.2 cm^3/g. However, beyond the 10% concentration, the specific volume begins to decrease. Furthermore, β-GRF addition affects various sensory aspects of bread including crumb, crust, and texture characteristics. Despite these alterations, the bread compared to the control remains within acceptable sensory parameters and the final 3g β-glucan/100g bread meets the criteria for a health claim related to cholesterol reduction. Overall, these findings can contribute to the development of functional foods enriched with β-glucan and promote human health.

REFERENCES

1. Aoe, S., Morita, T., & Ohno, N. (Eds.). (2022). Beta-Glucan in Foods and Health Benefits. MDPI. https://doi.org/10.3390/books978-3-0365-5108-1
2. Mohebbi, Z., Homayouni, A., Azizi, M. H., & Hosseini, S. J. (2017). Effects of beta-glucan and resistant starch on wheat dough and prebiotic bread properties. In Journal of Food Science and Technology (Vol. 55, Issue 1, pp. 101–110). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-017-2836-9
3. Xu, S., Gong, Y., Rafique, H., He, T., & Hu, X. (2021). Effect of oat β-glucan addition on the staling properties of wheat-oat blended flour Chinese steamed bread. In Bioactive Carbohydrates and Dietary Fibre (Vol. 26, p. 100285). Elsevier BV. https://doi.org/10.1016/j.bcdf.2021.100285
4. Regulation (EC) No 1924/2006 of the European Parliament and of the council of 20 December 2006 on nutrition and health claims made on foods.
5. Bozbulut, R., & Sanlier, N. (2019). Promising effects of β-glucans on glyceamic control in diabetes. In Trends in Food Science & Technology (Vol. 83, pp. 159–166). Elsevier BV. https://doi.org/10.1016/j.tifs.2018.11.018
6. Garcia-Gimenez, G., Russell, J., Aubert, M. K., Fincher, G. B., Burton, R. A., Waugh, R., Tucker, M. R., & Houston, K. (2019). Barley grain (1,3;1,4)-β-glucan content: effects of transcript and sequence variation in genes encoding the corresponding synthase and endohydrolase enzymes. In Scientific Reports (Vol. 9, Issue 1). Springer Science and Business Media LLC. https://doi.org/10.1038/s41598-019-53798-8
7. Zhu, F., Du, B., & Xu, B. (2016). A critical review on production and industrial applications of beta-glucans. In Food Hydrocolloids (Vol. 52, pp. 275–288). Elsevier BV. https://doi.org/10.1016/j.foodhyd.2015.07.003
8. Maheshwari, G., Sowrirajan, S., & Joseph, B. (2017). Extraction and Isolation of β-Glucan from Grain Sources—A Review. In Journal of Food Science (Vol. 82, Issue 7, pp. 1535–1545). Wiley. https://doi.org/10.1111/1750-3841.13765
9. Ferrari, B., Finocchiaro, F., Stanca, A. M., & Gianinetti, A. (2009). Optimization of air classification for the production of β-glucan-enriched barley flours. In Journal of Cereal Science (Vol. 50, Issue 2, pp. 152–158). Elsevier BV. https://doi.org/10.1016/j.jcs.2009.04.007
10. Messia, M. C., De Arcangelis, E., Candigliota, T., Trivisonno, M. C., & Marconi, E. (2020). Production of ß-glucan enriched flour from waxy barley. In Journal of Cereal Science (Vol. 93, p. 102989). Elsevier BV. https://doi.org/10.1016/j.jcs.2020.102989
11. Skendi, A., Papageorgiou, M., & Biliaderis, C. G. (2010). Influence of water and barley β-glucan addition on wheat dough viscoelasticity. In Food Research International (Vol. 43, Issue 1, pp. 57–65). Elsevier BV. https://doi.org/10.1016/j.foodres.2009.08.012
12. Yu, L., Ma, Y., Zhao, Y., Pan, Y., Tian, R., Yao, X., Yao, Y., Cao, X., Geng, L., Wang, Z., Wu, K., & Gao, X. (2021). Effect of Hulless Barley Flours on Dough Rheological Properties, Baking Quality, and Starch Digestibility of Wheat Bread. In Frontiers in Nutrition (Vol. 8). Frontiers Media SA. https://doi.org/10.3389/fnut.2021.785847
13. McCleary, B. V., & Codd, R. (1991). Measurement of (1 → 3),(1 → 4)-β-<scp>D</scp>-glucan in barley and oats: A streamlined enzymic procedure. In Journal of the Science of Food and Agriculture (Vol. 55, Issue 2, pp. 303–312). Wiley. https://doi.org/10.1002/jsfa.2740550215
14. AACC International. Approved Methods of Analysis, 11th Ed. Method 54-21. Farinograph Method for Flour. Cereals & Grains Association, St. Paul, MN, U.S.A. Retrived from https://www.cerealsgrains.org/resources/Methods/tools/Documents/54-21-01.pdf.
15. AACC International. Approved Methods of Analysis, 11th Ed. Method 10-05.01. Guidelines for Measurement of Volume by Rapeseed Displacement. St. Paul, MN, U.S.A. Retrived from https://www.cerealsgrains.org/resources/Methods/Pages/10BakingQuality.aspx.
16. ISO 13299:2016 Sensory analysis – Methodology – General guidance for establishing a sensory profile Retrived from: https://www.iso.org/obp/ui/en/#iso:std:iso:13299:ed-2:v1:en
17. Benito-Román, Ó., Alonso, E., Palacio, L., Prádanos, P., & Cocero, M. J. (2014). Purification and isolation of β-glucans from barley: Downstream process intensification. In Chemical Engineering and Processing: Process Intensification (Vol. 84, pp. 90–97). Elsevier BV. https://doi.org/10.1016/j.cep.2013.12.006
18. Cajzek, F., Bertoncelj, J., Kreft, I., Poklar Ulrih, N., Polak, T., Požrl, T., Pravst, I., Polišenská, I., Vaculová, K., & Cigić, B. (2019). Preparation of β-glucan and antioxidant-rich fractions by stone milling of hull-less barley. In International Journal of Food Science & Technology (Vol. 55, Issue 2, pp. 681–689). Wiley. https://doi.org/10.1111/ijfs.14322

