



Physicochemical and digestive properties of low-sodium bread enriched with *Agaricus bisporus* and *Pleurotus ostreatus* co-products

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ABSTRACT

The work explored the use of *Agaricus bisporus* (ABSF) and *Pleurotus ostreatus* (POSF) co-products as functional ingredients to partially or fully replace NaCl in bread (eight formulations, 0.5–6%; 50–100% replacement). Chemical, physicochemical, sensory, and *in vitro* digestion analyses were performed. The bread with ABSF (BAB) and POSF (BPO) showed higher protein, dietary fiber, K and Ca content than control bread, with sodium reduced from 2164.75 mg/100 g to 59.70 mg/100 g. POSF reduced the specific volume in breads in a dose-dependent manner; bread with 6% POSF showed lower specific volume, higher hardness, and reduced overall sensory acceptability. Protein digestibility reached ~85% across breads and bread with 3% POSF showed the lowest predicted glycemic index. Mg and K bioaccessibility decreased, indicating that strategies to improve mineral bioaccessibility are needed. In conclusion, mushroom co-products, can reduce sodium, and support public health and circular economy strategies.

1. Introduction

Edible mushrooms are rich in bioactive compounds, including β -glucans, ergosterol, ergothioneine, phenolic compounds, terpenoids, and polysaccharide–protein complexes (Cirlincione et al., 2026; Ramos et al., 2019; Umaña et al., 2020; Valchev, 2020). These attributes, coupled with their high-quality protein and distinct umami flavor, have driven a steady expansion in global production, reaching a market value of approximately US\$ 46.5 million in 2022. (Food and Agriculture Organization of the United Nations, 2023; Ramos et al., 2019). While species such as *Agaricus bisporus*, *Lentinula edodes*, and the *Pleurotus* genus (including *P. eryngii*, *P. pulmonarius*, and *P. sajor-caju*, but especially *P. ostreatus*) dominate 27% of the market, their commercialization generates significant volumes of co-products (Raman et al., 2021; Wang et al., 2021). These include spent mushroom substrate, stems, and specimens that do not meet commercial size or shape standards. Stems alone can represent up to 20% of the total yield (Papoutsis et al., 2020); however, they retain the same valuable nutritional profile and bioactive agents found in the fruiting bodies. Consequently, both whole mushrooms and these nutrient-dense co-products are successfully incorporated into meat

analogues, dairy, and bakery products to enhance their functional value (Navarro-Simarro et al., 2024; Salehi, 2019; Wang et al., 2021).

Bread is regarded as one of the most important dietary food products in several countries (Lu et al., 2021; Zhang et al., 2019). Therefore, it represents a suitable food matrix for fortification, enrichment, and, more generally, reformulation strategies aimed at improving the nutritional health of the population (Lu, Brennan, Serventi & Brennan, 2018; Lu et al., 2021; Riis et al., 2021). High salt (NaCl) intake is considered a major health concern worldwide because of its association with elevated blood pressure and thus increased risk of cardiovascular diseases (CVDs) (Riis et al., 2021; Toft et al., 2020). It is estimated that the global average salt intake ranges from 9 to 12 g/day, which is approximately double the maximum intake recommended for adults by the World Health Organization (WHO), set at 5 g/day of NaCl (corresponding to 2 g/day of sodium) (World Health Organization, 2023). Processed foods are the main source of salt in the diet of Western countries (70% of total salt intake), with bread being one of the main contributors, along with cheeses, spreads, and processed meat and fish (Ma et al., 2024; Riis et al., 2022). Thus, reducing NaCl in bread may be an effective approach to lower salt consumption across the population (Riis et al., 2021, 2022).

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However, salt is extensively used in the food industry because of its low cost, availability, and techno-functional properties (Gorman et al., 2023; Tyl et al., 2024). Concretely, in bread NaCl affects the fermentation rate through osmotic pressure: in dough, the salt concentration in the free water available to yeast is considerably higher than the total salt content of the dough, which inhibits yeast activity, allows the development of the gluten network, extends the shelf life, and affects flavor and dough rheology (Belz et al., 2012; Gorman et al., 2023; Silow et al., 2016; Tyl et al., 2024). The main approaches to reducing salt concentration in bread have consisted of gradual reduction, the addition of salt replacers, physical modifications, and flavor enhancers (Dunteman et al., 2021; Gorman et al., 2023; Ma et al., 2024). The most common flavor enhancer is umami substances, including monosodium glutamate (MSG) and 5' nucleotides (Ma et al., 2024). Most of these strategies impact manufacturing costs; physical modification methods may require new processing equipment, and the addition of a clean label or unfamiliar ingredients such as MSG could reduce sales (Dunteman et al., 2021). Umami substances are found in edible mushrooms and also in ABS and POS stems, mainly L - glutamic acid (L -Glu) and its salts, MSG (Harada-Padermo et al., 2020). L-Glu has also been found in ABS and POS, so it could be interesting to revalue this agricultural waste as umami ingredients to enhance food flavor (Bermúdez-Gómez et al., 2024a). The potential of mushroom-derived powders as salt replacers lies in the synergistic effect between free amino acids (such as L-glutamate) and 5'-nucleotides. This synergy enhances saltiness perception and overall flavor intensity, enabling a reduction in sodium chloride (NaCl) without compromising sensory acceptability (Fibri et al., 2024). Authors such as Sakai et al. (2024) found that the inclusion of enzymes that produce glutamate-like substances enhances saltiness perception in vegetable soups. In line with this, the partial substitution of salt with mushroom powder (e.g., *Pleurotus ostreatus*) was also shown to enhance saltiness perception in potato chips (Fibri et al., 2024).

Mushroom powder addition in bread has been characterized only for wheat flour substitution (Losoya-Sifuentes et al., 2022; Lu et al., 2021; Ndung'u et al., 2015; Salehi, 2019; Sławińska et al., 2022; Zhang et al., 2019). The novelty of this study lies in the fact that the addition of ABS and POS is oriented towards the production of low-salt bread, which could result in an interesting solution to the technological and sensory challenge of salt reduction in bread while contributing to sustainability and the circular economy by revaluing agricultural waste. To our knowledge, in the scientific literature, it is possible to find studies related to the reduction of salt by the addition of mushroom powder only in meat products; these studies refer to the fruiting body and not to the co-product generated after the mushroom harvesting (Botella-Martínez et al., 2023; Cerón-Guevara et al., 2020, 2021). Overall, this work aimed to assess for the first time the effects of the incorporation of flours obtained from *Agaricus bisporus* stems (ABSF) and *Pleurotus ostreatus* stems (POSF) as sodium chloride replacers on the chemical, physicochemical, protein digestibility, starch digestibility, mineral bioaccessibility and sensory properties of bread.

2. Materials and methods

2.1. Mushroom stem flour

The preparation of mushroom stem flours followed the methodology outlined by Bermúdez-Gómez et al., 2024b. In summary, stems of *Agaricus bisporus* (Sylvan A15 strain) were provided by Cultivos Riojal (Autol, La Rioja, Spain), stored at 4 °C for up to 5 days, cleaned through abrasive peeling, and then dehydrated using Klarstein Master Jerky 550 dehydrator (Chal-Tec GmbH, Berlin, Germany) at 50 °C for 24 h. Similarly, *Pleurotus ostreatus* (Sylvan SPOPO strain) stems were sourced from Micotec SA (Autol, La Rioja, Spain), kept at 4 °C for no more than 5 days, and dried under the same conditions. The dehydrated samples were then ground using an ultracentrifugal mill (ZM 200 model, Retsch™, Düsseldorf, Germany) to obtain a flour with a particle

diameter of less than 180 µm and stored in hermetic polyethylene bags at room temperature in the dark. The two fractions obtained were *A. bisporus* stem flour (ABSF) and *P. ostreatus* stem flour (POSF).

2.2. Manufacture of low-sodium bread

Bread was manufactured according to a traditional formula containing 60% refined wheat flour (9.44% moisture, 11.7% protein, and 1.4% fat), 37% water, 1% sodium chloride, and 2% yeast. This mixture was used as a control. Eight low-sodium bread formulations were developed as shown in Table 1. The salt was substituted in two levels: i) 50% with 0.5% ABSF or POSF and 0.5% sodium chloride, ii) 100% with 0% sodium chloride and 1% ABSF or POSF, and iii) 100% with 3% and 6% of ABSF or POSF by the reduction of wheat flour. These breads with ABSF and POSF were named BAB_{0.5}, BAB₁, BAB₃, BAB₆, BPO_{0.5}, BPO₁, BPO₃, and BPO₆, respectively. The selected salt replacement levels were defined based on previous literature (Dunteman et al., 2021; Losoya-Sifuentes et al., 2022; Sławińska et al., 2022; Zhang et al., 2019). The 100% replacement level was evaluated to explore the potential limit of salt substitution using mushroom stem flours. Higher flour concentrations (3% and 6%) were also tested to assess whether increased levels of mushroom-derived compounds, particularly those associated with umami flavor, could help compensate for the absence of sodium chloride. Additionally, these levels allowed the evaluation of the technological impact of incorporating these flours under conditions of complete salt removal. Higher percentages were not explored because they limited technological feasibility.

Breads were prepared following the process described in Supplementary Figure 1. Wheat flour, water, sodium chloride (and/or ABSF/POSF), and yeast were mixed in an automatic bread maker (Silvercrest automatic bread maker, La Rioja, Spain) to produce a smooth dough (300 g per treatment). Then, the dough was kneaded, rounded, and fermented (first fermentation) at room temperature inside the baker compartment. In the next step, the dough was kneaded again to break the air bubbles, rounded, and fermented (second fermentation) at room temperature to reach at least double its size. Finally, the dough was divided into breads of approximately 50 g (6 breads per treatment) and stored at -20 °C for no longer than 10 days prior to baking. This step was taken to allow subsequent analyses to be performed under comparable conditions. All formulations, including the control, were subjected to the same freezing and storage conditions. The breads were then baked at 185 °C for 40 min in an electric oven (HR-38 N RM7 silver, Grunkel, La Rioja, Spain).

