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APPLICATION OF MEDICINAL MUSHROOM EXTRACTS IN SHELF-STABLE GLUTEN-FREE PRODUCTS: PHYSICOCHEMICAL, ANTIOXIDANT AND SENSORY EVALUATION

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ABSTRACT

Global demand for functional gluten-free products has driven the search for new bioactive ingredients. This study evaluated the impact of medicinal mushroom extracts from shiitake (*Lentinula edodes*) and maitake (*Grifola frondosa*) on the physicochemical, bioactive, and sensory properties of gluten-free pasta and rice-flour-based crackers. The total phenolic content was determined using the Folin-Ciocalteu method, while antioxidant activity was assessed via the DPPH radical scavenging assay. Chemical composition, including crude protein and ash content, was analysed according to AOAC standards, and sensory quality was evaluated by a trained panel using a hedonic scale. The results confirmed that the addition of extracts significantly increased ($p<0.05$) the total phenolic content and antioxidant activity in both product types. Furthermore, the incorporation of extracts led to a significant increase in ash content, reflecting an enhanced mineral profile, while a slight relative decrease in crude protein proportion was observed in certain variants. In pasta, a crucial interplay between mushroom extracts and egg fortification was demonstrated; egg-based variants showed markedly better texture and overall sensory acceptance than egg-free samples. Regarding the rice crackers, the 5% shiitake extract variant was identified as optimal for overall impression. The study suggests that shiitake and maitake extracts are promising ingredients for developing functional gluten-free foods with high nutritional value and a favourable sensory profile.

Keywords: gluten-free foods, rice flour, *Lentinula edodes, Grifola frondosa*, antioxidants, sensory analysis

INTRODUCTION

The growing global demand for gluten-free products has stimulated the search for ingredients that can optimize their quality. Rice flour remains a fundamental raw material in this segment due to its neutral flavour, low allergenicity, and high digestibility **(Culetu *et al*., 2021; Park and Kim, 2023)**. However, it's high starch content and lack of gluten present significant technological challenges, including substantial cooking losses and suboptimal textures **(Marana *et al*., 2023)**.
In this context, mushrooms such as shiitake (*Lentinula edodes*) and maitake (*Grifola frondosa*) represent promising functional ingredients. They are rich in proteins and minerals (K, Mg, P) and enrich cereal-based products with dietary fibre, which may influence glycaemic response and increase antioxidant capacity **(Lu *et al*., 2018; Rani *et al*., 2024)**. According to literature, bioactive compounds in these mushrooms, particularly beta-glucans, have been associated with various biological activities, including antioxidant and immunomodulatory properties **(Pinya *et al*., 2019; De Giani *et al*., 2021; Łysakowska *et al*., 2023)**.
Regarding physical properties, mushroom polysaccharides may interact with rice starch, thereby influencing structural integrity and resistance to overcooking **(Heo *et al*., 2014; Lu *et al*., 2016)**. Furthermore, mushroom extracts contain volatile compounds that can modify the sensory profile of gluten-free formulations **(Hiraide, 2006; Jiang *et al*., 2023)**. Their inclusion also affects the colour of the final products, an important factor for consumer acceptance **(Muresan *et al*., 2017)**.
The application of these extracts may provide technological advantages across various shelf-stable products. In gluten-free pasta, they can help mitigate stickiness, while in savoury snacks like crackers, they may act as structural modifiers affecting crispness and oxidative stability **(Sulieman *et al*., 2019; Szydłowska-Tutaj *et al*., 2023a)**. The integration of these mushrooms into gluten-free formulations thus opens new possibilities for developing functional foods that meet criteria for nutritional value and sensory appeal **(Bell *et al*., 2022)**.
The aim of the present study was to develop innovative rice-based gluten-free products (pasta and crackers) and to evaluate the impact of *Lentinula edodes* and *Grifola frondosa* extract addition at various concentrations on their technological, physicochemical, antioxidant, and sensory properties.

MATERIAL AND METHODS

Raw materials

The primary raw material for the production of gluten-free pasta was finely ground rice flour (Mlyn Zrno, Slovakia) with the following nutritional composition per 100g: energy value 1532 kJ/366 kcal; carbohydrates 80.13 g (of which sugars 0.12 g); fibre 2.4 g; proteins 5.95 g; fat 1.42 g; (of which saturates 0.38 g); and salt 0 g. Other ingredients (olive oil, eggs, tapioca starch, vinegar, sodium bicarbonate, and salt) were purchased from the local retail network.
Commercially available powdered extracts of medicinal mushrooms were utilized as functional ingredients: shiitake (*Lentinula edodes*) and maitake (*Grifola frondosa*) (Supplier: One Pharma s.r.o., Slovakia; Origin: Natural Field, China).