19. Huang, Z., Wang, J. J., Chen, Y., Wei, N., Hou, Y., Bai, W., & Hu, S.-Q. (2020). Effect of water-soluble dietary fiber resistant dextrin on flour and bread qualities. In Food Chemistry (Vol. 317, p. 126452). Elsevier BV. https://doi.org/10.1016/j.foodchem.2020.126452

20. Zeng, F., Hu, Z., Yang, Y., Jin, Z., & Jiao, A. (2023). Regulation of baking quality and starch digestibility in whole wheat bread based on β-glucans and protein addition strategy: Significance of protein-starch-water interaction in dough. In International Journal of Biological Macromolecules (p. 128021). Elsevier BV. https://doi.org/10.1016/j.ijbiomac.2023.128021

21. Li, Z., Gao, W., Liang, J., Fan, H., Yang, Y., Suo, B., & Ai, Z. (2023). Mechanism underlying the weakening effect of β-glucan on the gluten system. In Food Chemistry (Vol. 420, p. 136002). Elsevier BV. https://doi.org/10.1016/j.foodchem.2023.136002

22. Selaković, A., Nikolić, I., Dokić, L., Šoronja-Simović, D., Šimurina, O., Zahorec, J., & Šereš, Z. (2021). Enhancing rheological performance of laminated dough with whole wheat flour by vital gluten addition. In LWT (Vol. 138, p. 110604). Elsevier BV. https://doi.org/10.1016/j.lwt.2020.110604

23. Lante, A., Canazza, E., & Tessari, P. (2023). Beta-Glucans of Cereals: Functional and Technological Properties. In Nutrients (Vol. 15, Issue 9, p. 2124). MDPI AG. https://doi.org/10.3390/nu15092124

24. Mohebbi, Z., Homayouni, A., Azizi, M. H., & Hosseini, S. J. (2017). Effects of beta-glucan and resistant starch on wheat dough and prebiotic bread properties. In Journal of Food Science and Technology (Vol. 55, Issue 1, pp. 101–110). Springer Science and Business Media LLC. https://doi.org/10.1007/s13197-017-2836-9