2.2.1. Kneading and fermentation times

Kneading and fermentation times for the control bread were optimized by testing three kneading times (5, 10, and 15 min), as well as different durations for the first fermentation (10, 15, and 20 min) and the second fermentation (10, 15, and 20 min). The tested kneading and

Table 1

Formulations of control bread and formulations with low-sodium content by the addition of *Agaricus bisporus* stem flour (ABSF) and *Pleurotus ostreatus* stem flour (POSF).

Ingredient g / 100 g bread	CT	BAB _{0.5} / BPO _{0.5}	BAB ₁ / BPO ₁	BAB ₃ / BPO ₃	BAB ₆ / BPO ₆
Wheat Flour	60	60	60	58	55
Water	37	37	37	37	37
Salt	1	0.5	0	0	0
ABSF or POSF	0	0.5	1	3	6
Yeast	2	2	2	2	2

CT—bread with all sodium, white wheat, and without mushroom powder; BAB_{0.5}/BPO_{0.5}—bread with 0.5% addition of ABSF or POSF and 0.5% of salt; BAB₁/BPO₁—bread with 1% addition of ABSF or POSF without salt; BAB₃/BPO₃—bread with 3% addition of ABSF or POSF without salt; BAB₆/BPO₆—bread with 6% addition of ABSF/POSF without salt.

fermentation times were selected based on values previously reported in the literature (Dizlek & Özer, 2021; Parenti et al., 2025). Specific volume was used as the critical control parameter to determine the conditions that yielded breads with optimal physical characteristics (Cappelli et al., 2019; Lu, Brennan, Serventi, & Brennan, 2018; Parenti et al., 2025). Subsequently, the same approach was applied to BPO_{0.5} and BAB_{0.5} formulations. Finally, kneading and fermentation times for the formulations with 100% salt replacement were selected based on the optimal conditions identified for the 50% replacement breads and adjusted to obtain enriched breads with physical characteristics as comparable as possible to those of the control bread. Additionally, shorter processing times (8 and 10 min from kneading and 5 from first fermentation) were evaluated, as longer times made dough handling more difficult. For the formulations with 3% and 6% mushroom stem flour (BPO3, BAB3, BPO6, and BAB6), the kneading and fermentation times were kept constant at the values previously optimized for the corresponding 100% salt replacement formulations (BPO1 and BAB1, respectively). This approach was adopted to avoid introducing additional processing variables, allowing the technological impact of increasing mushroom stem flour concentration to be evaluated independently of the effects associated with salt absence. The whole process was repeated 3 times on different days, (three independent batches per formulation). **Supplementary Figure 2** shows the results of the elaboration process.

2.3. Chemical parameters of bread

2.3.1. Proximate composition

The moisture, ash, total fat on samples were determined using standardized methods recommended by the Association of Official Analytical Chemists (AOAC) (AOAC, 2000). Crude fats were extracted following the Folch method described by Eggers and Schwudke (Eggers & Schwudke, 2016). The dietary fiber was analyzed according to the gravimetric-enzymatic methods following the AOAC methods 991.43 (AOAC, 1995). Total carbohydrates were calculated by subtracting moisture, fat, protein, and ash from 100%. For each formulation per batch, three determinations were performed ($n = 9$).

2.3.2. Minerals profile

The mineral profile of samples was determined using inductively coupled plasma-mass spectrometry (ICP-MS, Shimadzu MS-2030, Shimadzu, Kyoto, Japan), following the method described by Muñoz-Bas et al. (2023). First, the samples were digested with hydrogen peroxide at 69% (w/v) and nitric oxide in a microwave extraction system (Mars 6 PFAS, CEM Corporation, Charlotte, North Carolina). The standard compounds were diluted and utilized to calibrate the ICP-MS for mineral analysis in bread samples. ICP-MS operated under the following conditions: carrier gas 0.70 L/min; plasma gas 9.0 L/min; auxiliary gas 1.10 L/min; radio frequency 1.2 kW; and energy filter 7.0 V. All analyses were performed in triplicate per batch of each treatment ($n = 9$), and results were expressed as mg/100 g of bread.

2.4. Physico-chemical properties of bread

2.4.1. pH, water activity, and instrumental color

The pH of the bread was measured using a portable pH-meter Crison GLP 21 (Crison Instrument S.A., Barcelona, Spain) equipped with a puncture electrode probe. For each formulation per batch, three determinations were performed ($n = 9$). Similarly, water activity (a_w) was determined using a Novasina Thermoconstanter Sprint TH-500 (Novasina, Pfäffikon, Switzerland) at 25 °C. The analyses were performed in triplicate per batch from each treatment ($n = 9$). The instrumental color was measured in the crumb of all baking bread with a BGD 551 colorimeter (Biuged Precise Instruments Co., Guangzhou, China) with the following specifications: illuminating D₆₅, 8° observer angle, SCI mode, 8 mm aperture for illumination, and 4 mm aperture. Prior to use,

calibration according with the standard manual was performed. The CIE Lab color space was selected to assess the coordinates: lightness (L^*), redness (a^*) and yellowness (b^*). From these coordinates, chroma (C^*) was calculated using Eq. 1, while the color difference (ΔE^*) of each formulation. The control was measured using Eq. 2. For each formulation per batch, six determinations were performed ($n = 18$).

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (1)$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (2)$$

2.4.2. Specific volume and texture profile analysis

The bread volume of all formulations was measured by rapeseed displacement methodology according to the American Association of Cereal Chemists (AACC) expressed as cm³/g of bread (AACC Method (10-05. 01), 1998). The analyses were performed in triplicate per batch from each treatment ($n = 9$). Texture profile analysis (TPA) of the breads was determined by the American Association of Cereal Chemists (AACC) Method 74-09.01 with minor modifications (Claus, 1995). TPA of all bread formulations was realized in a TA-XT2i Texture Analyzer (Stable Micro Systems, Surrey, UK). Each bread sample was evaluated by compressing three central crumb slices (50 mm thick) individually, applying a 40% strain at a test speed of 1 mm/s, with a post-test speed of 5 mm/s and an activation threshold of 0.049 N. The parameters determined were hardness (N), springiness (mm), cohesiveness, and chewiness (N × mm). For each formulation per batch, six determinations were performed ($n = 18$).

2.5. Sensorial analysis

Bread control and low-sodium samples were submitted to a panel of 70 people (53% female and 47% male; with an age range from 19 to 54 years old). Untrained consumers were recruited among students and staff at Miguel Hernandez University and the Mushroom Technological Research Center of La Rioja (CTICH). Before the analysis began, all participants were informed about the characteristics of the product they would be tasting and the nature of the analysis. They also provided written informed consent. This study was approved by the Responsible Research Office at Miguel Hernández University (OIR- Reg. 220504213907, Ref. PRL.DTA.MVM.01.22 UMH, Elche, Alicante, Spain). The sensory evaluation was carried out with bread samples 1 h after baking. Coded samples of bread (1 × 1 × 1 cm) with a random 3-digit number were given to the evaluation panel. All panelists tested all the samples. Water was used to rinse the mouth before and after each sample test. The panelists were asked to score on a nine-point hedonic scale, where 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely, the following attributes: appearance, crumb color, crust hardness, crumb sponginess, aroma, taste, salinity, and overall acceptability.

2.6. In vitro digestion

In vitro gastrointestinal digestion was done following the harmonized INFOGEST protocol (V 2.0) (Brodtkorb et al., 2019). Prior to initiating the digestion process, the bread samples were ground and passed through a 200 μm mesh sieve to simulate the particle size typically resulting from mastication. In brief, 500 mg of milled bread was mixed with 500 μL of distilled water to obtain pasta with tomato pasta consistency. Then, simulated salivary fluid with salivary alpha-amylase (75 U/mL) (α -Amylase from human saliva, Type XIII-A, 940 U/mg protein) was added, followed by incubation in a rotation mixer (ELMI Intelli-Mixer™ RM-2S; ELMI; Riga, Latvia) place in a thermal incubation, 37 °C and 60 rpm. After 2 min, 1.6 mL of simulated gastric fluid and HCl (1 M, 0.25 M) until pH 3.0 was incorporated into the oral samples to stop oral digestion. After that, 100 μL of porcine pepsin solution (2000 U/mL)

(Sigma-Aldrich P7012) was added. Then the samples were incubated in the same conditions as the oral phase for 2 h. Finally, the gastric phase was mixed with 1.7 mL of simulated intestinal fluid, 8 μ L of CaCl₂ (3 M), 0.5 mL of bile (Bile bovine B3883-25G), and 1 mL of pancreatin (Sigma Pancreatin P7545 8 x USP specifications). The pH was adjusted to 7.0 with NaOH (1 M, 0.25 M), and the samples were incubated for 2 h at 37 °C and 60 rpm of agitation. A heat shock (100 °C, 5 min) was carried out to inactivate digestive enzymes in each stage. For the respective five points studied, an individual *in vitro* digestion was performed as recommended by Brodtkorb et al., 2019. The simulated digestion of 5 different endpoints was achieved in duplicate for the 3 breads selected after sensorial analysis. After inactivating digestive enzymes, the digested samples were centrifuged at 10,000 rpm for 10 min.

2.6.1. *In vitro* protein digestibility (IVPD)

In vitro protein digestibility (IVPD) was expressed as the percentage of the protein content before digestion and the protein content after digestion. The precipitate of the centrifugation was considered as the insoluble part that is potentially non-absorbable, while the supernatant contains the soluble and potentially absorbable proteins. Then the protein content in the precipitate was analyzed by the Kjeldahl method in the same way as in proximate composition. The degree of digestibility was calculated following Eq. 3 at three points of the intestinal phase incubation (140, 210, and 240 min).

$$\text{IVPD (\%)} = 100 \times \frac{P_i - P_d}{P_i} \quad (3)$$

where P_i is the protein content of the sample without treatment before digestion, and P_d is the protein content in the sample precipitate after intestinal digestion.

2.6.2. Minerals bioaccessibility

The minerals bioaccessibility of all breads were estimated as the percentage (%) of the amount of minerals release to breads to the soluble phase after enzymatic digestion relative to the initial mineral content of the breads (Eq. 4). Minerals were determined in the supernatant fraction obtained after the centrifugation of the intestinal phase (240 min), following the methodology described in 2.3.2. section.

$$\text{Mineral bioaccessibility (\%)} = \frac{M_d}{M_i} \times 100 \quad (4)$$

where M_i is the mass of the mineral in the sample before digestion; M_d is the mass of the mineral after intestinal digestion.

