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Lion's Mane (*Hericium erinaceus* (Bull.) Pers.) as a functional component for wheat bread production: influence on physicochemical, antioxidant, and sensory properties

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Abstract. Given the high global consumption of wheat bread, a significant challenge is to improve its nutritional and health value. The aim of the study was to evaluate the possibility of using Lion's Mane for fortifying wheat bread. The impact of Lion's Mane addition at levels of 3, 6, 9, and 12% to wheat flour on the farinographic properties of dough, physicochemical characteristics, quality parameters, and sensory attributes of bread were analyzed. The results showed that the Lion's Mane addition improved water absorption and stability of the dough and reduced softening, positively influencing the dough and bread yields while slightly reducing the bread volume. Importantly, the Lion's Mane fortification significantly boosted the nutritional profile of the bread, particularly the polyphenol content (from 0.13 to 2.24 mg GAE g^{-1} d.m.) and antioxidant activity, providing a promising approach for bakery product enhancement. The research demonstrates the significant potential of Lion's Mane to be used for fortification of bakery products.

Keywords: medicinal mushrooms, bakery products, dough consistency, nutritional value, antioxidant capacity

1. INTRODUCTION

The contemporary interest in mushrooms arises not only from their nutritional and culinary importance but also from their beneficial impact on health and scientifically confirmed therapeutic properties. Some mushroom species, *e.g.* Lion's Mane (LM), Reishi, Chaga, Turkey Tail, Shiitake, and Cordyceps, are referred to as medicinal mushrooms and are gaining popularity as dietary supplements and sources of bioactive substances (Łysakowska *et al.*, 2023; Venturella *et al.*, 2021). Medicinal mushrooms are a rich source of bioactive compounds, including (1,3) (1,6)-β-D-glucans, triterpenes, active peptides, polyphenols, sterols, and mine-ral components (Landi *et al.*, 2022; Sousa *et al.*, 2023; Thongbai *et al.*, 2015; Villares *et al.*, 2012), which exhibit immunomodulatory, anticancer, antioxidant, and antidiabetic properties (Elkhateeb *et al.*, 2019; Panda and Luyten, 2022; Song *et al.*, 2020). In recent studies, fortification of wheat bread with *Ganoderma lucidum* (Reishi) has been shown to improve its nutritional value, notably increasing the dietary fiber and mineral content while influencing the bread volume and sensory attributes (Łysakowska *et al.*, 2024). A similar approach could be applied to Lion's Mane fortification potentially enhancing the antioxidant properties and nutritional value of bakery products.

Hericium erinaceus (Bull.) Pers., known colloquially as Lion's Mane, Yamabushitake, or Hóutóugū, has long been used in traditional Chinese medicine (Blagodatski *et al.*, 2018; Gong *et al.*, 2020). The dried mushroom contains 7.03% of water, 57.0% of carbohydrates, 22.3% of protein, 3.5% of lipids, 3.3-7.8% of dietary fiber, and 7.1% of ash (Łysakowska *et al.*, 2023). The predominant

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polysaccharide in Lion's Mane is (1-3), (1-6)-β-glucan (Ma *et al.*, 2021). Additionally, this mushroom contains other active polysaccharides and glycoproteins, polyphenols, steroids, alkaloids, lactones, monounsaturated fatty acids, and essential amino acids (Friedman, 2015; Ghosh *et al.*, 2021). Numerous studies have shown that Lion's Mane exhibits anti-inflammatory properties, thereby acting against inflammatory processes, delaying aging, and preventing the development of many diseases such as cardiovascular diseases, cancer, and diabetes (Anusiya *et al.*, 2021; Pandita and Pandita, 2023; Phan *et al.*, 2015; Singh *et al.*, 2022). The presence of hericenones and erinacines in Lion's Mane fruiting bodies stimulates the production of nerve growth factor (NGF) in the brain and thus limits the development of neurodegenerative diseases, *e.g.* Alzheimer's and Parkinson's diseases, and reduces the occurrence of anxiety and depression (Chong *et al.*, 2020). Recent studies have demonstrated that the aforementioned active substances reduce the cytotoxicity of β-amyloid (Aβ) and protect nerve cells from death induced by oxidative stress or endoplasmic reticulum stress (Szućko-Kociuba *et al.*, 2023; Włodarczyk *et al.*, 2020).

Due to the beneficial effects of Lion's Mane on health, attempts have been made to enrich various products, including durum wheat pasta, with this mushroom (Szydłowska-Tutaj *et al.*, 2023). However, there are still no data in the literature regarding the possibility of using Lion's Mane for fortifying wheat bread, which is one of the most commonly consumed grain products (Miettona *et al.*, 2022). Wheat bread, produced from low-extraction refined flour, has limited nutritional value due to its high content of digestible carbohydrates, mainly starch (80.3% d.m.), and relatively low content of protein (13.16% d.m.), dietary fiber (6.77% d.m.), mineral elements (0.72% d.m.), and biologically active compounds (Goel *et al.*, 2021; Wirkijowska *et al.*, 2023; Bredariol *et al.*, 2020). Introducing a mushroom component into the recipe of wheat bread offers the possibility of obtaining a product with functional properties, but the added component may have a negative impact on the physicochemical properties and sensory quality of the product.

The aim of the present research was to evaluate the effect of adding Lion's Mane powder on the rheological parameters of dough, the baking value of flour, and the physicochemical properties and sensory quality of bread, including its antioxidant potential. The fortification of wheat bread with Lion's Mane mushroom may offer an interesting and promising alternative to traditional additives used in baking.

2. METHODOLOGY

2.1. Materials and methods

2.1.1. Raw materials

Wheat flour type 750 produced by Polskie Młyny (Warsaw, Poland) was used in this study. Its characteristics included ash content of 0.74% d.m., wet gluten content of 27.5% ± 1.0, gluten index of 99.0 ± 0.3, falling number 304 s ± 6, and an average particle size equivalent to 0.12 mm.

As a substitute for wheat flour, powdered fruiting body of Lion's Mane (LM) was used (NatVita, Mirków, Poland). The fruiting body was ground into powder with a particle size of 200-300 μm, and each batch underwent microbiological and physicochemical testing before being incorporated, including analyses for heavy metal content and microbial contamination. This mushroom was chosen given its high quality confirmed by information about the certification, expiry date, and country of origin.

Both the flour and the mushroom powder were carefully stored in dark airtight containers, maintaining a temperature below 25°C and relative humidity in the range of 60-65%. These precise storage conditions were maintained both before the baking process and before the chemical analysis of the raw materials and bread.

2.1.2. Chemical reagents

H_2SO_4, HCl, K_2SO_4, H_3PO_4, $K_2S_2O_8$, $AlCl_3$ methyl red, Quercetin, Folin-Ciocalteu reagent, DPPH solution, ABTS solution, MES, TRIS (Sigma-Aldrich, Germany); $CuSO_4$, HNO_3 (Honeywell, USA); NaOH (WARCHEM, Poland), H_3BO_3, CH_3COONa (Alfa Aesar, USA); hexane, H_2O_2 (Thermo Fisher Scientific, USA); Na_2SO_4, Na_2CO_3 (Merck, Germany); Total Dietary Fiber Assay Kit (Megazyme, Ireland), Ethanol, Acetone (STANLAB, Poland); gallic acid (Cayman Chemical, USA).

2.2. Farinographic characteristics of dough

Various farinographic parameters of wheat flour type 750 and wheat flour partially substituted with 3, 6, 9, and 12% of powdered Lion's Mane (LM) mushroom were analyzed. In this study, the LM powder was used to replace a portion of the wheat flour, as shown in Table 1. Specifically,

Table 1. Bread ingredients expressed in percentages

Bread samples	Quantity (%)				
	Flour	Powdered (LM)	Water	Yeast	Salt
CON	100	–	58.4	3	1.5
BS3	97	3	59.2		
BS6	94	6	60.6		
BS9	91	9	63.1		
BS12	88	12	66.7		

CON – wheat bread without additives; BS3, BS6, BS9, BS12 – wheat bread with the addition of 3, 6, 9, and 12% Lion's Mane, respectively.

the wheat flour was substituted by the LM powder at levels of 3, 6, 9, and 12% by weight of the total flour content. These substitutions reduced the amount of the wheat flour proportionally.