25. Torbica, A., Mocko Blažek, K., Belović, M., & Janić Hajnal, E. (2019). Quality prediction of bread made from composite flours using different parameters of empirical rheology. In Journal of Cereal Science (Vol. 89, p. 102812). Elsevier BV. https://doi.org/10.1016/j.jcs.2019.102812

26. Škrbić, B., Milovac, S., Dodig, D., & Filipčev, B. (2009). Effects of hull-less barley flour and flakes on bread nutritional composition and sensory properties. In Food Chemistry (Vol. 115, Issue 3, pp. 982–988). Elsevier BV. https://doi.org/10.1016/j.foodchem.2009.01.028

27. Andrzej, K. M., Małgorzata, M., Sabina, K., Horbańczuk, O. K., & Rodak, E. (2019). Application of rich in β-glucan flours and preparations in bread baked from frozen dough. In Food Science and Technology International (Vol. 26, Issue 1, pp. 53–64). SAGE Publications. https://doi.org/10.1177/1082013219865379

28. Ortiz de Erive, M., He, F., Wang, T., & Chen, G. (2020). Development of β-glucan enriched wheat bread using soluble oat fiber. In Journal of Cereal Science (Vol. 95, p. 103051). Elsevier BV. https://doi.org/10.1016/j.jcs.2020.103051

29. Kurek, M. A., Wyrwisz, J., Brzeska, M., Moczkowska, M., Karp, S., & Wierzbicka, A. (2018). Effect of different beta-glucan preparation pretreatments on fortified bread quality. In Food Science and Technology (Vol. 38, Issue 4, pp. 606–611). FapUNIFESP (SciELO). https://doi.org/10.1590/fst.06917

30. Abdel-Gawad, A., Youssef, M., Abou-Elhawa, S., & Abdel-Rahman, A. (2018). Different Moisture Contents of Tempered Hull Barley and Hull-Less Barley Grains Prior to Milling 2. Effect on Physical and Sensory Properties of Bread Baked from these Barley Flours. In Journal of Food and Dairy Sciences (Vol. 2018, Issue 0, pp. 77–90). Egypts Presidential Specialized Council for Education and Scientific Research. https://doi.org/10.21608/jfds.2018.77757

31. Gill, S., Vasanthan, T., Ooraikul, B., & Rossnagel, B. (2002). Wheat Bread Quality as Influenced by the Substitution of Waxy and Regular Barley Flours in Their Native and Extruded Forms. In Journal of Cereal Science (Vol. 36, Issue 2, pp. 219–237). Elsevier BV. https://doi.org/10.1006/jcrs.2001.0458

32. Cicero, A. F. G., Fogacci, F., Veronesi, M., Strocchi, E., Grandi, E., Rizzoli, E., Poli, A., Marangoni, F., & Borghi, C. (2020). A Randomized Placebo-Controlled Clinical Trial to Evaluate the Medium-Term Effects of Oat Fibers on Human Health: The Beta-Glucan Effects on Lipid Profile, Glycemia and inTestinal Health (BELT) Study. In Nutrients (Vol. 12, Issue 3, p. 686). MDPI AG. https://doi.org/10.3390/nu12030686

Funds:

This research received no external funding.

Acknowledgments:

We would like to thank you Tina Stokelj providing beta-glucan analyses.

Conflict of Interest:

The authors declare no conflict of interest.

Ethical Statement:

This article does not contain any studies that would require an ethical statement.

Contact Address:

***Boris Kovac**, University of Primorska, Faculty of Health Sciences, Department of dietetics, Polje, 42, 6310 Izola, Slovenia,
Tel.: 00386 56626465
E-mail: boris.kovac@fvz.upr.si
ORCID: https://orcid.org/0009-0008-0033-3450

Andreja Bregar, Mlinotest d.d., Quality department, Tovarniška, 14., 5270, Ajdovščina, Slovenia,
Tel.:00386 53644500
E-mail: andreja.bregar@mlinotest.si
ORCID: https://orcid.org/0009-0001-6896-5517

Corresponding author: *