2.6.3. Kinetic of starch digestion and predicted glycemic index

The adapted protocol by Lucas-González et al. (2024) to previous reported protocol to Goñi et al. (1997) was used to determine kinetic starch digestion and predicted glycemic index. In brief, two aliquots of 0.45 mL were taken from each studied digestive endpoint (2, 120, 140, 210 and 240 min) and mixed with sodium acetate (100 mM) plus ClCa₂ (5 mM) pH 5.0 (Dilution 1:4), and amyloglucosidase (20 μ L; 60 U/mL) to complete the digestion of maltose and oligosaccharides. The samples were incubated at 50 °C for 30 min. After that, the samples were diluted (1:10). Finally, aliquots of 50 μ L were mixed with GOPOD reactive (1.5 mL) and incubated for 30 min at 50 °C. Then the absorbance of the samples was measured at 510 nm. Blank was carried out by substituting samples with sodium acetate (100 mM) plus ClCa₂ (5 mM) pH 5.0. Three glucose patterns (1 mg/mL) were included in each reaction. To avoid over-starch hydrolysis estimation after *in vitro* gastrointestinal digestion, a blank with a sample but without enzymes was used for each batch. Eq. 5 was used to calculate the percentage of starch after *in vitro* digestion.

$$\% \text{Starch} = \Delta A \times F \times \frac{VD}{0.05} \times D \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} \quad (5)$$

where,

A = Absorbance sample.

F = factor to convert absorbance values to μ g of D-glucose (100 μ g of D-glucose divided by the GOPOD absorbance value for 100 μ g of D-glucose).

VD = Digestion phase volume (mL).

D = Dilution factor.

W = Sample dry weight (mg).

162/180 = Factor to convert from free glucose, as determined, to anhydroglucose, as occurs in starch.

Finally, to assess the percentage of hydrolyzed starch content, total starch content was carried out using the AOAC Official Method 996.11 with a previous alcohol washing with the help of a total starch kit (Megazyme, Bray, Ireland). Throughout the intestinal phase, starch fractions were classified according to the chronological markers established by Englyst et al. (1992). rapidly digestible Starch (RDS) was measured as the glucose released after 20 min of intestinal digestion, while slowly digestible starch (SDS) was calculated as the glucose released between 20 and 120 min. Resistant starch (RS) was determined as the starch fraction remaining unhydrolyzed after 120 min. Predicted glycemic index (pGI) was calculated as the area under the curve (AUC) of each studied bread formulation, with the help of the first-order equation of the hydrolytic process (Eqs. 5 and 6), using the control (white bread) as reference food. Eqs. 7 and 8 proposed by Goñi et al. was also used for calculating the pGI (Goñi et al., 1997). The concentrations obtained at 210 min were used as the final reaction time.

$$C = C_{\infty}(1 - e^{-kt}) \quad (5)$$

$$\text{AUC} = C_{\infty}(t_{\infty} - t_0) - (C_{\infty}/k)[1 - e^{-k(t_{\infty}-t_0)}] \quad (6)$$

$$I = \text{AUC}_{\text{BAB}} \text{ or } \text{BPO}/\text{AUC}_{\text{white bread}} \times 100 \quad (7)$$

$$\text{pGI} = 39.71 + 0.549 \text{ HI} \quad (8)$$

where,

C = % hydrolyzed starch.

C_{∞} = % hydrolyzed starch at final time.

k = kinetic reaction constant.

t_{∞} = final reaction time (210 min).

t_0 = start reaction time.

2.7. Statistical analysis

The full process (bread manufacture and all analysis) was replicated three times (three independent batches) on separate days. The results were reported as average \pm standard error of the means. A mixed model was applied considering the treatments as a main factor (fixed effect) and repeated experiments as a random effect. The Tukey post-hoc test was performed at a 95% significance level to explore significant differences among bread samples. Analysis was conducted to determine relationships between the results using Pearson correlation analysis. Principal Component Analysis (PCA) was performed as a multivariate statistical method to reduce data dimensionality and explore the relationship among the chemical, physicochemical, and sensory variables of the different bread formulations. A biplot representation was generated to simultaneously visualize the spatial distribution of the samples and the contribution of the original variables to each principal component. PCA was performed using GraphPad Prism 10 (GraphPad, CA, USA). Statistical analyses were carried out using the statistical package SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Bread making process

Salt reduction and the incorporation of ingredients rich in dietary

fiber can modify dough stability, gluten network development, and gas retention capacity (Belz et al., 2012; Lu, Brennan, Serventi, & Brennan, 2018; Silow et al., 2016). In addition, kneading and fermentation times are key parameters influencing gluten network formation and dough aeration (Cappelli et al., 2019; Hackenberg et al., 2019; Dizlek & Özer, 2021). For this reason, these processing conditions were optimized for the different formulations using specific volume as the main control parameter (Supplementary Table 1).

For the control bread and the formulations BPO_{0.5} and BAB_{0.5}, kneading and fermentation times were initially set at 10 min kneading, 15 min first fermentation, and 15 min second fermentation (10:15:15), based on values commonly reported in the literature for wheat bread processing (Cappelli et al., 2019; Dizlek & Özer, 2021; Parenti et al., 2025). Around these reference conditions, additional times above and below the selected values were tested while keeping the other parameters constant. The results (Supplementary Table 1) confirmed that the 10:15:15 combination provided the optimal conditions in terms of specific volume.

For the formulations with 100% salt replacement, shorter processing times were also evaluated. The absence of sodium chloride can affect dough structure and fermentation control, making longer processing times more difficult to manage during kneading and proofing (Belz et al., 2012; Silow et al., 2016). In these formulations, the optimal processing conditions varied depending on the type of mushroom stem flour used. The reference conditions (10:15:15) were first evaluated and then the second fermentation time was reduced, resulting in improved specific volume when the second fermentation was shortened to 10 min ($p < 0.05$). Subsequently, the first fermentation time was optimized while maintaining the previously adjusted parameters, with higher volumes obtained at 10 min for BAB₁ and 5 min for BPO₁ ($p < 0.05$). Finally, kneading time was optimized while maintaining the selected fermentation conditions. In this case, BAB₁ maintained the same kneading time as the control (10 min), whereas BPO₁ required a shorter kneading time (6 min) to obtain specific volumes as close as possible to those of the control (Supplementary Table 1). The differences observed between both species may be attributed to differences in flour composition and hydration properties. POSF is characterized by a higher β -glucan content and lower chitin content compared to ABSF, which conversely contains more chitin and a lower number of β -glucans (Bermúdez-Gómez et al., 2024a; Vetter, 2007). These compositional differences are technologically relevant, as they influence the hydration behavior of the fiber fractions and their interaction with the gluten network. β -Glucans, being soluble dietary fibers, exhibit high water-holding capacity and contribute to increased viscosity of the aqueous phase, which can limit water availability for gluten hydration and development. In contrast, chitin is an insoluble fiber with a high water-binding capacity but does not form viscous gels; instead, it physically competes for water without contributing to network development (Djordjević et al., 2022; Hu et al., 2022). Consequently, ABSF presents significantly higher water holding capacity than POSF, while swelling properties are similar but slightly superior for POSF (Bermúdez-Gómez et al., 2024a). These distinct hydration properties imply that, during dough mixing, POSF may induce greater competition for water than ABSF, potentially limiting gluten hydration and delaying or impairing the formation of a continuous gluten network. These phenomena have been also reported on bread enriched with AB and PO (Lu, Brennan, Serventi, & Brennan, 2018; Losoya-Sifuentes et al., 2022). This could explain the need for shorter kneading times in BPO₁ compared to BAB₁ to achieve comparable specific volume. Whenever no significant differences in specific volume were observed between tested conditions, the processing time closest to the control was selected to minimize potential bias and ensure that differences observed among formulations were mainly associated with salt replacement and flour incorporation rather than processing conditions.

3.2. Chemical parameters

3.2.1. Proximate composition

The proximate composition of the bread formulated in the present work is shown in Table 2. The moisture content did not exhibit significant differences among the studied bread ($p > 0.05$). The addition of ABSF and POSF appeared to increase the protein amount of the bread compared to the control (9.77 g/100 g), especially with BAB₆ (10.77 g/100 g) ($p < 0.05$). The same fact has been observed in various studies in which bread containing fruiting bodies powder of PO at 5%, 7.5%, and 10% and AB at 4% have been characterized (Ndung'u et al., 2015; Salehi, 2019; Zhang et al., 2021). According to the results (Table 2), there were no significant differences in protein level between 1% and 3% of ABSF and 1%, 3%, and 6% of POSF ($p > 0.05$). These findings indicate that incorporating BAB at the studied concentrations did not modify the protein content of the bread. Furthermore, while mushroom stem flours contain a complete profile of essential amino acids—unlike wheat flour, which lacks sufficient lysine and threonine—their incorporation could in principle improve the protein quality of the bread. However, at the levels tested, this potential effect is limited and unlikely to have a significant impact. (Bermúdez-Gómez et al., 2024a; Losoya-Sifuentes et al., 2022). Additionally, the protein proportion in BAB₆ was higher than in BPO₆ ($p < 0.05$), supporting the hypothesis that the increase in protein amount is associated with the incorporation of different flours. This is consistent with the protein composition of the flours, as ABSF contains higher protein content (14.36 g of protein/100 g) than, POSF (9.42 g of protein/100 g) (Bermúdez-Gómez et al., 2024a). The fat level was approximately 1 g/100 g of bread in all formulations, like previously reported in bread made with the same basic ingredients (1.54 g/100 g) (Zhang et al., 2021). Other authors have found an increment in ash with the addition of mushroom flour since it was a source of minerals (Salehi, 2019; Zhang et al., 2021). On the contrary, in this study, ash concentration in all bread formulations with ABSF and POSF was lower than in the control ($p < 0.05$), a reduction intensified with the 100% salt substitution. This fact correlated with sodium reduction (Table 2) with a $r = 0.85$ in bread with ABSF and $r = 0.91$ in POSF.