The analyzed farinographic parameters included dough development time (DDT), water absorption (WA), stability time (ST), degree of dough softening (DS), and farinograph quality number (FQN). Measurements were conducted using a Farinograph-E (Brabender, model 8110142, Duisburg, Germany) according to the AACC 54-21 procedure. Each parameter was measured three times in each sample to ensure accuracy.

2.3. Bread production process

Five bread recipes were prepared with different proportions of wheat flour, including a control bread (CON) made from wheat flour only, and breads enriched with powdered LM mushroom at 3, 6, 9, and 12% by weight of wheat flour, designated BS3, BS6, BS9, and BS12, respectively (as shown in Table 1, which outlines the full recipe proportions).

The full bread recipe included 600 g of wheat flour or a mixture of wheat flour with powdered LM mushroom, 9 g (1.5%) of table salt, 18 g (3%) of pressed yeast (*Saccharomyces cerevisiae*), and the amount of water precisely determined based on the farinographic water absorption (WA) measured at a consistency of 500 Brabender units. This approach allowed precise monitoring of the impact of the ingredient ratios on the final bread characteristics.

To prepare the bread, the methodology described by Wirkijowska *et al.* (2020) was employed. The bread dough manufacturing process followed a one-phase method. Initially, the ingredients were combined in a BEAR Varimixer Teddy 5 L device (Varimixer A/S, Copenhagen, Denmark), first at a low speed for about 3 minutes and then at a higher speed until complete gluten development, as indicated by farinographic analysis. The fermentation process took place in a proofing chamber (Tefi Klima pro 100, Debag, Germany) at 30°C and 85 ± 2% relative humidity for 90 min. After 60 min, an intermediate dough punching step was performed, after which the dough was divided into portions weighing 290 g ± 5 g. Subsequently, the portions were manually shaped and placed in baking molds with dimensions of 18 × 7.5 × 7.0 cm. Three loaves were prepared for each bread variant.

The dough was left for 30 min in the proofing chamber maintained at 30°C and 85 ± 2% relative humidity. The fermentation time was monitored for each sample. The fermented dough was then baked in a bakery oven (Helios pro 100, Debag, Germany) at 230°C for 30 min. After baking, the loaves were allowed to cool for 1 hour at room temperature; then, they were individually placed in polyethylene bags and stored in room conditions (20°C, 50% humidity) before proceeding to the quality assessment. This protocol ensures the reliability of the research process and a comprehensive analysis of bread characteristics.

2.4. Evaluation of bread quality

Five hours post-cooling, the bread characteristics were analyzed, including bread yield assessment calculated using Eq. (1), crust loss determined by Eq. (2), bread volume measured with the mustard seed displacement method (AACC Method 10-05.010), specific volume (cm^3 100 g^{-1}) calculated as the ratio of bread volume to its mass, and crumb moisture content determined according to AACC Method 44-15.02. All analyses were conducted in triplicate:

$$BY = \frac{W_1}{W_2}\,100\%, \tag{1}$$

$$BL = \frac{W_3 - W_1}{W_3}\,100\%. \tag{2}$$

In the equations, BY represents the bread yield, W_1 denotes the mass of the baked bread (measured at 1 h after removal from the oven chamber), W_2 is the mass of the flour used for a specific loaf of bread; BL stands for baking loss, and W_3 is the mass of the dough weighed directly before being placed in the oven.

2.5. Porosity of bread

The bread crumb porosity was analyzed using a digital microscope VHX-7000N (Keyence Corporation, Osaka, Japan) at a magnification of x 2000. The analysis of the porous structure involved an assessment of the number and size of voids and their uniformity throughout the internal space of the bread. The results of the porosity measurements served as a significant indicator of bread quality, facilitating the assessment of its texture and consistency.

2.6. Evaluation of bread colour parameters

The colour parameters of the bread were evaluated following the methodology described by Wirkijowska *et al.* (2023). The measurement of the breadcrumb colour was conducted using a spherical spectrophotometer (Chroma Meter CR 5, Konica Minolta, Sakai Osaka, Japan), and the L^*, a^*, and b^* values were expressed in the CIE Lab colour space. L^* values indicate colours from black to white (0-100), a^* values express redness when positive and greenness when negative, and b^* values indicate yellowness when positive and blueness when negative. The spectrophotometer was calibrated using white and black standard tiles, and measurements were performed 10 times for each sample. The total colour difference (ΔE^*) between the control sample and the fortified bread loaves was calculated according to Eq. (3). Additionally, the whiteness index (WI), yellowness index (YI), and browning index (BI) were calculated for each bread sample from the L^*, a^*, and b^* values using Eqs (4), (5), and (6), respectively:

$$\Delta E^* = \sqrt{(L_c^* - L_i^*)^2 + (a_c^* - a_i^*)^2 + (b_c^* - b_i^*)^2}, \quad (3)$$

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^2 + b^2}, \quad (4)$$

$$YI = 142.83 \cdot \frac{b^*}{L^*}, \quad (5)$$

$$BI = \frac{100\,(x-0.31)}{0.17}, \quad (6)$$

where: L_c^*, a_c^*, b_c^* – represent the values for the control sample (CON); L_i^*, a_i^*, b_i^* – represent the values for tested samples enriched with (LM) mushroom; *WI* – whiteness index, *YI* – yellowness index, *BI* – browning index.

The Nix Colour Sensor program was used to analyze the *L**, *a**, and *b** values. The *L**, *a**, *b** values were inputted into the program, which used them to conduct colour conversion. The conversion process was based on mathematical algorithms that analyze colour parameters and transform them into appropriate values representing the hue of the colour. Consequently, the program generated the final colour hue.

2.7. Texture profile analysis (TPA) of bread

The study involved two mechanical tests to evaluate the texture of the bread crumb and the strength of the crust. All analyses were conducted using the Zwick/Roell Z0.5 strength testing machine (BT1-FR0.5TN.D14, Ulm, Germany) with a maximum force of 500 N.

For the texture profile analysis (TPA) of the crumb, the top portions of the loaves were removed, and each loaf was sliced into 20 mm wide pieces. From these slices, samples in the form of rectangular prisms with dimensions of 30 × 30 × 20 mm were extracted for further analysis. The double compression test was carried out using a flat cylindrical disc with a diameter of 100 mm at a constant head speed of 1 mm s^{-1}. The samples were compressed to 50% of their initial height. The force-deformation curves obtained from this test were used to determine several key parameters, including hardness (N), elasticity (-), cohesiveness (-), and chewiness (N). The TPA measurements were conducted 24 and 48 h after baking, with each sample being measured seven times to ensure accuracy and repeatability.

In addition to the crumb analysis, the strength of the bread crust was determined using a puncture test. A flat penetrometer with a diameter of 5 mm was used to puncture the crust in the upper part of the loaf. The entire bread loaf was placed on a stationary base, and the test was conducted at a speed of 1 mm s^{-1} until crust failure occurred. Failure was defined as a 20% drop in the registered force after reaching the maximum value. The result of the test was expressed as the maximum force (N) recorded during the puncture of the crust.

2.8. Chemical analysis of raw materials and bread

The raw materials and bread underwent thorough chemical analyses (moisture, ash, protein, fat, and dietary fiber content) following the AACC and AOAC methods.

For moisture analysis, 3 g samples were subjected to drying using Method AACC 44-15A in a laboratory dryer maintained at a constant temperature of 103°C ± 1°C until a constant mass was achieved.

The ash content was determined using method AACC 08-01. Precisely measured 3 g samples were placed in porcelain crucibles and subjected to combustion in a muffle furnace at 550°C for 7 h. After cooling, the samples were weighed, and the ash content was calculated.

To determine the total protein content, the KjeltecTM 8400 apparatus (Foss Analytical AB, Höganäs, Sweden) was used with automated Kjeltec Auto equipment from Tecator. The nitrogen content was converted to protein using a conversion factor of N × 5.7.

The total fat content was determined following acid hydrolysis, after which continuous extraction was carried out using a Soxtec TM8000 apparatus (Foss Analytical AB, Höganäs, Sweden) with hexane as a solvent. The analyses were conducted three times to ensure reliability of results.