Preparation of gluten-free pasta

In this experiment, six variants of gluten-free pasta were designed, differing in the addition of mushroom extract and the type of binding agent used (eggs vs. a combination of olive oil and tapioca starch). The percentage of extract addition was determined based on preliminary technological trials. The amount of added water was adjusted based on the dough's consistency, depending on the ingredients used (Table 1).

Table 1 Formulation of gluten-free pasta variants (per 100 g of composite flour)

Sample	Rice Flour (g)	Shiitake (g)	Maitake (g)	Egg (g)	Tapioca Starch (g)	Water (mL)	Olive Oil (mL)	Salt (g)
P0	100	-	-	-	25	50	2,5	0,2
PS15	85	15	-	-	25	45	2,5	0,2
PM5	95	-	5	-	25	60	2,5	0,2
P0-E	100	-	-	25	-	20	-	0,2
PS15-E	85	15	-	25	-	5	-	0,2
PM5-E	95	-	5	25	-	10	-	0,2

Legend: P0 – egg-free pasta (control); PS15 – pasta with 15% shiitake extract; PM5 – pasta with 5% maitake extract; P0-E – egg pasta; PS15-E – egg pasta with 15% shiitake extract; PM5-E – egg pasta with 5% maitake extract

The preparation of the six dough variants followed a standardized laboratory procedure. Initially, the dry ingredients (rice flour, mushroom extracts, starch, and salt) were accurately weighed and blended using a laboratory mixer to ensure homogeneity. Subsequently, the liquid components (olive oil or whole eggs) were added according to the specific formulation, and the mixture was homogenized again.

The final stage of dough formation involved the gradual addition of hot water (80-90 °C). This step was crucial for the partial gelatinization of rice starch, which served as a structural binder to form a cohesive, compact dough mass.

The prepared dough was processed using a professional pasta extruder (Italgi GINA, Italy) equipped with a spiral-shaped (fusilli) matrix. The extruded pasta samples were spread onto stainless steel trays and dried in a convection dryer (VEVOR, China) at 70 °C for 6 hours. After drying, a portion of each sample was cooked for sensory evaluation, while the remaining samples were stored in airtight containers for further objective physicochemical and antioxidant analyses.

Preparation of gluten-free crackers

The crackers were formulated using rice flour as a base, supplemented with mushroom extracts and common baking ingredients. All formulations included olive oil, wine vinegar, sodium bicarbonate, water, and salt. Five distinct variants were developed, as detailed in Table 2. The control sample (C0) consisted of 100% rice flour, while the enriched samples contained 5% or 10% of shiitake *(Lentinula edodes*) or maitake (*Grifola frondosa*) extracts. For samples with 5% enrichment, water addition was increased to compensate for hydration differences.

Table 2 Recipe for the preparation of gluten-free crackers (per 100 g rice flour)

Simple	Rice Flour (g)	Shiitake (g)	Maitake (g)	Olive Oil (mL)	Vinegar (mL)	Sodium bicarbonate (g)	Water (mL)	Salt (g)
C	100	-	-	10	1,5	1	60	1,5
CS5	95	5	-	10	1,5	1	65	1,5
CM5	95	-	5	10	1,5	1	65	1,5
CS10	90	10	-	10	1,5	1	60	1,5
CM10	90	-	10	10	1,5	1	60	1,5

Legend: C – control crackers; CS5 – crackers with 5% shiitake extract; CM5 – crackers with 5% maitake extract; CS10 – crackers with 10% shiitake extract; CM10 – crackers with 10% maitake extract

The dough preparation involved precise weighing and initial blending of ingredients using a laboratory mixer, followed by manual kneading to ensure a perfectly homogeneous consistency. The resulting dough was rolled out to a uniform thickness of 0.2 cm. The sheets were cut into square shapes (3×3 cm) using a rotary cutter. The crackers were then placed on a baking sheet lined with a silicone mat. To ensure even baking and prevent irregular rising, the surfaces were lightly perforated and brushed with water. The baking process was carried out in a professional deck oven (MIWE condo, Germany) at 200 °C for 15 minutes. After cooling at room temperature, the samples were stored in moisture-proof packaging for subsequent analyses.