There was no significant difference ($p > 0.05$) in the total carbohydrate proportion (53.83–58.79 g/100 g of bread) due to the inclusion of mushroom stem flours; moreover, as observed in previous studies, this was the main component on studied formulations (Losoya-Sifuentes et al., 2022; Salehi, 2019). Wheat flour, ABSF, and POSF are rich in carbohydrates; however, their main component in wheat flour is non-resistant starch, while in ABSF and POSF, it was D-glucans (Bermúdez-Gómez et al., 2024a; Losoya-Sifuentes et al., 2022). Furthermore, a rise in total dietary fiber (TDF) was observed in BAB₃ (4.33 g/100 g) and BAB₆ (5.04 g/100 g) compared to the control (2.95 g/100 g of bread) ($p < 0.05$). All bread formulations with 100% salt substitution using POSF, including those with 1%, showed higher TDF than the control ($p < 0.05$). A similar effect on dietary fiber content has been observed in previous studies (Salehi, 2019; Zhang et al., 2021). In this study, POSF led to an enhancement in fiber amount even at a 1% inclusion level, highlighting its potential as a valuable source of dietary fiber. Moreover, BPO₆ contained a sufficient dietary fiber proportion (7.18 g/100 g of bread) to meet the criteria for a high-fiber designation according to nutritional guidelines (Commission Regulation, 2012). This improvement could be attributed to the high fiber content naturally present in POSF (59.84 g/100 g), which exceeds that of ABSF (37.17 g/100 g), with insoluble dietary fiber (IDF) accounting for more than 90% in both (Bermúdez-Gómez et al., 2024a). Furthermore, IDF was consistently higher than soluble fiber (SDF) across all formulations containing mushroom co-product flour (Table 2). This rise seems to drive the observed increase in TDF, supporting the hypothesis that the incorporation of ABSF and POSF, which are flours rich in IDF, significantly contributed to the fiber concentration of the reformulated bread. It is important to highlight that white wheat bread has a very low fiber presence, primarily due to the refining process of wheat flour, which removes fractions rich in dietary

Table 2

Proximate composition and mineral content of low-sodium bread by the addition of *Agaricus bisporus* stem flour (ABSF) and *Pleurotus ostreatus* stem flour (POSF).

Parameter	CT	BAB _{0.5}	BAB ₁	BAB ₃	BAB ₆	BPO _{0.5}	BPO ₁	BPO ₃	BPO ₆
Moisture	33.07 ^a ± 0.73	32.35 ^{ab} ± 0.42	31.38 ^b ± 0.43	31.64 ^{ab} ± 0.54	33.46 ^a ± 0.16	28.99 ^c ± 0.24	30.59 ^b ± 0.31	29.09 ^c ± 0.78	32.10 ^{ab} ± 0.40
Protein	9.77 ^e ± 0.02	10.14 ^{cd} ± 0.00	10.28 ^b ± 0.05	10.39 ^b ± 0.08	10.77 ^a ± 0.05	10.01 ^d ± 0.05	10.33 ^b ± 0.01	10.20 ^{bc} ± 0.01	10.25 ^b ± 0.03
Fat	1.03 ^c ± 0.04	0.86 ^c ± 0.02	0.91 ^c ± 0.04	1.04 ^c ± 0.01	0.96 ^c ± 0.05	1.33 ^a ± 0.07	1.40 ^a ± 0.04	1.24 ^{ab} ± 0.14	1.27 ^{ab} ± 0.02
Ash	1.66 ^a ± 0.02	1.19 ^b ± 0.05	0.58 ^d ± 0.00	0.83 ^c ± 0.01	1.11 ^b ± 0.03	1.08 ^b ± 0.06	0.42 ^e ± 0.01	0.68 ^d ± 0.01	0.86 ^c ± 0.03
CH	54.47 ^c ± 0.79	55.28 ^c ± 0.66	56.84 ^{abc} ± 0.66	56.10 ^{abc} ± 0.52	53.83 ^c ± 0.41	58.60 ^{ab} ± 0.22	57.23 ^{abc} ± 0.33	58.79 ^a ± 1.10	55.53 ^{bc} ± 0.44
TDF	2.95 ^d ± 0.00	2.47 ^d ± 0.15	2.42 ^d ± 0.03	4.33 ^{bc} ± 0.19	5.04 ^b ± 0.29	3.81 ^{cd} ± 0.31	4.75 ^{bc} ± 0.19	4.48 ^{bc} ± 0.27	7.18 ^a ± 0.03
IDF	2.71 ^{ef} ± 0.07	2.44 ^f ± 0.13	2.21 ^f ± 0.11	4.09 ^{bc} ± 0.33	4.87 ^b ± 0.20	3.29 ^{de} ± 0.26	3.93 ^{cd} ± 0.08	3.88 ^{cd} ± 0.16	6.52 ^a ± 0.06
SDF	0.29 ^c ± 0.00	0.04 ^d ± 0.00	0.11 ^d ± 0.01	0.13 ^d ± 0.01	0.25 ^c ± 0.03	0.65 ^b ± 0.03	0.78 ^a ± 0.04	0.79 ^a ± 0.02	0.65 ^b ± 0.04
Ca	159.22 ^d ± 5.92	225.97 ^c ± 0.91	216.43 ^c ± 2.24	279.93 ^b ± 5.02	394.28 ^a ± 0.87	164.81 ^d ± 3.83	166.68 ^d ± 5.46	156.83 ^d ± 9.97	153.76 ^d ± 3.07
Cu	1.13 ^d ± 0.02	1.34 ^c ± 0.02	1.40 ^c ± 0.00	1.78 ^b ± 0.10	1.96 ^a ± 0.02	0.97 ^d ± 0.01	1.40 ^c ± 0.01	1.27 ^{cd} ± 0.02	1.72 ^b ± 0.02
Fe	6.29 ^{de} ± 0.40	5.68 ^e ± 0.04	10.28 ^{bc} ± 0.06	10.55 ^b ± 0.14	26.31 ^a ± 0.13	5.50 ^e ± 0.20	7.38 ^d ± 0.19	5.78 ^e ± 0.09	9.30 ^c ± 0.40
K	678.88 ^g ± 6.18	630.92 ^h ± 4.55	769.75 ^e ± 5.99	1217.55 ^d ± 7.62	1823.46 ^b ± 7.14	783.02 ^e ± 2.83	736.46 ^f ± 4.02	1343.34 ^c ± 2.84	1876.47 ^a ± 4.38
Mg	92.34 ^f ± 1.65	106.27 ^e ± 1.64	116.13 ^d ± 0.47	134.07 ^c ± 2.76	163.57 ^a ± 1.38	114.80 ^d ± 3.13	102.34 ^e ± 1.08	120.00 ^d ± 0.84	148.12 ^b ± 1.09
Mn	2.52 ^{abc} ± 0.02	2.59 ^{ab} ± 0.13	2.64 ^a ± 0.07	2.59 ^{ab} ± 0.06	2.65 ^a ± 0.03	2.68 ^a ± 0.14	2.62 ^a ± 0.05	2.25 ^{bc} ± 0.03	2.17 ^c ± 0.04
Na	2164.75 ^a ± 29.86	1691.98 ^b ± 22.42	86.31 ^c ± 4.99	91.15 ^c ± 0.74	121.51 ^c ± 0.81	1629.88 ^b ± 19.79	59.70 ^c ± 2.90	66.66 ^c ± 2.21	91.16 ^c ± 2.23
P	391.50 ^e ± 10.01	423.37 ^e ± 5.19	469.34 ^{cd} ± 2.69	518.64 ^b ± 13.10	595.10 ^a ± 7.45	443.57 ^{de} ± 12.03	454.48 ^{de} ± 4.90	502.01 ^{bc} ± 3.39	524.75 ^b ± 1.98
Zn	5.60 ^{bc} ± 0.40	4.07 ^e ± 0.13	5.45 ^{bcd} ± 0.06	6.19 ^b ± 0.26	8.74 ^a ± 0.04	4.46 ^{de} ± 0.02	5.85 ^{bc} ± 0.07	4.87 ^{cd} ± 0.12	4.98 ^{cd} ± 0.17

Results are reported as mean ± SEM (n = 9). Mean values within the same row followed by different superscript letters (a–h) are significantly different when subjected to Tukey's test ($p < 0.05$). CH—carbohydrates, IDF—insoluble dietary fiber, SDF—soluble dietary fiber, TDF—total dietary fiber. Results of proximate composition are expressed as g/100 g of bread and minerals as mg/100 g of bread.

fiber, such as the germ and bran of the grain (Losoya-Sifuentes et al., 2022).

3.2.2. Mineral profile

According to the results shown in Table 2, the mineral profile was influenced by the concentration of the incorporated flours and the mushroom species of these flours ($p < 0.05$). The main mineral in the control was sodium, which was gradually decreased by substituting salt in the bread formulations ($p < 0.05$). Sodium was reduced in BAB_{0.5} and BPO_{0.5}, by 21.84 and 24.71%, respectively. Furthermore, as expected, the most pronounced sodium reduction was observed in bread without salt ($p < 0.05$). Additionally, no significant differences in sodium levels were detected between the addition of ABSF and POSF. These results underscore the potential health benefits of the bread formulations under study, confirming their classification as low-sodium products (Commission Regulation, 2012). The bread obtained contributes to healthier dietary patterns and aligns with public health recommendations for sodium reduction (Riis et al., 2021).

In general, an increase in the content of the microelements is observed in those breads with a higher percentage of ABSF and POSF, except for manganese and sodium ($p < 0.05$). To the best of our knowledge, there are no references regarding the mineral profile of bread with AB; however, in cookies, there is an increase in minerals (Salehi, 2019). Potassium was the most abundant microelement in the low-sodium bread formulations, followed by phosphorus, calcium, and magnesium. It is worth noting that the concentration of potassium was substantially higher in bread containing ABSF and POSF, increasing proportionally with the substitution level of wheat flour and salt, aligned with the reported in previous studies ($p < 0.05$) (Ndung'u et al., 2015; Srivastava et al., 2024). This observation could be attributed to the high potassium content in the mushroom stem flours (Bermúdez-Gómez et al., 2024a). Potassium intake has been inversely associated with hypertension and a wide range of cardiovascular conditions, including stroke, myocardial infarction, and cardiovascular mortality (Castro & Raji, 2013). These results highlight the potential

cardiovascular benefits of low-sodium bread with the addition of ABSF and POSF.