Enzymatic methods (AACC 32-05, AACC 32-21, AOAC 991.43, and AOAC 985.29) were employed to determine the content of total dietary fiber (TDF), insoluble dietary fiber (IDF), and soluble dietary fiber (SDF). The process involved sequential enzymatic digestion of 1 g dry samples using thermostable α-amylase, protease, and amyloglucosidase. The enzymes and analytical procedures were developed by Megazyme International Ireland Ltd. (Wicklow, Ireland).

Digestible carbohydrates were calculated as the difference between 100% and the content of water, protein, fat, dietary fiber, and ash (USDA).

The energy value was measured in kilocalories (kcal) per 100 g of wet bread, using Atwater coefficients. Proteins and carbohydrates contributed 4 kcal g^{-1}, fats provided 9 kcal g^{-1}, and total dietary fiber was calculated at 2 kcal g^{-1}. The entire analysis process ensured a comprehensive assessment of the composition and nutritional value of the bread.

2.9. Determination of the content of mineral elements

In the study, the content of Mg, K, Fe, Zn, Mn, Se, Pb, and Cd was determined using flame atomic absorption spectrometry (FAAS) in accordance with the standard PN-EN ISO 6869:2002. In turn, the chromium content was analyzed using inductively coupled plasma mass

spectrometry (ICP-MS). For phosphorus determination, a spectrophotometric method with the use of a Shimadzu UV-1800 spectrophotometer was employed.

2.10. Extraction of polyphenols from raw materials and bread

The extraction of raw materials and bread was carried out using 70% ethanol. 10 grams of each material were mixed with 90 ml of ethanol and heated in a water bath for 10 hours at a temperature of 40°C, following the methodology described by Kozłowska *et al.* (2015). Subsequently, filtration was conducted to separate the raw material from the solvent using filter paper. The fresh extracts were used to determine the total polyphenol and flavonoid content and antioxidant capacity.

2.11. Total polyphenol and flavonoid content in raw materials and bread

The total polyphenol and flavonoid content was determined following the methodology provided by Krawęcka *et al.* (2022). In brief, the polyphenol content was measured using the Singleton and Rossi method with the Folin-Ciocalteu reagent. A 0.1 ml sample was mixed with the reagent and 20% (w/w) sodium carbonate and incubated for 30 min; the absorbance was measured at 700 nm using a Thermo Spectronic Helios Epsilon (Thermo Electron, Waltham, Massachusetts, USA). Gallic acid (1-150 mg l^{-1}) served as a standard, and the results were expressed as gallic acid equivalents in mg g^{-1} of tested sample.

The total flavonoid content was determined using the method proposed by Quettier-Deleu *et al.* (2000). A 2 ml sample of the extract was mixed with a 5% (w/w) solution of aluminum chloride, and the absorbance was measured at 405 nm after 30 min. Quercetin (0.25-20 mg l^{-1}) was used as a standard, and the results were expressed as quercetin equivalents in µg g^{-1} of tested sample.

2.12. Antioxidant activity of raw materials and bread against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$

An ethanol solution of DPPH at a concentration of 6 x 10^{-5} mol dm^{-3} was prepared and then diluted with ethanol to achieve a value of A = 0.70 at a wavelength of λ = 515 nm. The DPPH solution was stored in a light-free environment at room temperature.

To 1.8 ml of the methanol solution of DPPH with a concentration of 6 x 10^{-5} mol dm^{-3}, 100 µl of the test solution was added and thoroughly mixed. After 30 min, the absorbance was measured at 515 nm. Ethanol was used instead of the test sample as a control. The entire analysis was repeated three times. The radical-scavenging activity (RSA) was expressed as a percentage of DPPH neutralization:

$$RSA\ (\%) = (1 - A_t / A_0)\ \text{x}100, \qquad (7)$$

where: A_0 – absorbance of the control sample at time 0, A_t – absorbance of the test sample after 30 min.

0.1920 g of ABTS and 0.0343 g of potassium persulfate were precisely weighed and then transferred quantitatively to a 50-ml volumetric flask. The solution was kept in a light-free environment for 16 h. After this time, the ABTS solution was diluted with methanol to achieve an absorbance of A = 0.75 at a wavelength of λ = 734 nm.

Subsequently, 100 µl of the test solution was added to 1.8 ml of the ABTS solution with a concentration of 6 x 10^{-5} mol dm^{-3} and thoroughly mixed. Changes in the concentration of ABTS radical cations were determined spectrophotometrically after 30 min incubation with the test extracts. After 30 min, the absorbance was measured at a wavelength of 734 nm. Water was used as a control instead of the test solution. The analysis was conducted in three replications. The radical-scavenging activity (*RSA*) was expressed as the percentage of $ABTS^{\cdot+}$ free radical neutralization using the Eq. (7).

2.13. Sensory analysis

The sensory analysis of the bread was conducted using a five-point scale. The sensory panel consisted of 11 trained panelists selected based on their regular consumption of bread, good health status, and absence of allergic reactions to gluten products. All studies were approved by the Bioethical Commission (Resolution No. UKE/09/2023).

The following attributes were assessed: external appearance, odor, colour, elasticity, porosity, and flavour. The bread samples were mechanically sliced into 1 cm thick slices, coded, and served in random order to ensure the objectivity of the assessment. The tests were carried out in a controlled laboratory environment for sensory evaluation, which complied with ISO 8589:2007 standards regarding lighting, temperature, and humidity to ensure optimal conditions for objective sensory assessment (ISO 8589:2007). The selection, training, and monitoring of the sensory panelists followed the guidelines outlined in ISO 8586:2012 (ISO 8586:2012).

Each panelist was provided with detailed descriptors for each sensory attribute to ensure consistency in evaluations. For example, elasticity was measured by gently pressing the crumb and observing its ability to return to its original shape, while porosity was evaluated by visually examining the size and distribution of air pockets within the crumb. Flavour was assessed after chewing the bread, with particular attention paid to the intensity and balance of taste as well as any lingering aftertaste.

2.14. Statistical analysis

The gathered data underwent meticulous statistical analysis to derive meaningful conclusions. Mean values and their corresponding standard deviations were computed, providing a comprehensive summary of the dataset. Statistical significance was assessed using one-way analysis

of variance with replication (ANOVA). STATISTICA 13 software by Statsoft was employed for the analysis, and a significance level of $p < 0.05$ was adopted.

3. RESULTS AND DISCUSSION

3.1. Farinographic properties of the dough

Farinographic analysis of flour is a crucial tool in optimizing the bread production process, allowing precise selection of dough manufacturing parameters. In the farinographic studies, the influence of the addition of the mushroom component to the wheat flour on its properties, particularly water absorption and dough consistency, was examined. The results of these studies are presented in Table 2.

The farinographic analysis of wheat dough enriched with *Ganoderma lucidum* showed a significant impact of the mushroom on the rheological properties of the dough, such as water absorption capacity and dough stability (Łysakowska *et al.*, 2024). A similar approach was used in this study to assess the parameters of dough fortified with Lion's Mane.

The basic farinographic characteristics of dough obtained from wheat flour and wheat flour with the addition of the fungal component LM are presented in Table 2. The research showed that the incorporation of LM into the flour led to significant changes in dough rheology. With the addition level of 9% or higher, a significant ($p \leq 0.05$) increase in water absorption and extension of dough development time were observed. These results are consistent with the findings reported by Wu *et al.* (2016), who supplemented wheat bread with the pine mushroom (*Sparassis crispa* (Wulf.)). The increase in water absorption was attributed by the authors to the greater disintegration of the gluten network structure, facilitating water absorption by starch granules. Conversely, Nie *et al.* (2019) investigated the water absorption of flour enriched with *Flammulina velutipes* (mushroom) and emphasized that the higher water absorption capability was related to the high dietary fiber content in mushroom powders. Hydroxyl groups present in fiber structures bind water through hydrogen bonds. These conclusions align with the observations shown by Wirkijowska *et al.* (2023), who additionally emphasized that high water absorption resulting from increased dietary fiber content had a positive impact on bread yield and production profitability. The addition of LM had a positive effect on dough stability time (ST), which increased by up to 75 at the 3% fortification with the fungal component. Both the extension of the dough development time (DDT) and the dough ST demonstrate a positive impact of LM on the technological value of flour. The results of farinographic tests clearly indicate an improvement in the technological properties of the dough after the addition of LM, such as increased stability and a higher farinograph quality number (FQN). The farinograph also allows precise determination of water absorption and the optimal water addition needed for the dough, which is crucial for ensuring proper dough consistency and machinability during