Physicochemical composition and water activity

The physicochemical properties of the raw materials (compound flours and mushroom extracts) and the finished products were determined using standardized methods. The nitrogen content (crude protein) was determined according to ICC standard No. 105/2. The mineral content (ash content) was determined according to ICC standard No. 104/1 and dry matter determination by the method (ICC Standard No. 110/1). In the finished products (crekers), dry matter content was determined using a halogen moisture analyser (KERN DBS 60-3, KERN & SOHN GmbH, Balingen, Germany). The measurements were performed using the thermogravimetric method with a 400 W halogen quartz glass heater. The drying process was carried out at 130 °C with a weighing accuracy of 0.001 g until constant weight was reached. The water activity (a_w) was measured using a digital water activity meter (LabMaster-aw, Novasina AG, Lachen, Switzerland) at a controlled temperature (25±0.5 °C). Compositional analyses, including crude protein and ash content, were performed in technical duplicates (n=2), while other physical parameters were measured in triplicate (n=3). The statistical robustness of these determinations was ensured by a strict internal quality protocol; if the variation between parallel samples exceeded the established internal repeatability limits for the specific matrix, the analysis was repeated to ensure the precision and reproducibility of the procedures. All data are expressed as mean values ± standard deviation.

Antioxidant activity (DPPH radical scavenging assay)

The antioxidant properties of raw materials and finished products were determined spectrophotometrically using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay according to **Brand-Williams *et al.* (1995)**, with modifications for microplate application as described by **Kovacikova *et al.* (2025)**. The results were expressed as Trolox Equivalent Antioxidant Capacity (TEAC, mg TE·g^{-1}).

Total polyphenolic content

The total polyphenolic content was determined using the Folin-Ciocalteu reagent method **(Xiong *et al.*, 2020)**, with adjustments for microplate adaptation **(Kovacikova *et al.*, 2025)**. The results were expressed as Gallic Acid Equivalent (GAE, mg GAE·g^{-1}).

All measurements for both assays were performed in octuplicate (n=8), and the data are expressed as mean values ± standard deviation.

Sensory evaluation

Sensory analysis was conducted in accordance with ISO 6658:2017 by a trained panel (n = 12 for pasta; n = 16 for crackers). Evaluation included: Preference traits (Overall appearance, consistency /texture, aroma, taste, and overall impression) and Intensity traits (Aroma intensity, mushroom aroma, taste intensity, and mushroom taste). A 9-point hedonic scale was used, with 9 indicating a strong presence and 1 indicating a total absence for intensity traits. Panellists were encouraged to provide additional qualitative comments for a comprehensive perception analysis.

Statistical analysis

All chemical analyses, including Total Antioxidant Activity (TAA) and Total Polyphenolic Content (TPC), were performed in at least three independent replicates. Data processing was conducted using Microsoft Excel 365 (Microsoft Corporation, Redmond, WA, USA). Statistical significance was evaluated using XLSTAT 2020.5.1 (Lumivero, USA). One-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test was applied to identify significant differences between samples. The threshold for statistical significance was set at $p \leq 0.05$.

The results of the sensory analysis were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test at a confidence level of 0.05 ($p<0.05$). This specific statistical analysis was performed using Statistica Cz version 14 (TIBCO Software, Inc., Palo Alto, CA, USA) and Microsoft Excel 365.

RESULTS AND DISCUSSION

Evaluation of raw materials

The primary objective of this analysis was to evaluate the nutritional and bioactive properties of the raw materials, which dictate the subsequent technological behaviour of the gluten-free matrices. Although rice flour exhibited a significantly higher crude protein content (5.54%) than the mushroom extracts, its functional contribution is limited by the absence of a gluten-forming network. Rice proteins, primarily oryzenins, are characterized by high hydrophobicity, which often results in poor structural integrity. In this context, the mushroom extracts (1.32–1.88% protein) introduce non-gluten proteins and polysaccharides that may act as structural modifiers within the starch matrix.

The mineral fraction (ash content) showed a reverse trend, with shiitake and maitake extracts serving as potent mineral fortifiers compared to the rice base (Table 3). Technologically, the increased ash content can alter the dough's ionic strength, potentially affecting starch hydration and the system's rheological properties. These observations align with those of **Olawuyi and Lee (2019)**, who reported significantly higher ash levels in *L. edodes* powder than in rice flour.