Calcium rose from 159.22 mg/100 g in control to 394.28 mg/100 g in BAB₆ ($p < 0.05$), which provides 50% of calcium's nutrient reference values, demonstrating the potential of ABSF as a source of calcium in the food industry (Lewis, 2019). On the other hand, with the addition of POSF, this microelement did not show significant differences compared to the control ($p > 0.05$). The absence of enrichment in calcium observed in POSF could be attributed to the substantial difference in calcium content observed between ABSF (700 mg/100 g) and POSF (21.56 mg/100 g) (Bermúdez-Gómez et al., 2024a). The same tendency for calcium proportion has been shown in previous studies, in which PO was added to bread at 5% and 10% (Ndung'u et al., 2015). Copper, magnesium, and phosphorus concentrations were higher in both BAB₆ and BPO₆ than in the control ($p < 0.05$), with BAB₆ consistently showing greater values than BPO₆ ($p < 0.05$). These results align with the prior characterization of the flours, where ABSF had a generally higher mineral composition than POSF (Bermúdez-Gómez et al., 2024a). The rise in copper, magnesium, and phosphorus with the incorporation of mushroom flour in bread has also been reported in previous studies (Ndung'u et al., 2015; Srivastava et al., 2024). The iron amount was also found to increase with the addition of the flours under study ($p < 0.05$), consistent with findings from previous research (Ndung'u et al., 2015; Srivastava et al., 2024). This enhancement became more pronounced with higher concentrations of both flours, however, BAB₆ showed a greater effect than BPO₆ ($p < 0.05$). Specifically, iron presence rose from 6.29 mg/100 g in control to 26.31 mg/100 g in BAB₆, exceeding the daily reference values for this mineral as established by the Codex Alimentarius (Lewis, 2019). No significant differences were observed in Mn across the formulations ($p > 0.05$). Finally, zinc was only higher than the control at BAB₆ ($p < 0.05$). In general, the results highlight the potential of adding ABSF and POSF to improve the nutritional profile of bread through mineral enrichment. Although the increase in key minerals such as calcium and iron could be promising, it is necessary to study their bioavailability. Notably, the bread formulations also demonstrate a reduced sodium

content, with a significant increase in potassium, offering a dual benefit for health by lowering sodium intake while enhancing potassium levels (Castro & Raji, 2013).

3.3. Physico-chemical parameters

3.3.1. pH and water activity (a_w)

pH and Water Activity (a_w) values are shown in Table 3. All the samples analyzed had values of a_w ranging between 0.90 and 0.95. These a_w values were within the range reported for other bread formulated with food co-products such as broccoli stalks and potato peel (Curti et al., 2016; Lafarga et al., 2019). On the other hand, the pH was higher in BAB₃, BAB₆, and BPO₆ compared to the control, with BAB₆ exhibiting a higher pH than BPO₆ ($p < 0.05$). This could be related to the initial pH of both flours, which was 6.18 for ABSF and 6.07 for POSF (Bermúdez-Gómez et al., 2024a). An increase in pH may influence bread texture, often resulting in loaves with greater volume (Sari et al., 2017). In addition, sensory characteristics may also be affected, as higher pH levels can lead to the perception of an alkaline taste (Sari et al., 2017). Therefore, it is essential to analyze both parameters to assess the implications of the pH values observed in the bread examined in this study.

3.3.2. Specific volume

According to the results shown in Table 3, the specific volume was influenced by the concentration of the incorporated flours and the mushroom species of these flours ($p < 0.05$). Specific volume values varied from 1.84 to 3.74 cm³/g. The highest value was observed in the control sample, while the lowest value was achieved for BPO₆ ($p < 0.05$). The specific volume decreased with the addition of the stem flours from both species ($p < 0.05$). This trend has also been observed in previous studies, where the use of fruiting body flours from AB and PO at concentrations between 2% and 20% led to a gradual decrease in specific volume (Losoya-Sifuentes et al., 2022; Sławińska et al., 2022). Salt improves bread quality by regulating fermentation and forming a stable gluten network that traps gas bubbles and prevents their coalescence, which explains the reduced bread volume observed without salt (Silow et al., 2016). Specific volume is widely utilized as a key parameter in evaluating bread quality (Silow et al., 2016). Based on the results achieved, salt replacement with ABSF had a lesser impact on bread quality, resulting in a smaller reduction in specific volume than bread containing POSF ($p < 0.05$). However, complementary analyses, such as crumb structure, moisture retention, or rheological characterization, would be required to confirm this interpretation. Specifically, the volume of BAB₃ (2.81) was higher than that of all formulations with 100% salt replacement incorporating POSF ($p < 0.05$). The volume reduction with POSF in this study (51% in BPO₆) was greater than the 31% decrease reported in bread enriched with 10% PO flour (Losoya-Sifuentes et al., 2022). The differences observed in this study compared to previous

research may be due to earlier studies replacing only wheat flour, whereas this study involved the substitution of both wheat flour and salt. The difference between bread with ABSF and POSF could be related to the IDF content. Djordjević et al., 2022 reported that IDF negatively affects bread volume due to its hydration capacity. During the kneading process, competition for water occurs between starch and IDF, resulting in a disorganized structure that hinders the retention of increasingly larger gas bubbles due to the absence of salt. In the present study, IDF and specific volume were negatively correlated, with a $r = -0.68$ in bread with ABSF and $r = -0.83$ in POSF.

3.3.3. Color measurements

Table 3 shows the L*, a*, b*, C*, and ΔE for the breads elaborated by adding mushroom stems flours. The visual perception of color by consumers is a critical factor influencing product acceptability and varies mainly depending on the composition of raw materials, as well as the specific conditions of dough preparation, fermentation, and baking processes (Zhang et al., 2019). According to the results, the highest L* value was reported for the control, while the lowest value was achieved for BAB₆ ($p < 0.05$). In general, crumbs showed lower L* values in bread with a higher concentration of ABSF and POSF ($r = -0.96$ in bread with ABSF and $r = -0.93$ in POSF). When comparing the L* values of each bread at the same concentration of both species, lower lightness was observed in BAB compared to BPO ($p < 0.05$). The crumb of bread enriched with AB and PO powder was also characterized in other studies by lower L* values and higher a* and b* parameters than the bread with 100% wheat flour (Losoya-Sifuentes et al., 2022; Sławińska et al., 2022; Zhang et al., 2019, 2021). This observation, as suggested by previous authors, could be associated with the color of the incorporated flours (Losoya-Sifuentes et al., 2022; Zhang et al., 2019). However, structural changes in the dough caused by salt reduction and formulation differences may also influence crumb lightness by modifying gas retention and the internal crumb structure, which can affect light reflection (Djordjević et al., 2022; Zhang et al., 2019).

Contrary to L* values, the a*, b*, and C* of the formulations increased with the addition of the mushroom stems flours. While some authors have attributed changes in crumb color exclusively to the initial parameters of the raw materials, this suggestion does not account for the increase in a* observed in BPO. Specifically, the a* value in POSF was reported as 0.18, whereas wheat flour has exhibited values as high as 1.89 (Bermúdez-Gómez et al., 2024a; Hidalgo et al., 2017). Therefore, the color changes observed in breads enriched with ABSF and POSF may also be related to the presence of reducing sugars and free amino acids in mushroom stems, which can promote the formation of melanoidins through Maillard reactions during baking, as well as to the oxidation of phenolic compounds (Losoya-Sifuentes et al., 2022; Sławińska et al., 2022). The highest total color difference ΔE was recorded in BAB₆, followed by BPO₆ ($p < 0.05$). In general, replacing sodium chloride by

Table 3

Physicochemical parameters of bread elaborated by the addition of *Agaricus bisporus* stem flour (ABSF) and *Pleurotus ostreatus* stem flour (POSF).

Sample	pH	a_w	Specific Volume	Color				
				L*	a*	b*	C*	ΔE
Control	5.77 ^c ± 0.03	0.93 ^{bc} ± 0.00	3.74 ^a ± 0.08	61.18 ^a ± 1.21	2.07 ^e ± 0.09	12.07 ^d ± 0.24	12.18 ^e ± 0.26	–
BAB _{0.5}	5.83 ^{bc} ± 0.01	0.95 ^a ± 0.00	3.09 ^b ± 0.09	54.22 ^d ± 1.24	2.81 ^{de} ± 0.09	11.87 ^d ± 0.14	12.20 ^e ± 0.15	6.35 ^d ± 0.89
BAB ₁	5.87 ^{bc} ± 0.02	0.94 ^{ab} ± 0.00	2.75 ^c ± 0.11	55.48 ^e ± 0.70	3.22 ^d ± 0.10	13.15 ^{cd} ± 0.18	13.55 ^{de} ± 0.19	5.95 ^d ± 0.43
BAB ₃	6.00 ^{ab} ± 0.01	0.95 ^a ± 0.00	2.81 ^{bc} ± 0.08	47.41 ^e ± 0.37	4.65 ^c ± 0.06	15.14 ^{bc} ± 0.16	15.84 ^{bc} ± 0.16	14.34 ^b ± 0.45
BAB ₆	6.06 ^a ± 0.01	0.93 ^{bc} ± 0.00	2.54 ^{cd} ± 0.06	43.23 ^f ± 0.39	5.70 ^b ± 0.06	16.21 ^b ± 0.14	17.18 ^b ± 0.15	18.78 ^a ± 0.43
BPO _{0.5}	5.81 ^{bc} ± 0.01	0.94 ^{ab} ± 0.00	2.75 ^c ± 0.13	58.37 ^b ± 0.97	3.18 ^d ± 0.16	13.14 ^{cd} ± 0.11	13.50 ^{de} ± 0.11	2.37 ^e ± 0.21
BPO ₁	5.80 ^{bc} ± 0.01	0.93 ^c ± 0.00	2.31 ^d ± 0.07	54.87 ^e ± 0.47	3.50 ^d ± 0.11	14.06 ^e ± 0.21	14.49 ^{cd} ± 0.22	6.80 ^d ± 0.38
BPO ₃	5.88 ^{bc} ± 0.01	0.92 ^d ± 0.00	2.11 ^{de} ± 0.07	50.78 ^f ± 0.46	4.82 ^{bc} ± 0.06	16.52 ^b ± 0.14	17.21 ^b ± 0.14	11.64 ^c ± 0.55
BPO ₆	5.91 ^b ± 0.02	0.90 ^e ± 0.00	1.84 ^e ± 0.06	48.60 ^f ± 0.82	6.76 ^a ± 0.09	19.23 ^a ± 0.16	20.38 ^a ± 0.16	15.95 ^b ± 0.18