Table 2. Water absorption of flour and farinographic parameters of dough

Sample	WA (%)	DDT (min)	ST (min)	DS (FU)	FQN (mm)
CON	$57.9^{a} \pm 1.07$	$2.3^{a} \pm 0.12$	$6.05^{a} \pm 0.15$	$55^{d} \pm 0.9$	$71^{a} \pm 1.16$
BS3	$59.6^{ab} \pm 1.10$	$2.6^{a} \pm 0.15$	$10.6^{c} \pm 0.25$	$27^{c} \pm 0.44$	$107^{b} \pm 1.75$
BS6	$61^{ab} \pm 1.12$	$2.12^{a} \pm 0.3$	$9.65^{b} \pm 0.23$	$28^{c} \pm 0.46$	$102^{b} \pm 1.67$
BS9	$63.1^{bc} \pm 1.16$	$7.08^{b} \pm 0.12$	$10.47^{c} \pm 0.25$	$17^{b} \pm 0.28$	$119^{c} \pm 1.94$
BS12	$66.6^{c} \pm 1.23$	$8.08^{c} \pm 0.13$	$9.67^{b} \pm 0.23$	$14^{a} \pm 0.23$	$114^{c} \pm 1.86$

CON – control sample (100% wheat flour); BS3, BS6, BS9, BS12 – samples enriched with 3, 6, 9, and 12% of Lion's Mane. Data are presented as mean (n = 3) ± standard deviation. Means within the column followed by different letters are significantly different (Tukey test, $p \leq 0.05$). Tested parameters: WA – water absorption, DDT – dough development time, ST – dough stability time, DS – degree of dough softening, FQN – farinograph quality number.

Table 3. Physical properties of bread samples

Sample	Dough yield (%)	Bread yield (%)	Total baking loss (%)	Volume of 100 g of bread
CON	$162.17^{a} \pm 0.5$	$140.78^{a} \pm 1.31$	$13.19^{b} \pm 0.81$	$350.79^{d} \pm 2.69$
BS3	$162.67^{ab} \pm 0.3$	$141.65^{a} \pm 0.6$	$12.92^{ab} \pm 0.37$	$345.55^{cd} \pm 2.33$
BS6	$164.17^{ab} \pm 0.4$	$143.69^{a} \pm 1.33$	$12.47^{ab} \pm 0.81$	$341.08^{c} \pm 2.41$
BS9	$166.17^{ab} \pm 0.3$	$147.52^{b} \pm 1.33$	$11.09^{a} \pm 0.8$	$329.98^{b} \pm 2.35$
BS12	$167.50^{b} \pm 0.3$	$149.83^{b} \pm 0.61$	$10.30^{a} \pm 0.36$	$321.84^{a} \pm 2.05$

CON – control sample (100% wheat flour); BS3, BS6, BS9, BS12 – samples enriched with 3, 6, 9, and 12% of Lion's Mane. Data are presented as mean (n = 3) ± standard deviation. Means within the column followed by different letters are significantly different (Tukey test, $p \leq 0.05$).

Table 4. Analysis of the texture profile of bread crust and crumb

Sample	Crust hardness (N)		Hardness (N)		Springiness		Chewiness (N)		Cohesiveness	
	24 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	48 h
CON	$19.24^{cdB}\pm 1.53$	$2.15^{aA}\pm 0.25$	$2.26^{aA}\pm 0.53$	$0.95^{bA}\pm 0.05$	$0.96^{dA}\pm 0.04$	$2.15^{aA}\pm 0.25$	$2.26^{aA}\pm 0.53$	$0.95^{bA}\pm 0.05$	$0.96^{dA}\pm 0.04$	$15.64^{bA}\pm 1.23$
BS3	$19.94^{dB}\pm 0.74$	$2.33^{abA}\pm 0.33$	$3.00^{aA}\pm 0.37$	$0.93^{bB}\pm 0.03$	$0.90^{cA}\pm 0.05$	$2.33^{abA}\pm 0.33$	$3.00^{aA}\pm 0.37$	$0.93^{bB}\pm 0.03$	$0.90^{cA}\pm 0.05$	$14.26^{abA}\pm 1.54$
BS6	$18.90^{cdB}\pm 1.61$	$3.11^{bA}\pm 0.16$	$4.65^{bB}\pm 0.44$	$0.94^{bB}\pm 0.03$	$0.87^{bcA}\pm 0.02$	$3.11^{bA}\pm 0.16$	$4.65^{bB}\pm 0.44$	$0.94^{bB}\pm 0.03$	$0.87^{bcA}\pm 0.02$	$13.48^{aA}\pm 1.23$
BS9	$16.72^{bcB}\pm 1.77$	$4.82^{cA}\pm 0.49$	$4.85^{bA}\pm 0.45$	$0.91^{bB}\pm 0.05$	$0.84^{abA}\pm 0.01$	$4.82^{cA}\pm 0.49$	$4.85^{bA}\pm 0.45$	$0.91^{bB}\pm 0.05$	$0.84^{abA}\pm 0.01$	$13.32^{aA}\pm 1.40$
BS12	$14.88^{abA}\pm 0.68$	$7.97^{dA}\pm 1.01$	$8.05^{cA}\pm 0.78$	$0.82^{aA}\pm 0.02$	$0.81^{aA}\pm 0.03$	$7.97^{dA}\pm 1.01$	$8.05^{cA}\pm 0.78$	$0.82^{aA}\pm 0.02$	$0.81^{aA}\pm 0.03$	$14.74^{abA}\pm 1.88$

CON – control sample (100% wheat flour); BS3, BS6, BS9, BS12 – bread enriched with 3, 6, 9, and 12% of Lion's Mane. Data are presented as mean (n=7) ± standard deviation. Different lowercase letters (a-d) within the column and different uppercase letters (A-B) within the row indicate statistically significant differences (Tukey test, $p \leq 0.05$).

mixing. This positive impact of LM on the technological value of flour reflects its ability to enhance dough stability and reduce softening. However, the reduction in the bread volume (Table 3) and the firmer texture (Table 4) of bread enriched with LM may stem from the differing impact of this additive on the dough properties and the final product. While the farinograph provides valuable insights into the dough behavior during mixing and shaping, it does not directly measure parameters related to fermentation and gas retention, which are crucial for the final quality of the bread. The results of the farinographic evaluation (DDT, ST, DS) show that a strong gluten network was formed in the dough enriched with the mushroom powder, which can limit dough growth during fermentation and negatively affect the porosity of the bread. Nie *et al.* (2019) recorded an opposite trend when enriching wheat dough with 2.5-15% *Flammulina velutipes* (mushroom powders). This indicates that the species of mushroom used for fortification and its chemical composition are crucial in shaping dough consistency. As suggested by Welc-Stanowska *et al.* (2023), the presence of phenolic acids in the raw material may be significant. The authors demonstrated that hydrogen bonds between the polypeptide chain and phenolic acid functional groups stabilized the gluten network, positively influencing dough consistency and stability. On the other hand, Teterycz and Sobota (2023) emphasize that the presence of mineral elements plays a significant role in shaping dough rheological parameters. As reported by the authors, increased levels of magnesium, potassium, or sodium ions may stimulate interactions between polypeptide chains and induce greater dough resistance to mixing while limiting its softening.

3.2. Evaluation of bread quality characteristics

An important aspect in the bakery technology is the analysis of bread baking parameters, including the determination of dough and bread yields, total baking losses, and bread volume per 100 g.