The bioactive potential of these materials is closely linked to their polyphenolic profiles. As reported by numerous studies **(Huang *et al*., 2010; Kosińska *et al*., 2012; Cai *et al*., 2019)**, a significant portion of total antioxidant activity (TAA) is directly correlated with polyphenol concentration. The specific bioactive profiles of the extracts used in this research – shiitake and maitake – were previously characterized in our study **(Bojňanská *et al*., 2025)**.

The striking differences in TAA and TPC between the extracts establish a critical baseline for the technological stability of the final products. Maitake extract demonstrated a significantly higher antioxidant potential (87.928±5.986 mg·g⁻¹ of DM) and polyphenolic content (10.648±0.730 mg·g⁻¹ of DM) compared to shiitake (3.095±0.139 mg·g⁻¹ of DM for TAA and 0.842±0.116 mg·g⁻¹ of DM for TPC, respectively). From a technological standpoint, these polyphenols may act as natural stabilizers, protecting the lipid fraction against oxidation during thermal processing. These results are consistent with those of **Sharpe *et al.* (2021)**, who reported that G. frondosa extracts typically exhibit much higher scavenging activity and phenolic concentration than L. edodes extracts.

In summary, while rice flour provides the starch-rich structural and protein base, the mushroom extracts function as metabolic and structural modifiers, providing minerals and a robust antioxidant defence system, both of which are necessary for maintaining the quality of gluten-free formulations.

Evaluation of gluten-free pasta

The incorporation of mushroom extracts significantly influenced the nutritional and structural framework of the developed gluten-free pasta. Beyond being a rich source of bioactive compounds such as polysaccharides and phenolic acids (**Lu *et al*., 2021; Sławińska *et al*., 2022**), these extracts act as biopolymer modifiers within the starch matrix.

In our study, the protein content showed distinct trends across the base formulations. In egg-fortified variants, the addition of extracts resulted in protein levels of 8.82–8.89% (Table 3), aligning with findings by **Ng *et al.* (2022)** and **Szydlowska-Tutaj *et al*. (2023b)**. Technologically, the interaction between egg proteins and mushroom polysaccharides is crucial; the egg proteins provide the necessary cohesive network that rice flour lacks, while the mushroom components integrate into this matrix, potentially increasing its density.

In contrast, a slight decrease in nitrogenous substances was observed in non-egg variants (PS15, PM5) compared to the control (P0). This phenomenon, which partially contrasts with **Ndung'u *et al.* (2015)**, can be attributed to the specific form of the additive. Unlike whole mushroom powders, the concentrated extracts used here are primarily composed of soluble carbohydrates and minerals, which may cause a "dilution effect" on the total protein fraction within the dry matter (DM).

The ash content, an indicator of mineral content, confirmed the synergistic effect of the extracts and the egg component. The highest value was recorded for egg-based pasta containing shiitake extract (PS15-E; 0.89%), reflecting the high mineral content of *L. edodes.* From a technological perspective, increased mineral content (ionic strength) is known to influence hydration equilibrium and starch network interactions during dough processing. These physico-chemical changes likely contribute to the modified cooking quality and textural profiles observed in mushroom-enriched pasta, as reported by **Szydlowska-Tutaj *et al.* (2021)** and **Lu *et al.* (2016).**

Table 3 Crude protein and ash content of raw materials, gluten-free pasta, and gluten-free crackers

Sample	Crude Protein (%)	Ash Content (%)
Rice	5.84±0.13	0.30±0.08
Shiitake	1.33±0.16	4.60±0.23
Maitake	1.88±0.06	2.94±0.01
P0	6.51±0.07	0.32±0.05
PS15	5.23±0.14	0.38±0.03
PM5	4.66±0.08	0.33±0.04
P0-E	8.29±0.53	0.50±0.13
PS15-E	8.89±0.01	0.89±0.17
PM5-E	8.82±0.10	0.80±0.20
C	5.51±0.09	0.13±0.04
CS5	5.66±0.07	0.61±0.04
CS10	5.59±0.14	0.84±0.04
CM5	5.50±0.14	0.71±0.01
CM10	4.97±0.11	0.78±0.03

Legend: P0 – egg-free pasta (control); PS15 – pasta with 15% shiitake extract; PM5 – pasta with 5% maitake extract; P0-E – egg pasta; PS15-E – egg pasta with 15% shiitake extract; PM5-E – egg pasta with 5% maitake extract; C – control crackers; CS5 – crackers with 5% shiitake extract; CM5 – crackers with 5% maitake extract; CS10 – crackers with 10% shiitake extract; CM10 – crackers with 10% maitake extract. Values are expressed as mean ± standard deviation (n=2). All results are reported on a 100% dry matter basis.