Results are reported as mean ± SEM ($n = 18$). Mean values within the same column followed by different superscript letters (a–h) are significantly different when subjected to Tukey's test ($p < 0.05$). CT—bread with all sodium, white wheat, and without mushroom powder, BAB_{0.5}—bread with 0.5% of ABSF, BAB₁—bread with 1% of ABSF, BAB₃—bread with 3% of ABSF, BAB₆—bread with 6% of ABSF, BPO_{0.5}—bread with 0.5% of POSF, BPO₁—bread with 1% of POSF, BPO₃—bread with 3% of POSF, BPO₆—bread with 6% of POSF. L*: Lightness, a*: redness, b*: yellowness, C*: chroma, ΔE*: color difference.

adding mushroom stem flours resulted in ΔE values exceeding the three-unit threshold necessary to be perceptible to the human eye, except for BPO_{0.5} (Bermúdez-Gómez et al., 2024a).

Overall, these results suggest that the main driver of the observed color changes was the level and type of mushroom stem flour incorporated into the formulation, although structural differences in the crumb and reactions occurring during baking may also have contributed to the final color attributes.

3.3.4. Textural profile analysis

The effect of different types and concentrations of mushroom stem flours on the textural properties of bread is presented in Fig. 1. Bread hardness increased significantly from control to BPO₆ 35.34 ($p < 0.05$), while no significant difference was found between the control and BAB_{0.5} ($p > 0.05$). These findings are consistent with previous studies in which wheat flour was partially replaced with fruiting body powder from both AB and PO at higher concentrations than 0.5% (Lu, Brennan, Serventi, & Brennan, 2018; Sławińska et al., 2022; Srivastava et al., 2024; Zhang et al., 2019). Hardness is a crucial parameter for evaluating bread quality (Lu et al., 2021), and salt plays a key role in dough rheology by reinforcing the non-covalent crosslinks within the gluten network, thereby enhancing texture (Lu, Brennan, Serventi, & Brennan, 2018; Pashaei et al., 2022). Additionally, replacing wheat flour with other gluten-free flours may disrupt the proper formation of the gluten matrix during fermentation and baking (Sławińska et al., 2022). Considering the impact of salt and wheat flour in bread, the results suggested that the incorporation of POSF had a greater influence on bread texture, as BPO₁, BPO₃, and BPO₆ showed a higher hardness compared to BAB₁, BAB₃, and BAB₆ ($p > 0.05$). Moreover, an inverse correlation between bread hardness and specific volume was observed, which aligns with previous reports ($r = -0.75$ in bread with ABSF and $r = -1.00$ in POSF) (Lu, Brennan, Serventi, & Brennan, 2018; Sławińska et al., 2022; Srivastava et al., 2024). Springiness was higher in all breads containing ABSF (0.79–0.91 mm) compared to the control (0.66 mm), while only BPO₃ (0.84 mm) showed this tendency ($p < 0.05$). That finding differs from previous studies, which reported a decrease in

springiness when reducing wheat flour by the addition of fruiting body powder from AB and PO (Lu, Brennan, Serventi, & Brennan, 2018; Sławińska et al., 2022; Srivastava et al., 2024; Zhang et al., 2019). In terms of cohesiveness, only 100% salt replacement by POSF (0.49–0.52) showed significant differences relative to the control (0.64) ($p < 0.05$). The same decrease in cohesiveness has been reported in previous studies with the addition of fruiting body powder of PO (Srivastava et al., 2024). Finally, chewiness increased in BAB₃, BAB₆, and BPO₆ compared to the control ($p < 0.05$). The increment in chewiness in bread containing AB has been documented in previous research; however, no significant differences with white bread were observed for bread containing PO (Srivastava et al., 2024; Zhang et al., 2019).

The TPA profiles observed are likely a synergistic result of the unique chemical composition of the added ingredients, the reduction in sodium chloride levels, and the modified kneading and fermentation protocols employed for the reformulated doughs (Pashaei et al., 2022; Sławińska et al., 2022). The insoluble dietary fiber presence, especially in BPO, could interfere in the starch-gluten matrix developed due to the competition for the available water between IDF and starch (Djordjević et al., 2022). Overall, these findings highlight the influence of both the type and concentration of mushroom stem flour on bread texture and suggest that it is important to further assess whether these changes may affect sensory acceptability.

3.4. Sensory acceptability

The sensory evaluation of all bread formulations is shown in Fig. 2. Despite some significant differences observed among certain bread formulations, most formulations with sodium chloride substitution generally did not affect sensory quality.

In the present study, the crumb sponginess of BPO₆ received a lower score compared to BPO_{0.5} and BAB₁ ($p < 0.05$). This could be attributed to the reduction in volume, which implies lower crumb porosity. The taste evaluation revealed lower score in BPO₆ than BPO_{0.5}, BAB_{0.5}, and the control. The absence of sodium chloride in bread has been associated with tastelessness and the presence of yeasty or sourdough-like flavors

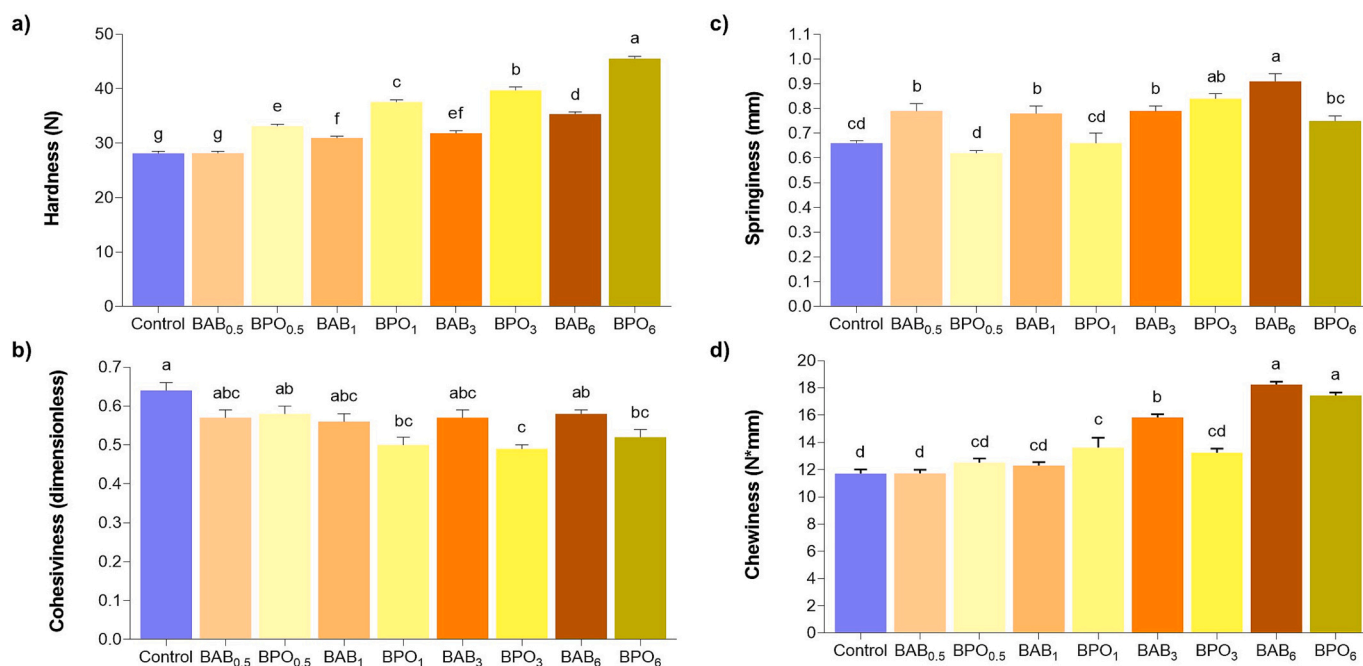


Fig. 1. Texture profile analysis of bread elaborated by the addition of *Agaricus bisporus* stem flour (ABSF) and *Pleurotus ostreatus* stem flour (POSF). (a) Hardness, (b) Springiness, (c) Cohesiveness (d) Chewiness. CT—bread with all sodium, white wheat, and without mushroom powder, BAB_{0.5}—bread with 0.5% of ABSF, BAB₁—bread with 1% of ABSF, BAB₃—bread with 3% of ABSF, BAB₆—bread with 6% of ABSF, BPO_{0.5}—bread with 0.5% of POSF, BPO₁—bread with 1% of POSF, BPO₃—bread with 3% of POSF, BPO₆—bread with 6% of POSF. Results are reported as mean \pm SEM.321.

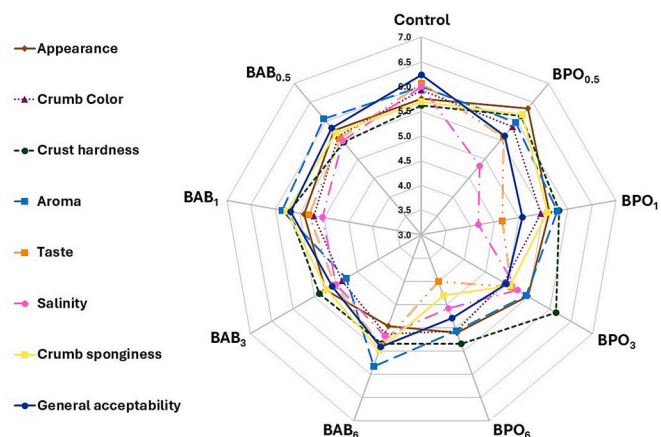


Fig. 2. Radar plot of sensory results of bread elaborated by the addition of *Agaricus bisporus* stem flour (ABSF) and *Pleurotus ostreatus* stem flour (POSF). CT—bread with all sodium, white wheat, and without mushroom powder, BAB_{0.5}—bread with 0.5% of ABSF, BAB₁—bread with 1% of ABSF, BAB₃—bread with 3% of ABSF, BAB₆—bread with 6% of ABSF, BPO_{0.5}—bread with 0.5% of POSF, BPO₁—bread with 1% of POSF, BPO₃—bread with 3% of POSF, BPO₆—bread with 6% of POSF.