Table 3 presents the results of the physical properties of bread loaves. The evaluation of the baking parameters showed that both the dough yield and the bread yield increased significantly ($p \leq 0.05$) by approximately 5 and 9% points, respectively, in samples enriched with 12% of the LM powder. The increased bread yield and the reduced baking losses recommend LM as a component enhancing bread production profitability. However, it should be noted

Fig. 1. Samples of wheat bread (CON) and bread (BS) enriched with 3, 6, 9 and 12% Lion's Mane.

that the addition of the LM powder led to a statistically significant decrease ($p \leq 0.05$) in specific bread volume (Table 3, Fig. 1), already noticeable at a fortification level of 6%. Similar trends were observed by Lu *et al.* (2016), who introduced white mushroom and shiitake mushroom powder into wheat bread. Similarly, Skendi *et al.* (2010) reported that incorporation of ß-glucan isolate into wheat flour led to a reduction in specific bread volume with increasing substitution levels. Yuan *et al.* (2017) observed that supplementation of bread with a fungal component has a negative effect on bread volume, mainly by weakening the gluten network crucial for retaining gases generated during fermentation. Enzymes present in fungal powder can hydrolyze proteins and starch in dough, further reducing the elasticity of the gluten network and weakening the dough structure. Incorporation of high-fiber fungal powder, devoid of gluten proteins, seems to contribute to a reduction in dough resistance to extensional stress occurring mainly during dough fermentation. Despite the greater resistance to mechanical stress (higher ST and lower DS, Table 2), dough enriched with a fungal component exhibits reduced gas retention capacity, including carbon dioxide produced during fermentation. Additionally, the altered dough recipe composition may negatively influence the fermentation process, potentially reducing gas production by yeast. As a result, bread with added fungal flour is characterized by lower volume and poorer porosity compared to traditional wheat bread (Fig. 2). Similar results were obtained by Yuan *et al.* (2017), who enriched bread with *Auricularia auricula* (mushroom) supplementation.

3.3. Evaluation of bread colour parameters

Studies on colour parameters are extremely important in assessing bread quality due to their impact on bread perception and consumer acceptance. In this study, the colour of wheat bread crumb with the addition of LM was compared to the colour of the control sample.

The statistical analysis revealed that the addition of LM, even at the level of 6%, significantly ($p < 0.05$) decreased the L^* value (brightness) and darkened the bread (Table 5). Simultaneously, with the increase in the level of wheat flour substitution with the fungal component in the range from 0 to 12%, a significant increase in the a^* (from 0.51 to 7.17) and b^* (from 12.27 to 27.14) values was observed, consequently leading to an increase in the bread browning index (from 22.11 to 77.61) and the yellowness index (from 28.5 to 72.58) with a simultaneous decrease in the whiteness index. The analysis of the total colour difference (ΔE) showed that even the smallest level of the fungal powder addition caused a significant change in the crumb colour. ΔE for sample BS3 (with the lowest fungal powder addition) had a value of 6.07, which was greater than 3, indicating a significant difference in the colour from the control sample, according to Pathare *et al.* (2013).

Typically, the LM fruiting body is white, but over time, the colour may change to yellow or brownish hues. In a study conducted by Kim (2020), the lack of data on the presence of pigments in LM was emphasized. The hypothesis suggests that the change of the fungus colour may be related to the processing into powder. Thermal processing may induce Maillard reactions, leading to the formation of melanoidins ranging from brown to black, which are responsible for the yellow-brown colour of the powder.

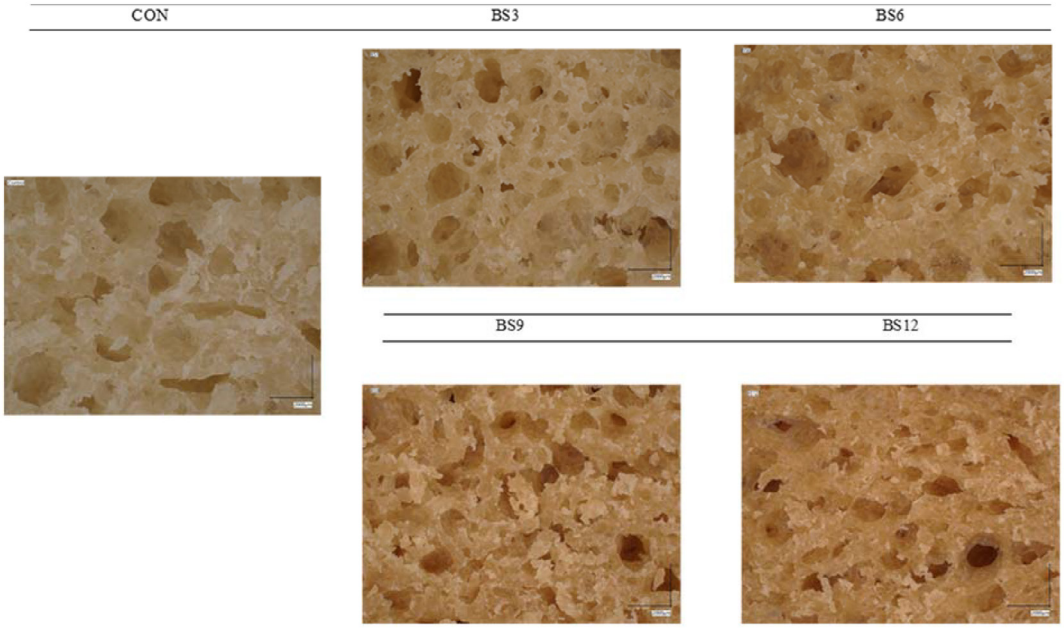

Fig. 2. Porosity of fresh crumb of wheat bread (CON) and bread enriched (BS) with 3, 6, 9 and 12% Lion's Mane.

Table 5. Colour of bread samples

Simple	Colour reading using the Nix Colour Sensor program	*L**	*a**	*b**	Index			
					Browning	Yellowness	White	Δ*E**
CON		61.49^{c} ± 0.58	0.51^{a} ± 0.37	12.27^{a} ± 0.46	22.11^{a} ± 0.98	28.5^{a} ± 1.08	59.58^{c} ± 0.55	–
BS3		60.17^{bc}± 1.07	1.84^{b} ± 0.12	17.96^{b} ± 1.81	36.53^{b} ± 1.51	42.66^{b} ± 1.41	56.26^{c} ± 1.07	6.07^{a} ± 0.57
BS6		57.97^{b} ± 1.2	3.67^{c} ± 0.23	22.97^{cd} ± 1.62	54.28^{c} ± 1.8	57.26^{c} ± 2.1	51.88^{b} ± 5.39	13.13^{b} ± 1.86
BS9		57.07^{b} ± 1.91	5.29^{d} ± 0.46	24.96^{c} ± 0.92	62.23^{d} ± 3.5	62.52^{d} ± 2.44	50.03^{b} ± 1.55	14.45^{b} ± 1.03
BS12		53.43^{a} ± 1.35	7.17^{e} ± 0.28	27.14^{d} ± 1.02	77.61^{e} ± 2.05	72.58^{e} ± 1.34	45.61^{a} ± 0.97	18.28^{c} ± 0.56

CON – control sample (100% wheat flour bread); BS3, BS6, BS9, BS12 – bread enriched with 3, 6, 9, and 12% of Lion's Mane. Colour parameters include: *L** – lightness, *a** – red-green shade, and *b** – blue-yellow shade. Data are presented as mean (n = 10) ± standard deviation. Mean values for the same parameters followed by different letters are significantly different (Tukey test, $p \leq 0.05$).

Losoya-Sifuentes *et al.* (2021) observed a similar effect of mushroom addition on bread colour. They found that the addition of *Pleurotus ostreatus* caused a decrease in the *L** parameter and a significant increase in the *a** and *b** parameters. These changes were attributed to the darker colour of the mushroom powder compared to refined wheat flour and Maillard reactions leading to the formation of melanoidins giving brown colour to bread during baking. Jia *et al.* (2023) further emphasized that, during baking, Maillard reactions and caramelization of sugars inside the loaf are limited due to lower temperature and less intense water evaporation compared to the outer layers of the bread. Therefore, the bread crumb colour is mainly dependent on the colour of raw materials.

In the context of the present research on the addition of LM to bread, it is important to consider whether the introduction of mushrooms may increase the amount of substrates needed for Maillard reactions. Lion's Mane, like many other mushrooms, contains reducing sugars and amino acids, such as lysine, which are key substrates for Maillard reactions. By adding LM to bread, it is possible to provide additional substrates needed for this chemical reaction. Ultimately, this leads to a change in bread colour and the formation of new aroma and flavour compounds, which may have a significant impact on consumer perception and acceptance of the product.