The functional profile (Table 4) revealed that maitake-fortified pasta (PM5 and PM5-E) exhibited superior TAA ($p<0.0001$). This confirms that bioactive molecules from *G. frondosa* remain stable and effective even within a complex, starch-heavy pasta matrix. Although the egg-based control (P0-E) showed high TPC ($p<0.05$), the mushroom extracts significantly boosted the overall radical-scavenging activity. This enhancement is likely due to the thermostability of fungal polyphenols and their ability to form complexes with the starch-protein network, protecting them during the drying process. These results align with those of **Szydlowska-Tutaj *et al.* (2023a)** and **Quan *et al.* (2023)**, confirming that mushroom enrichment substantially increases antioxidant capacity.

While the DPPH and Folin-Ciocalteu assays provide a reliable basis for comparing antioxidant potential and total phenolic content across samples, certain methodological considerations should be noted. The Folin-Ciocalteu method measures total reducing capacity, which may include minor contributions from other reducing agents in the mushroom matrix **(Sánchez-Rangel *et al*., 2013)**. Similarly, the DPPH assay assesses radical-scavenging capacity under specific in vitro conditions. Although these assays are standard in food science for comparative purposes, future research could include complementary methods (such as FRAP or ORAC) to provide broader functional characterization.

Table 4 Total antioxidant activity (TAA) and total polyphenol content (TPC) in enriched gluten-free pasta

	P0	PS15	PM5	P0-E	PS15-E	PM5-E
TAA (mg TE·g⁻¹)	2.200±0.051^{c}	2.361±0.128^{c}	3.769±0.318^{a}	2.238±0.310^{c}	2.630±0.072^{b}	3.703±0.230^{a}
TPC (mg GAE·g⁻¹)	1.796±0.432^{c}	2.353±0.356^{b}	2.633±0.197^{ab}	2.865±0.607^{a}	2.473±0.431^{ab}	2.518±0.452^{ab}

Legend: P0 – egg-free pasta (control); PS15 – pasta with 15% shiitake extract; PM5 – pasta with 5% maitake extract; P0-E – egg pasta; PS15-E – egg pasta with 15% shiitake extract; PM5-E – egg pasta with 5% maitake extract. Values are expressed as mean ± standard deviation (n=8). Means in the same row followed by different letters are significantly different (TAA: $p<0.0001$, TPC: $p<0.05$).

Evaluation of gluten-free crackers

The development of gluten-free rice crackers enriched with 5% and 10% mushroom extracts aimed to enhance the nutritional and bioactive profile of the starch-based matrix. The protein content in the crackers exhibited a specific trend: while 5% fortification (CM5; 5.66%) showed a slight increase compared to the control (5.51%), 10% fortification (CM10; 4.97%) led to a decrease. This confirms a "dilution effect" where the concentrated extract, rich in non-protein solids, displaces a portion of the rice flour protein. This contrasts with studies using whole mushroom powders **(Toan and Thu, 2018; Szydłowska-Tutaj *et al.*, 2023b)**, which highlights the different functional impacts of extracts versus fruiting body flours.

Technologically, the bioactive compounds in shiitake, such as beta-glucans, may influence the matrix by modifying protein solubility and digestibility **(Tu *et al.*, 2021a)**. Furthermore, the significant increase in ash content (max. 0.84% in CM10) confirms the mineral-fortifying potential of *G. frondosa* and *L. edodes* **(Regula and Gramza-Michałowska, 2009; Srivastava *et al.*, 2024)**. These mineral fractions, along with dietary fibre, may interact to enhance mineral retention during the baking process **(Zhao *et al.*, 2022; Tu *et al.*, 2021b).**

A critical observation was the downward trend in dry matter and the concurrent rise in water activity (a_w) as extract concentrations increased (Figure 1a, b). The highest a_w (0.880 in CM10) significantly exceeds the stability threshold of 0.6 **(Labuza and Altunakar, 2020; Rani *et al.*, 2024)**. This phenomenon can be attributed to the high hygroscopicity of the mushroom extracts. From a macromolecular perspective, the soluble fibres and low-molecular-weight carbohydrates in the extracts possess a high density of hydroxyl groups, which establish strong hydrogen bonds with water molecules.