(Cirlincione et al., 2026; Silow et al., 2016). Additionally, mushrooms contain volatile and non-volatile compounds, such as 5'-nucleotides, free amino acids, 1-octen-3-ol, organic acids, and soluble carbohydrates, which may influence how consumers perceive their flavor (Sławińska et al., 2022; Zhang et al., 2019). Notably, the EUC index (equivalent of monosodium glutamate, MSG) was generally higher in AB (179) than in PO (48.6), suggesting that ABSF could enhance the flavor of bread

without salt through its umami-related compounds (Poojary et al., 2017). Moreover, in the present study, a higher sodium concentration was observed in breads with ABSF compared to those with POSF. In terms of salinity, both BPO₆ and BPO₁ received lower scores than the control. Finally, in alignment with the results obtained for the other parameters analyzed, BPO₆ was the only formulation that showed significantly lower overall acceptability related to the control. Other authors have documented a similar effect by incorporating PO fruiting body powder into bread (Ndung'u et al., 2015; Srivastava et al., 2024).

3.5. Principal component analysis (PCA)

PCA was used to evaluate the relationships between chemical, physicochemical, and sensory properties of various bread formulations (Fig. 3). The first two principal components explained 73.62% of the total variability. Control samples showed strong positive associations with salinity, volume, taste, and overall acceptability, and negative associations with fiber (TDF) and hardness. Formulations with low levels of mushroom stem flour (e.g., BAB_{0.5}, BPO_{0.5}, and BAB₁) clustered close to the control, indicating minimal impact on evaluated physicochemical, nutritional, and sensory properties. In contrast, samples with higher levels of enrichment (BAB₃, BAB₆, BPO₃, BPO₆) showed greater deviations, especially in color, texture, and sensory acceptance. BPO₆ had high TDF and hardness but poor sensory qualities. Overall, PCA confirmed that mushroom species and enrichment level significantly affect bread characteristics, and careful optimization is needed. Based on these findings, BPO₃, BAB₆, and the control formulation were selected for further analysis of starch hydrolysis, protein digestibility, and mineral bioaccessibility.

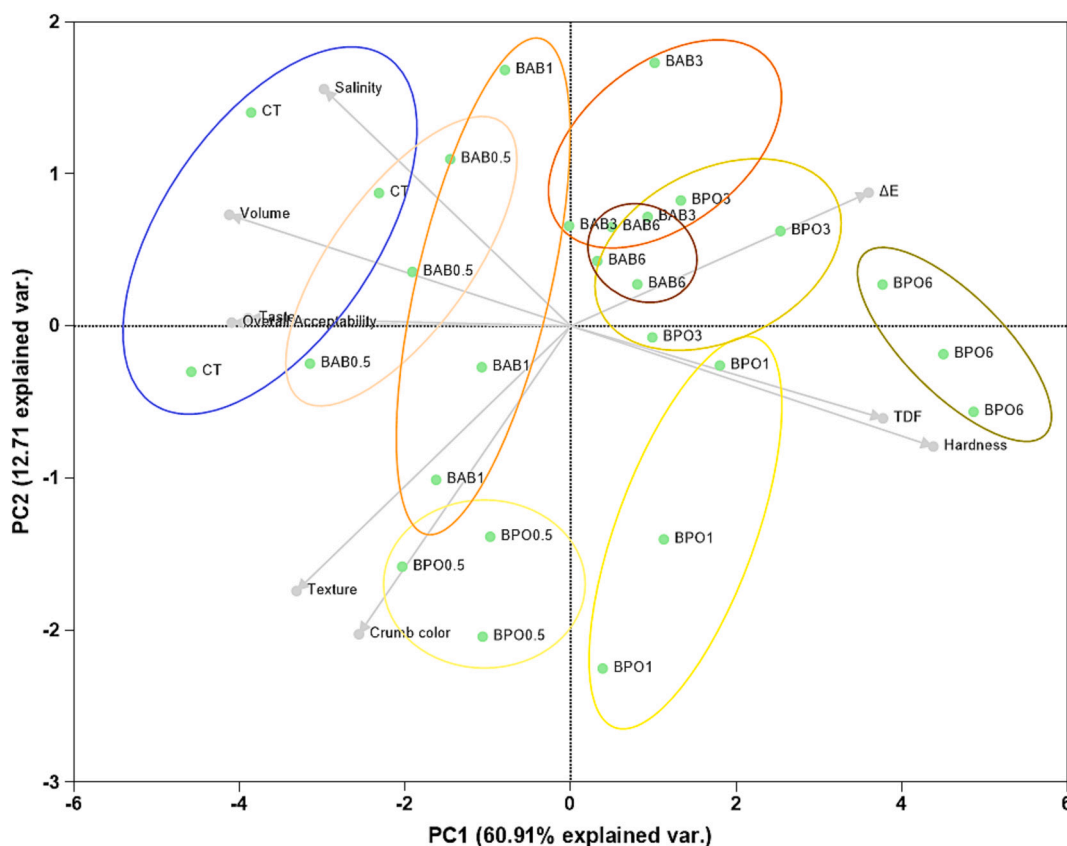


Fig. 3. Principal component analysis (PCA) biplot and component loadings (evaluated parameters). CT—bread with all sodium, white wheat, and without mushroom powder, BAB_{0.5}—bread with 0.5% of ABSF, BAB₁—bread with 1% of ABSF, BAB₃—bread with 3% of ABSF, BAB₆—bread with 6% of ABSF, BPO_{0.5}—bread with 0.5% of POSF, BPO₁—bread with 1% of POSF, BPO₃—bread with 3% of POSF, BPO₆—bread with 6% of POSF, TDF—Total dietary fiber, ΔE—Color difference.

3.6. *In vitro* protein digestibility (IVPD)

Protein quality is primarily defined by its digestibility and the absorption of amino acids, dipeptides, and tripeptides in the gastrointestinal tract (Santos-Sánchez et al., 2024). In this study, protein digestibility kinetics revealed formulation-dependent effects (Fig. 4). During the gastric phase, BPO₃ exhibited significantly lower IVPD than the control, whereas BAB₆ showed enhanced proteolysis ($p < 0.05$). However, at early intestinal digestion (140 min), both salt-free formulations, BPO₃ and BAB₆, displayed higher IVPD than the control ($p < 0.05$). Despite these kinetic differences, all formulations reached similar IVPD values at the end of the intestinal phase (84–85%), aligning with the findings of Constantini et al. in wheat bread (Constantini et al., 2022). This indicates that overall protein digestibility was not compromised by the incorporation of mushroom stem flours at the studied percentages.

These differences in protein hydrolysis kinetics are primarily driven by the food matrix and processing conditions. First, β -Glucans form viscous gels that reduce enzyme diffusion and entrap proteins, limiting gastric proteolysis (Boachie et al., 2021; Łysakowska et al., 2025). Second, the absence of salt necessitated shorter kneading and fermentation times for the BPO₃ dough (Fig. 1a) (Silow et al., 2016), resulting in a denser crumb structure (higher hardness, lower springiness) that may have further hindered enzyme access (Sciarini et al., 2017). Together, these factors explain the delayed gastric kinetic effect observed in BPO₃. Third, salt omission weakens the gluten network by reducing protein aggregation and β -sheet formation (Ukai et al., 2008),

creating a less cohesive matrix that is more susceptible to enzymatic hydrolysis. This effect combined with β -glucans competition with water, which also contributed to disturbing gluten-matrix network explains why both salt-free formulations (BPO₃ and BAB₆) showed higher IVPD than the control during the early intestinal phase (140 min). Interestingly, although chitin has been reported to reduce protein digestibility in insects (Manditsera et al., 2019), no gastric and intestinal delay was observed in BAB₆, likely due to the low inclusion level (6% ABSF) or the less crystalline structure than insect chitin (Izadi et al., 2025). Nevertheless, utilizing confocal laser scanning microscopy (CLSM) and dough rheology are needed to directly visualize gluten network modifications caused by mushroom flour incorporation and salt reduction.

3.7. Starch digestibility and predicted glycemic index

The nutritional profile and starch digestibility of bread samples were significantly altered by the incorporation of mushroom powders (ABSF and POSF) (Fig. 4). While total starch (TS) content remained relatively similar across samples, BPO₃ exhibited the highest value (49.21%), likely due to its lower moisture content (Supplementary Table 2). A critical finding was the significant reduction in RDS in both reformulated breads (<78%) compared to the control (85.22%, $p < 0.05$). Conversely, BPO₃ showed the highest level of RS at 16.40%, nearly double that of the control (8.96%). The *in vitro* digestion kinetics revealed that starch hydrolysis initiated during the oral phase (2 min), with the control bread showing the highest hydrolysis percentage. During the transition to the gastric phase, starch levels in control bread and BAB₆ remained stable; however, BPO₃ experienced a significant increase in hydrolyzed starch (HS%), reaching 42.91% ($p < 0.05$). This behavior in BPO₃ is attributed to polysaccharide–protein interactions that, while limiting protein accessibility, may render the starch granules more susceptible to enzymes under gastric conditions. Upon entering the intestinal phase, all samples peaked at 140 min, with BPO₃ and BAB₆ reaching stability earlier (210 min) than the control. Quantitatively, the hydrolysis kinetic constant (k) was significantly lower for BPO₃ compared to the control, correlating with a reduced pGI (Supplementary Table 2). The control bread maintained a high pGI of 94.61, whereas BPO₃ successfully reduced this value to 89.15 ($p < 0.05$). These results confirm that the synergy between POSF inclusion and modified processing parameters effectively modulates starch fraction distribution and slows down the overall digestive kinetics. The reduction in pGI through the incorporation of PO into wheat bread has been previously reported by Losoya-Sifuentes et al. (2022).