3.4. Texture profile analysis (TPA) of bread

The incorporation of LM into the bread recipe resulted in changes in the textural properties of the crust and crumb. It is noteworthy that the crust hardness decreased with an increase in the LM content (Table 5). However, statistically significant changes were observed only after the application of the 12% addition. During storage, the crust hardness generally decreased, with changes being more pronounced in the variant with the higher LM content. The decrease in crust hardness during storage is likely due to moisture migration from the crumb to the crust (Mironeasa *et al*., 2018).

In turn, crumb hardness increased after the incorporation of LM. The increase in the amount of the LM powder caused further changes in this parameter. Similar trends were observed in a study conducted by Sulieman *et al.* (2018), where the hardness of gluten-free bread crumb increased with the addition of lyophilized *Agaricus bisporus* mushroom powder. The increase in the hardness of wheat bread fortified with mushroom or plant-based powders may result from the inclusion of a raw material that reduces the formation of the gluten network. Typically, bread hardness increases linearly with increasing concentrations of plant additives (Liu *et al.*, 2022; Steffolani *et al.*, 2014; Wirkijowska *et al.*, 2022). The average values of hardness determined at 48 h after baking generally increased. Furthermore, during storage, the crumb hardness changed the most in the bread with the 6% LM addition (by 49.5% compared to the value of the parameter determined at 24 h after baking). The further increase in the LM content did not result in such significant changes.

The highest springiness, indicating the ability to regain shape after deformation, was exhibited by the control bread crumb. With the increasing LM content, the values of this parameter gradually decreased, reaching significantly lower values compared to the control sample ($p \leq 0.05$) only at the 12% LM fortification level. Slightly greater variability was observed for bread resilience after 48 h of storage. The values of this parameter were significantly lower in all breads containing *Hericium erinaceus*. Ulziijargal *et al.* (2013) also observed lower resilience values in bread crumb with the addition of LM compared to wheat bread. However, the data obtained by the authors after long-term

bread storage were interesting. The wheat bread exhibited significantly lower resilience at 6 days after baking than those enriched with *Hericium erinaceus*.

The addition of 3% of LM did not result in significant changes in crumb cohesiveness in our bread (both fresh and after 48 h of storage). Similar conclusions were reached by Ulziijargal *et al.* (2013), who added 5% of mushroom powder, including *Hericium erinaceus*, to the wheat bread recipe. It is also worth noting that, after 48 h of storage, the bread crumb with the addition of the mushroom component exhibited lower cohesiveness than the fresh bread. These changes are primarily associated with bread staling (Esteller *et al.*, 2004). The greatest changes in cohesiveness were observed for breads with the 3-9% LM addition.

Based on the results of statistical analysis, it was found that the fortification with up to 6% LM did not cause significant changes in bread crumb chewiness. Previous studies (Sulieman *et al.*, 2018) confirm that enrichment of bread with LM results in increased crumb chewiness, indicating an increase in the force required to crush the crumb before swallowing. Wirkijowska *et al.* (2023) also demonstrated that incorporation of plant-based materials with high fiber content (69% of dry matter) to wheat bread can increase its chewiness. In their study, a 12% addition of tomato waste significantly affected this characteristic of bread. However, the addition of pepper waste, characterized by dietary fiber content of 33.47% dry matter, did not cause significant changes in bread chewiness.

3.5. Sensory evaluation of bread characteristics

Sensory acceptance and consumer preferences are key determinants of the market success of new food products. Therefore, the developed bread variants underwent a detailed sensory evaluation to determine their acceptance by consumers and identify potential undesired sensory attributes. The results of the sensory evaluation demonstrated that the use of the mushroom component additive in quantities of 3-12% did not significantly deteriorate the external appearance of the bread (Table 6). Particularly, the appearance of samples BS3 and the control (CON) were rated the highest by the panelists (4.45), suggesting that the addition of the LM powder at this level does not negatively impact the visual perception of the product. Similar observations pertain to the evaluation of odor, which was highly rated for all samples, especially for sample BS3 and the control. The study confirmed the lack of a negative impact of the LM powder additive on the aromatic attractiveness of the bread.

In terms of colour, all samples, regardless of the mushroom component content, received a uniform rating (4.27), suggesting a neutral influence of the mushrooms on this sensory parameter. However, concerning elasticity and porosity, a decrease in the rating values was observed with an increase in the mushroom content. This was consistent with the results of bread quality characteristics, including reduced volume per 100 g of bread and decreased crumb porosity. The most significant differences were noticed in the taste category, where the control sample received the highest rating (4.54), while sample BS12 (with the highest mushroom addition) obtained a relatively low rating (2.81). These results indicate that a higher level of substitution with the mushroom component may have an adverse effect on the taste of the bread, which is a crucial aspect in terms of consumer acceptance. Different results were obtained by Ulziijargal *et al.* (2012), who investigated the impact of addition of different mushrooms on the sensory characteristics of bread. The authors showed that substitution of wheat flour with a 5% addition of *Antrodia camphorata, Agaricus blazei, Hericium erinaceus*, and *Phellinus linteus* negatively influenced the colour and appearance of bread but did not have an adverse impact on its taste.

3.6. Chemical composition of raw materials and bread

The chemical analysis of the raw materials and bread indicated statistically significant differences in the nutrient content between the tested samples (Table 7). Wheat flour type 750, which is a traditional raw material in bread production, was characterized by relatively low moisture content (9.4%) and ash content (0.69% d.m.), which is consistent with literature data presented for this type of soft wheat flour (Wirkijowska *et al.*, 2023; Biel *et al.*, 2016; Adhikari *et al.*, 2015). The protein (13.12% d.m.) and crude

Table 6. Results of the sensory evaluation of the bread

Sample	Evaluation parameters					
	Appearance	Smell	Colour	Flexibility	Taste	Overall rating
CON	$4.45^{a} \pm 0.5$	$4.36^{a} \pm 0.57$	$4.27^{a} \pm 0.71$	$4.18^{a} \pm 0.75$	$4.55^{b} \pm 0.42$	4.36
BS3	$4.45^{a} \pm 0.86$	$4.27^{a} \pm 0.88$	$4.36^{a} \pm 0.88$	$4.00^{a} \pm 0.88$	$4.00^{ab} \pm 0.88$	4.22
BS6	$4.18^{a} \pm 0.77$	$3.64^{a} \pm 0.75$	$4.27^{a} \pm 0.50$	$3.64^{a} \pm 0.67$	$3.91^{ab} \pm 0.75$	3.93
BS9	$3.91^{a} \pm 0.74$	$4.18^{a} \pm 0.75$	$4.27^{a} \pm 0.67$	$3.55^{a} \pm 0.86$	$3.18^{ab} \pm 0.57$	3.82
BS12	$4..36^{a} \pm 0.95$	$3.73^{a} \pm 0.88$	$4.27^{a} \pm 0.43$	$3.73^{a} \pm 0.54$	$2.82^{a} \pm 0.86$	3.78

CON – control sample (100% wheat flour bread); BS3, BS6, BS9, BS12 – bread enriched with 3, 6, 9, and 12% of Lion's Mane. Data are presented as mean (n = 11) ± standard deviation. Different letters (a-b) in the column indicate statistically significant differences (Tukey test, $p \leq 0.05$).