This high water-binding capacity, as reported by **Spim *et al.* (2020)**, prevents efficient moisture loss during baking. In a gluten-free rice matrix lacking a strong protein network to facilitate steam release, these extracts act as humectants, retaining water within the crumb. This poses a technological challenge, as elevated a_w levels may compromise microbiological stability and the characteristic crispness of the crackers. Consequently, the use of high extract concentrations (10%) necessitates adjustments to baking parameters (time/temperature) or to the formulation to ensure a shelf-stable final product.

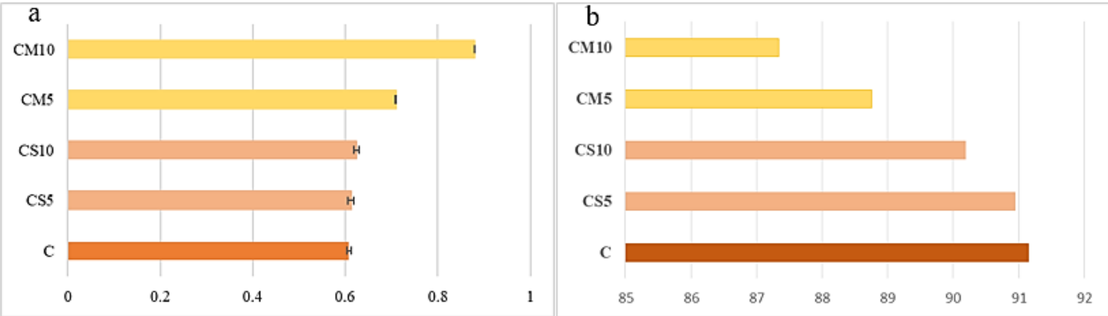

Figure 1 (a) Water activity in gluten-free crackers; (b) Dry matter content in gluten-free crackers
Legend: C – control crackers; CS5 – crackers with 5% shiitake extract; CM5 – crackers with 5% maitake extract; CS10 – crackers with 10% shiitake extract; CM10 – crackers with 10% maitake extract. Values are expressed as mean ± standard deviation (n=3).

The most prominent benefit of the fortification was the significant increase in Total Antioxidant Activity (TAA) and Total Polyphenolic Content (TPC) (p<0.0001 for TAA; p<0.05 for TPC). The 10% maitake variant reached the highest TAA (3.810±0.218 mg.g^{-1} of DM), while both 10% variants shared the highest TPC group (approx. 1.96 mg.g^{-1}) (Table 5).

Table 5 Total polyphenol content (TPC) and total antioxidant activity (TAA) in enriched gluten-free crackers

	C	CS5	CS10	CM5	CM10
TAA (mg TE·g^{-1})	2.586±0.0761^{a}	3.008±0.418^{bc}	3.134±0.372^{a}	2.829±0.128^{ab}	3.810±0.218^{a}
TPC (mg GAE·g^{-1})	1.401±0.204^{c}	1.600±0.219^{bc}	1.954±0.412^{a}	1.766±0.254^{ab}	1.973±0.205^{a}

Legend: C – control crackers; CS5 – crackers with 5% shiitake extract; CM5 – crackers with 5% maitake extract; CS10 – crackers with 10% shiitake extract; CM10 – crackers with 10% maitake extract. Values are expressed as mean ± standard deviation (n=8). Means in the same row followed by different letters are significantly different (TAA: p<0.0001, TPC: p<0.05).

These trends align with **Olawuyi and Lee (2019)** and **Toan and Thu (2018)**, where higher mushroom concentrations corresponded to increased bioactive potential. This suggests that fungal polyphenols possess high thermal stability during dry heat processing. Furthermore, the Maillard reaction during baking likely generated antioxidant melanoidins, contributing to the overall radical-scavenging capacity. While some literature suggests these antioxidants can improve sensory properties by masking off-flavours **(Sulieman *et al.*, 2019)**, our results indicate that at higher concentrations (10%), the technological drawbacks – specifically the increased water activity (a_w) and decreased dry matter – must be carefully balanced against the substantial functional gains to maintain the desired texture and shelf life.