Variations in starch degradation can be attributed to several factors i) processing; the lower hydrolysis rates, particularly in BPO₃, are linked to the reduced fermentation and kneading times used for these formulations. Shorter processing can limit starch gelatinization and mechanical starch damage, resulting in a more enzyme-resistant matrix (Constantini et al., 2022). ii) food macrostructure – starch may interact with proteins and dietary fibers, forming physical barriers that hinder enzyme accessibility and prevent gelatinization, and iii) texture – a compact, dense, and firm food matrix can reduce enzymatic activity by limiting substrate accessibility (Freitas et al., 2025; Lu, Brennan, Serventi, Liu, et al., 2018; Sciarini et al., 2017). Thus, the reduction in starch hydrolysis observed with the incorporation of mushroom powder could be associated with the presence of significant amounts of dietary fiber in the mushroom material (Bermúdez-Gómez et al., 2024a; Lu, Brennan, Serventi, Liu, et al., 2018). Similarly, no differences were found in the kinetic constant or in any of the other parameters analyzed (Supplementary Table 2). This discrepancy may be attributed to structural differences in bread, as reflected in the texture profile analysis (Fig. 1) (Freitas et al., 2025). The less BAB₆ was found to be less hard and compact than BPO₃, showing lower values for both hardness and specific volume. Comparable findings have been reported in previous studies, where the addition of fiber-rich ingredients to starch-based foods led to structural changes substantial enough actually to increase starch

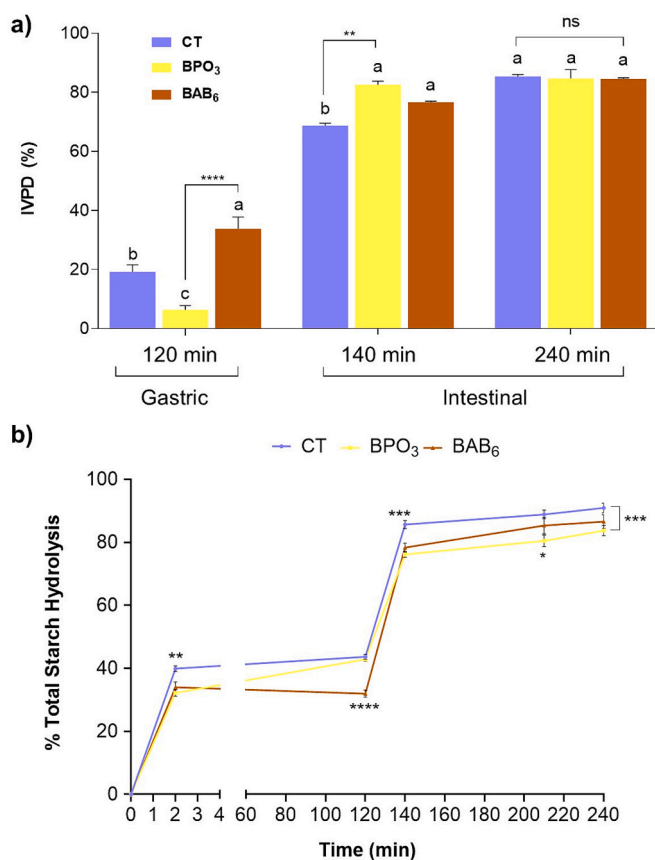


Fig. 4. A *In vitro* protein digestibility of studied breads B. Total starch hydrolysis rate of studied breads; CT—bread with all sodium, white wheat, and without mushroom powder, BPO₃—bread with 3% of POSF, BAB₆—bread with 6% of ABSF.

Asterisks denote statistically significant differences between samples. When not explicitly assigned to a specific comparison, significance is interpreted as a difference between the sample closest to the asterisk and both other samples (** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$).

hydrolysis (Lucas-González et al., 2021; Sciarini et al., 2017).

Differences in starch digestion rates and the associated physiological responses are important considerations when evaluating the nutritional effects of starch-based foods (Freitas et al., 2025). Diets with a high glycemic index—often characterized by rapidly digestible starchy foods—have been linked to an increased incidence of type 2 diabetes, stroke, coronary heart disease, certain cancers such as breast cancer, and obesity-related complications (Freitas et al., 2025). Conversely, low-GI starch sources are commonly found in healthier dietary patterns and are often emphasized in nutritional guidelines (European Food Safety Authority (EFSA), 2011).

3.8. Mineral bioaccessibility

The mineral bioaccessibility values of the samples are presented in Table 4. Significant differences ($p < 0.05$) were observed in the bioaccessibility of magnesium, manganese, and phosphorus between the different formulations. However, no significant differences were found in copper bioaccessibility ($p > 0.05$). The values obtained for Cu and Mn fall within the range previously reported for various wheat bread varieties, whereas those for Mg and P were lower than typically found in the literature (Cetiner et al., 2023). The reduced bioaccessibility of minerals is often attributed to their binding with phytic acid, a naturally occurring antinutrient in wheat (Wolters et al., 1993). During dough fermentation, the decreasing pH enhances the catalytic activity of phytase, the enzyme responsible for degrading phytic acid, thereby improving mineral bioaccessibility (Martins et al., 2017). It could be suggested that the fermentation time in the studied breads was insufficient to allow for adequate phytate degradation, resulting in minerals remaining bound to phytic acid and thus less mineral bioaccessibility. However, the influence of the mushroom flour source must be considered, as ABSF generally reported significantly lower mineral bioaccessibility for Mg and Mn compared to the control and BPO₃ ($p < 0.05$). This occurred despite the longer fermentation time for ABSF (10 min) compared to BPO₃ (5 min). Therefore, the chitin content in the mushroom stipe flours—which is present at higher levels in AB than in PO—may have been a primary factor in reducing mineral bioaccessibility. Similar effects have been observed in studies on chitin-rich insects; for instance, Manditsera al. (2019) reported that boiling edible insects reduced Fe and Zn bioaccessibility by approximately 50%, attributing this to mineral–chitin interactions. Similarly, Dello Staffolo et al. (2011) demonstrated that chitosan retained over 50% of iron *in vitro*, while Gordon and Williford (1983) observed decreased iron absorption in rats fed with chitin-supplemented diets. These results highlight the imperative necessity of evaluating the digestive behavior of reformulated foods to gain a comprehensive understanding of their overall impact on human nutrition. The data demonstrates that the initial mineral content of the breads does not necessarily correlate with their final nutritional value; rather, the matrix release in these products is a critical factor in determining mineral bioaccessibility.

4. Conclusions

This study demonstrates that edible mushroom co-products—specifically flours derived from the stems of *Agaricus bisporus* (ABSF) and *Pleurotus ostreatus* (POSF)—can be effectively used as functional ingredients in the development of low-sodium bread. Their incorporation not only enabled a significant reduction in sodium content but also improved the nutritional profile of bread, notably increasing protein, dietary fiber, and essential minerals such as iron, potassium, and calcium, particularly in formulations containing ABSF. From a technological and sensory standpoint, ABSF performed better than POSF, maintaining greater consumer acceptability without substantially compromising bread volume, texture, or taste. In contrast, POSF—especially at higher concentrations (e.g., BPO₆)—posed more technological challenges, with a marked decrease in specific volume, harder

Table 4

Minerals bioaccessibility of bread elaborated by the addition of *Agaricus bisporus* stem flour (ABSF) and *Pleurotus ostreatus* stem flour (POSF).

Sample	Minerals Bioaccessibility (%)			
	Cu	Mg	Mn	P
CT	11.57 ^a ± 0.62	22.83 ^a ± 0.81	7.38 ^a ± 0.44	12.63 ^a ± 0.22
BPO ₃	10.22 ^a ± 0.57	17.61 ^b ± 0.51	7.49 ^a ± 0.24	9.51 ^b ± 0.47
BAB ₆	9.92 ^a ± 0.12	13.78 ^c ± 0.45	2.94 ^b ± 0.03	9.37 ^b ± 0.05

Results are reported as mean ± SEM ($n = 9$). Mean values within the same column followed by different superscript letters (a–b) are significantly different when subjected to Tukey's test ($p < 0.05$). CT—bread with all sodium, white wheat, and without mushroom powder, BPO₃—bread with 3% of POSF, BAB₆—bread with 6% of ABSF.

texture, and lower sensory scores. In terms of digestibility, the addition of mushroom stem flours did not diminish protein digestibility, as all samples achieved comparable *in vitro* protein digestibility by the end of the intestinal phase. However, a slight decrease in mineral bioaccessibility was observed, highlighting the need to evaluate the digestibility behavior of reformulated foods to have a complete picture of human nutrition improvement. Future strategies such as optimizing fermentation conditions, incorporating phytase-active ingredients, or applying processing techniques that disrupt mineral–fiber complexes may help enhance mineral bioaccessibility. Furthermore, the inclusion of ABSF and POSF reduced starch hydrolysis rates and predicted glycemic index, suggesting additional benefits for glycemic control. Overall, these findings highlight the potential of ABSF and POSF as sustainable, functional ingredients for the formulation of healthier bakery products—aligned with global sodium-reduction strategies and the circular economic approach by valorizing agro-industrial co-products. Further research focusing on the microstructure of these reformulated breads is required to validate the current findings. Specifically, understanding how the absence of salt and the addition of fungal co-products alter the protein-polysaccharide network will provide a deeper insight into the observed shifts in nutrient digestibility and mineral release.

CRedit authorship contribution statement

Patricia Bermúdez-Gómez: Writing – original draft, Methodology, Formal analysis. **Margarita Pérez-Clavijo:** Validation, Supervision, Funding acquisition, Formal analysis. **Manuel Viuda-Martos:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Juana Fernández-López:** Supervision, Data curation, Conceptualization. **Raquel Lucas-González:** Supervision, Methodology, Data curation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2026.149358>.

Data availability

Data will be made available on request.

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