Table 7. Chemical composition of raw materials and bread samples

Sample	Moisture (%)	Ash (% d.m.)	Protein	Crude fat	TDF	IDF	SDF	Digestible carbohydrate (CHO)	Calories (kcal 100 g^{-1})
Wheat flour type 750	$9.4^{A}\pm$ 0.1	$0.69^{A}\pm$ 0.01	$13.12^{A}\pm$ 0.04	$0.45^{A}\pm$ 0.03	$5.3^{A}\pm$ 0.13	$2.4^{A}\pm$ 0.1	$2.9^{A}\pm$ 0.2	$71.04^{B}\pm$ 0.03	$351.30\pm$ 0.11^{B}
LM powder	$5.3^{B}\pm$ 0.14	$5.80^{B}\pm$ 0.1	$21.16^{B}\pm$ 0.01	$1.92^{B}\pm$ 0.09	$54.44^{B}\pm$ 0.11	$47.14^{B}\pm$ 0.38	$7.3^{B}\pm$ 0.49	$11.38^{A}\pm$ 0.05	$256.32\pm$ 0.05^{A}
CON	$41.46^{a}\pm$ 0.71	$2.20^{a}\pm$ 0.01	$10.83^{a}\pm$ 0.04	$1.23^{a}\pm$ 0.03	$7.22^{a}\pm$ 0.19	$4.26^{a}\pm$ 0.17	$2.95^{a}\pm$ 0.02	$78.52^{e}\pm$ 0.42	$224.16^{d}\pm$ 0.17
BS3	$42.11^{ab}\pm$ 0.23	$2.35^{b}\pm$ 0.09	$13.62^{b}\pm$ 0.05	$1.28^{ab}\pm$ 0.12	$10.67^{b}\pm$ 0.18	$7.7^{b}\pm$ 0.06	$2.97^{a}\pm$ 0.23	$72.08^{d}\pm$ 0.3	$217.47^{c}\pm$ 0.9
BS6	$43.04^{b}\pm$ 0.30	$2.4^{b}\pm$ 0.08	$13.95^{b}\pm$ 0.0b	$1.33^{ab}\pm$ 0.09	$10.84^{bc}\pm$ 0.36	$7.37^{b}\pm$ 0.49	$3.43^{b}\pm$ 0.13	$71.48^{c}\pm$ 0.24	$213.8^{b}\pm$ 0.8
BS9	$44.52^{c}\pm$ 0.2	$2.58^{c}\pm$ 0.08	$16.25^{c}\pm$ 0.09	$1.38^{bc}\pm$ 0.1	$11.8^{c}\pm$ 0.46	$8.4^{b}\pm$ 0.72	$3.41^{b}\pm$ 0.26	$67.99^{b}\pm$ 0.2	$206.93^{ab}\pm$ 0.16
BS12	$45.12^{c}\pm$ 0.15	$2.78^{d}\pm$ 0.4	$16.46^{c}\pm$ 0.08	$1.44^{c}\pm$ 0.07	$13.27^{d}\pm$ 0.8	$9.34^{c}\pm$ 0.80	$3.93^{c}\pm$ 0.01	$66.05^{a}\pm$ 0.22	$202.80^{a}\pm$ 0.2

CON – control sample (100% wheat flour); BS3, BS6, BS9, BS12 – bread enriched with 3, 6, 9, and 12% of Lion's Mane (LM); CHO – digestible carbohydrates (by difference). Data are presented as mean (n = 3) ± standard deviation. Different letters in the column indicate statistically significant differences between the results for raw materials (A-B) and bread samples (a-d) (Tukey test, $p \leq 0.05$).

fat (0.45% d.m.) content as well as the dietary fiber content (5.3% d.m.) divided into soluble (2.9% d.m.) and insoluble (2.4% d.m.) fractions indicate a chemical composition typical for wheat flour (Capuano *et al.*, 2017; Gill *et al.*, 2021).

The LM powder was characterized by relatively low moisture content (5.8% d.m.), typical for dried mushroom products (Łysakowska *et al.*, 2023). The high content of ash (5.8% d.m.), protein (21.16% d.m.), and dietary fiber (TDF) (54.44% d.m.), with a predominant insoluble fraction (IDF) (47.14% d.m.), confirm the high nutritional potential of LM. Dimopoulou *et al.* (2022) highlight the high protein content in *Hericium erinaceus*, which has been reported by these authors to be around 19.9% d.m.

The analysis of the chemical composition of the LM-supplemented bread revealed an increase in bread moisture from 41.46% in the control sample to 45.12% in sample BS12 (Table 6). This trend may result from the higher content of dietary fiber in the LM-enriched samples, which is characterized by high water absorption. Both the increased water content introduced into the dough and the lower losses during baking determine the higher final moisture content of the product. The increase in the ash content from 2.20% d.m. in CON to 2.78% d.m. in BS12 indicates higher mineral content in bread enriched with *Hericium erinaceus*. The addition of LM also led to an increase in protein content in the bread. Products with the 12% addition (BS12) were characterized by a 52% increase in the protein content compared to the control sample (CON). The LM-enriched bread also had significantly higher total dietary fiber content (TDF), including both insoluble (IDF) and soluble (SDF) fractions. At the 12% level of wheat flour substitution with the mushroom component, the TDF and IDF content in the bread increased approximately 2-fold, while the SDF content increased 1.3 times. The crude fat content in the bread was relatively low, ranging from 1.23 to 1.44% d.m. With the increase in the content of the analyzed macronutrients, a decrease in digestible carbohydrates from 78.52% d.m. in CON to 66.05% d.m. in BS12 was observed, accompanied by a decrease in the calorie content in the bread from 224.16 kcal 100 g^{-1} in CON to 202.80 kcal 100 g^{-1} in BS12.

3.7. Mineral composition

Mushrooms can be a good source of minerals in the human diet (Haro *et al.*, 2020). As bioaccumulators, they have the ability to concentrate macroelements (*e.g.* potassium, calcium, phosphorus, and magnesium), trace elements (*e.g.* iron, chromium, zinc, manganese, copper, and cobalt), and heavy metals (*e.g.* lead and cadmium). Such factors as mushroom species, growth stage, fruiting body morphology, and environmental conditions significantly influence the mineral profile of mushroom fruiting bodies (Gençcelep *et al.*, 2009; Mallikarjuna *et al.*, 2013). Table 8 shows the content of selected minerals, *i.e.* P, Mg, K, Fe, Zn, Mn, and Se, and heavy metals (Pb, Cd) in the mushroom powder, wheat flour, and bread. Additionally, recommended dietary allowances (RDA) values resulting from the consumption of 100 g of the product were calculated and adequate intake (AI)

Table 8. Content of selected minerals in raw materials and bread samples

Sample	Macroelements			Microelements				Heavy metals	
	P	Mg	K	Fe	Zn	Mn	Se	*Pb	*Cd
				Content (mg 100 g^{-1})					
Wheat flour type 750	131^{A}±31	22.3^{A}±0.1	171.0^{A}±0.5	2.50^{A}±0.08	0.704^{A}±0.014	0.825^{A}±0.07	0.0112^{A}±0.0003		
LM powder	412^{B}±68	48.0^{B}±0.2	2440.00^{B}±11	6.76^{B}±0.12	1.68^{B}±0.08	1.11^{B}±0.18	0.0121^{A}±0.0005		
CON	89.4^{a}±2.2	13.5^{a}±0.1	121^{a}±6	1.36^{a}±0.05	0.537^{a}±0.007	0.483^{a}±0.07	0.0081^{a}±0.0001	ND	
BS3	94.1^{ab}±2.4	14.9^{b}±0.1	153^{b}±5	1.44^{b}±0.06	0.544^{ab}±0.006	0.503^{ab}±0.24	0.0076^{a}±0.0002		
BS6	98.6^{bc}±1.2	15.6^{c}±0.1	194^{c}±8	1.47^{b}±0.08	0.555^{b}±0.002	0.506^{ab}±0.35	0.0076^{a}±0.0002		
BS9	101.3^{cd}±1.7	15.9^{d}±0.1	234^{d}±11	1.61^{c}±0.04	0.628^{c}±0.005	0.500^{ab}±0.36	0.0077^{a}±0.0003		
BS12	103.0^{d}±2.0	16.1^{c}±0.1	283^{c}±5	1.62^{c}±0.02	0.674^{d}±0.001	0.614^{b}±0.27	0.0079^{a}±0.0004		
RDA/AI (mg day^{-1})	700	375	2 000	14	10	2	0.055	0.428	0.06
				% RDA/AI					
CON	12.74	3.6	6.05	9.71	5.37	24.15	14.72		
BS3	13.44	3.97	7.65	10.28	5.44	25.15	13.81	–	
BS6	14.09	4.16	9.70	10.5	5.55	25.3	13.81	–	
BS9	14.47	4.24	11.7	11.5	6.28	25	14	–	
BS12	14.71	4.29	14.2	11.57	6.74	30.7	14.36	–	

CON – control sample (100% wheat flour bread); BS3, BS6, BS9, BS12 – bread enriched with 3, 6, 9, and 12% of Lion's Mane (LM). RDA – recommended dietary allowances, AI – adequate intake. *Daily allowable dose (WHO). Data are presented as mean (n = 3) ± standard deviation. Means in the same column (raw material or sample) with different letters are significantly different (Tukey test, $p \leq 0.05$). The mineral elements are presented for dry mass in the case of raw materials and for wet basis in the case of baked bread.

values are presented. It is recognized that the most common deficiencies in mineral nutrients in the human diet include iron and magnesium (Montowska *et al.*, 2019).