Sensory evaluation of pasta

The sensory profiles (Figures 2a, b) indicate that mushroom extracts significantly influenced the organoleptic properties of the gluten-free pasta (p<0.05). Non-egg variants generally received lower scores, with the control (P0) performing worst in consistency (1.83) and overall impression (3.08). The addition of 5% maitake (PM5) significantly improved these parameters, particularly smell (5.33) and appearance (4.75), confirming that mushroom volatiles can mask the neutral profile of rice flour, as noted by **Cordón *et al.* (2023).**

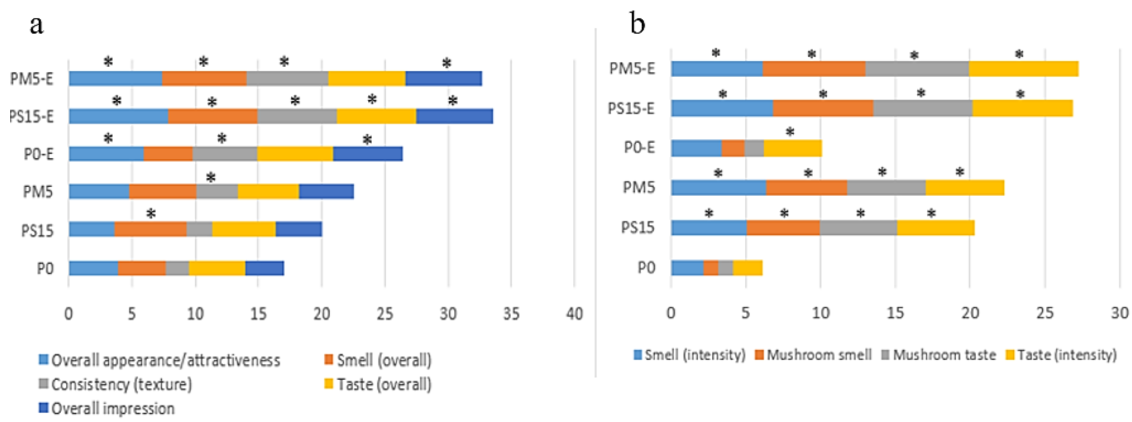

Figure 2 Sensory evaluation of gluten-free pasta with added shiitake and maitake extracts: (a) preferential properties and (b) intensity properties
Legend: P0 – egg-free pasta (control); PS15 – pasta with 15% shiitake extract; PM5 – pasta with 5% maitake extract; P0-E – egg pasta; PS15-E – egg pasta with 15% shiitake extract; PM5-E – egg pasta with 5% maitake extract; * p<0.05 indicates a statistically significant difference compared to the control.

Egg-fortified samples (PS15-E, PM5-E) were rated significantly higher across all attributes. The 15% shiitake variant (PS15-E) excelled in appearance (7.83), while 5% maitake (PM5-E) achieved the highest scores for taste (7.33) and texture (6.42). This improved consistency in egg-based formulations is likely due to the synergy between egg proteins and mushroom polysaccharides, which creates a more cohesive protein-starch matrix. This is supported by **Wang *et al.* (2020)**, who attributed increased firmness to interactions between mushroom lipids and the starch matrix, and by **Lu *et al.* (2016)** regarding enhanced thermomechanical properties.
Despite these improvements, a persistent surface stickiness after cooking was observed. This suggests that while mushroom components and eggs improve dough firmness, they do not fully prevent amylose leaching from the rice starch granules during boiling. This common technological challenge in gluten-free systems indicates that while the extracts enhance flavour and colour **(Ko and Joo, 2009)**, further optimization of the hydrocolloid matrix is required to achieve full consumer acceptance and eliminate post-cooking adhesiveness.

Sensory Evaluation of Crackers

The sensory profiles of the gluten-free rice crackers (Figures 3a, b) show that mushroom extracts significantly influence both preference and intensity attributes ($p<0.05$). The 5% shiitake variant (CS5) achieved the highest overall acceptance, with peak scores for appearance (8.19) and taste (6.81), identifying this concentration as the sensory optimum. In contrast, higher fortification (10%) led to decreased scores, particularly for CM10 (appearance: 6.06).
This decline in visual appeal is linked to significant surface darkening. While **Duta and Culetu (2015)** attribute browning in biscuits to protein content, our study suggests a different mechanism: the high concentration of natural fungal pigments and free reducing carbohydrates in the extracts, which accelerate non-enzymatic browning (Maillard reaction) during baking. This aligns with **Olawuyi and Lee (2019)**, who noted similar darkening in rice-based systems.

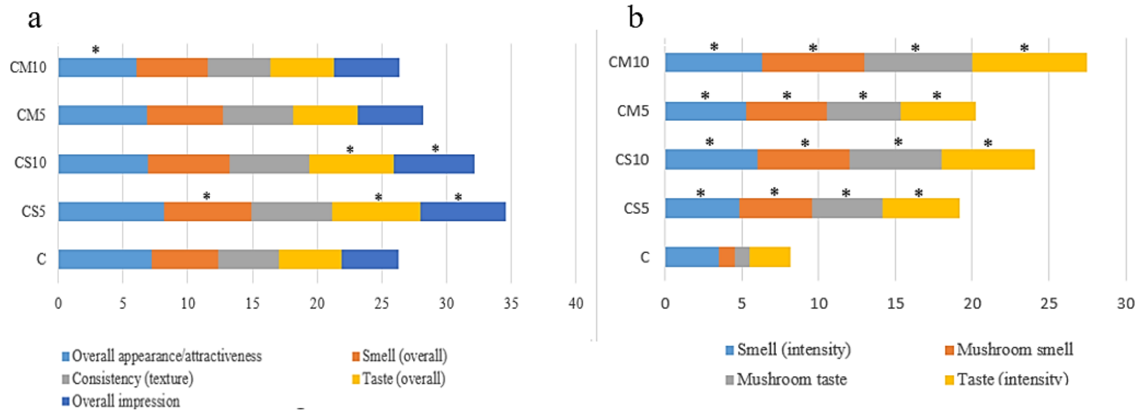

Figure 3 Sensory evaluation of gluten-free crackers with added shiitake and maitake extracts: (a) preferential properties and (b) intensity properties
Legend: C – control crackers; CS5 – crackers with 5% shiitake extract; CM5 – crackers with 5% maitake extract; CS10 – crackers with 10% shiitake extract; CM10 – crackers with 10% maitake extract; * $p<0.05$ indicates a statistically significant difference compared to the control.

Figure 4 Prepared gluten-free products with added shiitake and maitake extracts
Legend: P0 – egg-free pasta (control); PS15 – pasta with 15% shiitake extract; PM5 – pasta with 5% maitake extract; P0-E – egg pasta; PS15-E – egg pasta with 15% shiitake extract; PM5-E – egg pasta with 5% maitake extract; C – control crackers; CS5 – crackers with 5% shiitake extract; CM5 – crackers with 5% maitake extract; CS10 – crackers with 10% shiitake extract; CM10 – crackers with 10% maitake extract

The intensity of mushroom aroma and taste followed a direct dose-response trend, peaking in CM10 (taste: 7.50). Regarding texture, shiitake variants (CS5: 6.25; CS10: 6.19) performed best. This structural modification is attributed to the interaction between fungal fibre and rice starch, which affects water distribution and the degree of gelatinization within the dough matrix **(Tu *et al.*, 2021b)**.
Our findings correspond with **Bojňanská *et al.* (2024)**, confirming that shiitake is generally more acceptable in cracker formulations than maitake. While mushroom components up to 10% do not compromise the basic profile **(Ng *et al.*, 2022)**, the intense, specific flavours may contrast with consumer expectations for mild gluten-free products **(Stantiall and Serventi, 2018)**. Successful formulation thus requires a balance between functional enrichment and sensory acceptance, potentially through recipe adjustments to manage the intense flavour profiles of medicinal mushrooms **(Sangiorgio *et al.*, 2023)**.
Photographs of individual gluten-free products containing extracts from medicinal mushrooms are shown in Figure 4.

CONCLUSION

This study confirms that the incorporation of Lentinula edodes and Grifola frondosa extracts modifies the nutritional and bioactive profiles of gluten-free pasta and rice-based crackers. The addition of these extracts increased total phenolic content (TPC) and antioxidant activity in both product types.
Among gluten-free pasta, the 15% shiitake and 5% maitake egg-based variants achieved the highest consumer acceptance for appearance and flavour. In the case of rice-based crackers, the 5% shiitake variant was identified as optimal for overall impression. Higher concentrations (10%) led to significant surface darkening, which reduced visual scores despite the increased antioxidant potential.
In conclusion, mushroom extracts are suitable functional ingredients for gluten-free cereal products. Future research should focus on further optimizing the texture of non-egg formulations and managing browning intensity in baked snacks to balance the enrichment of bioactive compounds with sensory requirements.

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