The wheat flour used as a traditional bread ingredient exhibited relatively low concentrations of such elements as phosphorus (P) (131 mg 100 g^{-1}), magnesium (Mg) (22.3 mg 100 g^{-1}), potassium (K) (171 mg 100 g^{-1}), iron (Fe) 2.5 mg 100 g^{-1}), zinc (Zn) 0.704 mg 100 g^{-1}), manganese (Mn) 0.825 mg 100 g^{-1}), and selenium (Se) 0.0112 mg 100 g^{-1}. Heavy metals, such as cadmium (Cd) and lead (Pb), were not identified in this raw material (Table 8). Similar mineral contents in flour were reported by Martínez-Martín *et al.* (2023).

In comparison to the flour, the LM powder showed significantly higher concentrations of phosphorus (412 mg 100 g^{-1}), magnesium (48 mg 100 g^{-1}), potassium (2440 mg 100 g^{-1}), iron (6.76 mg 100 g^{-1}), zinc (1.68 mg 100 g^{-1}), manganese (1.11 mg 100 g^{-1}), and selenium (0.0121 mg 100 g^{-1}), suggesting its potentially beneficial influence on enriching bread with these mineral elements. Compared to the raw materials, the concentration of minerals in 100 g of the bread was lower due to the higher moisture content, but the analysis of the control bread (CON) and bread enriched with various concentrations of the mushroom (BS3, BS6, BS9, BS12) revealed significant differences ($p>0.05$) in the mineral content. The increase in the level of fortification with the mushroom component was accompanied by a gradual increase in the mineral content. BS12 was characterized by the highest levels of phosphorus (103 mg 100 g^{-1}), magnesium (16.1 mg 100 g^{-1}), potassium (283 mg 100 g^{-1}), iron (1.62 mg 100 g^{-1}), zinc (0.674 mg 100 g^{-1}), manganese (0.614 mg 100 g^{-1}), and selenium (0.0079 mg 100 g^{-1}). The enriched bread (BS3, BS6, BS9, BS12) covered RDA/AI for most minerals to a significantly greater extent. For example, in BS12, the percentage coverage of RDA/AI for potassium increased from 6.05 to 14.2%. However, the most significant increase in the percentage coverage of RDA/AI was observed for such minerals as manganese, phosphorus, and zinc. The significant increase in the mineral content in the enriched bread may have a significant impact on the nutritional value of the product. Particularly important is the increase in the levels of such minerals as iron and potassium, which play a crucial role in many physiological processes and are often deficient in typical diets (Jamova *et al.*, 2022).

Table 9. Polyphenol content and antioxidant activity of raw materials and bread samples

Sample	Flavonoids (mg QE g^{-1} d.m.)	Polyphenols (mg GAE g^{-1} d.m.)	Radical scavenging activity against (%)	
			$ABTS^{\cdot+}$	$DPPH^{\cdot}$
Wheat flour type 750	ND	$0.13^{A}\pm0.03$	$30.32^{A}\pm0.9$	$45.02^{A}\pm0.8$
(LM) powder	0.85 ± 0.05	$11.42^{B}\pm0.1$	$96.08^{B}\pm1.1$	$98.83^{B}\pm1.3$
CON	ND	$0.46^{a}\pm0.02$	$30.12^{a}\pm0.8$	$42.84^{a}\pm0.3$
BS3	ND	$0.87^{bc}\pm0.03$	$41.32^{b}\pm0.82$	$51.06^{b}\pm0.8$
BS6	ND	$0.98^{c}\pm0.02^{b}$	$52.32^{c}\pm0.34$	$54.69^{c}\pm0.72$
BS9	$0.01^{A}\pm0.01$	$1.16^{d}\pm0.05$	$60.88^{d}\pm0.2$	$57.38^{e}\pm0.35$
BS12	$0.02^{A}\pm0.01$	$2.24^{e}\pm0.08$	$76.77^{e}\pm0.24$	$60.36^{e}\pm0.24$

CON – control sample (100% wheat flour bread); BS3, BS6, BS9, BS12 – bread enriched with 3, 6, 9, and 12% of Lion's Mane (LM). Data are presented as mean (n = 3) ± standard deviation. Means in the same column followed by different letters are significantly different (Tukey test, $p \leq 0.05$).

3.8. Polyphenol content and antioxidant activity

Wheat flour type 750 exhibited polyphenol content of 0.13 mg GAE g^{-1} d.m. with antioxidant activity measured by its ability to inhibit the cation radicals $ABTS^{\cdot+}$ and $DPPH^{\cdot}$ at 30.32 and 45.02%, respectively. In comparison to the wheat flour, the LM mushroom powder had significantly higher total polyphenol content (0.13 *vs*. 11.42 mg GAE g^{-1} d.m.) and antioxidant activity reaching 96.08 and 98.83% against $ABTS^{\cdot+}$ and $DPPH^{\cdot}$, respectively. The results presented in Table 9 indicate a markedly greater antioxidant potential of LM mushroom compared to traditional wheat flour. Therefore, the incorporation of the LM mushroom powder into bread at levels ranging from 3% to 12% substantially increased the polyphenol content and antioxidant activity of the product. The polyphenol content increased from 0.87 mg GAE g^{-1} d.m. in the bread with the 3% addition (BS3) to 2.24 mg GAE g^{-1} d.m. in the bread with the 12% addition of the mushroom component (BS12). Concurrently, the antioxidant activity of the bread increased, reaching a maximum value of 76.77% and 60.36 RSA against $ABTS^{\cdot+}$ and $DPPH^{\cdot}$, respectively, at the highest level of mushroom substitution (BS12). The addition of the mushroom component, which is rich in phenolic compounds, significantly boosted the antioxidant activity of the bread. The presence of hydroxyl groups (-OH) in the structure of phenolic acids may be a source of the antioxidant activity of (LM) mushrooms (Chang *et al.*, 2021). Similar research findings were reported by Liu *et al.* (2022), who introduced powder from *Pleurotus eryngii* and *Cantharellus cibarius* mushrooms into bread. Breads enriched with the mushroom components exhibited higher total polyphenol content (TPC), suggesting a positive influence of these mushrooms on the antioxidant properties of the bread. The increase in TPC was not directly proportional to the amount of added powder, which may be due to partial degradation of polyphenols during bread baking. Therefore, introduction of medicinal mushrooms into bread production offers the possibility of creating functional products with health-promoting properties and high antioxidant potential (Liu *et al.*, 2022).

4. CONCLUSIONS

The present results provide evidence for the potential use of Lion's Mane (LM) mushrooms to enrich wheat bread. The incorporation of the mushroom component at the levels from 3 to 12% positively influenced the farinographic parameters of the dough, increasing water absorption, development time, stability, and Farinograph Quality Number, while reducing dough softening. The addition of LM mushrooms may help to optimize the bread production process by increasing dough and bread yields and reducing total baking loss. The textural properties of the crumb, such as hardness, chewiness, and cohesiveness, did not significantly change with additions up to 6%, and the sensory evaluation results (overall rating) indicated that even the 12% addition of the LM mushrooms yielded sensory acceptable products (overall rating >3.5). The increase in the LM mushroom content in the bread was associated with a change in crumb colour towards darker, more yellowish, and brownish hues. The chemical composition analysis demonstrated that bread supplemented with the mushroom was richer in protein, dietary fiber (IDF and SDF), and mineral elements, enhancing its nutritional value. Additionally, it was characterized by higher polyphenol content and greater antioxidant activity, which may be potentially important in the prevention of *e.g*. cardiovascular diseases and cancer.

In summary, the present results indicate that powdered LM mushroom can significantly improve the nutritional profile and antioxidant properties of wheat bread while maintaining its sensory acceptability. These studies open up new perspectives for the baking industry in terms of the production of functional products with the addition of medicinal mushrooms. The incorporation of mushroom

components, rich in biologically active compounds, into such a popular food product as wheat bread may contribute to improvement of the overall health status of the population and play a significant role in the prevention and treatment of non-communicable diet-related diseases.

CRediT author statement:

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