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Sensorial and chemical characterization of a new food ingredient of *Rhizopus oligosporus* biomass produced under solid and submerged state fermentation

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ABSTRACT

The current food system relies heavily on intensive crop and livestock production, raising sustainability concerns. Diversifying dietary protein sources is essential for reducing environmental impact and enhancing food system resilience. Filamentous fungi, such as *Rhizopus oligosporus*, offer a promising alternative due to their high protein content and functional properties. This study characterized the chemical and sensorial profile of *R. oligosporus* biomass produced through solid-state fermentation (SSF) and submerged state fermentation (SmF) assessing its potential as a new food ingredient.

GC–MS analysis revealed that SmF biomass contained significantly higher concentrations of flavor-active compounds such as 5-hydroxymethyl-2-furfural (18.81 μg g^{-1}), phenylethyl alcohol (2.41 μg g^{-1}), and ergosterol (25.03 μg g^{-1}), which are associated with caramelized, floral, and umami notes. In contrast, SSF biomass exhibited a milder, more neutral profile, with lower concentrations of these volatiles.

To assess consumer perception, a sensory evaluation was conducted with a panel of participants with gastronomic training ($n = 101$) using a CATA questionnaire and a 9-point hedonic scale. SmF biomass was more often associated with "meaty texture", "chewy", and "umami" attributes, and its acidity was significantly more liked (5.70 ± 1.99) compared to SSF (4.48 ± 1.90, $p < 0.05$). Natural Language Processing (NLP) identified four key sensory themes, 'texture,' 'umami,' 'meatiness,' and 'fermentation', highlighting consumer perceptions and guiding potential applications. Despite moderate hedonic scores (4.26–4.82), the biomass specially produced via SmF, demonstrated a fibrous, meat-like texture and a complex aroma profile, supporting its integration into both savory and sweet food formulations. The study demonstrates that fermentation tecnique significantly modifies the chemical composition of fungal biomass, directly impacting sensory perception. These findings underscore the potential of fungal biomass as a versatile ingredient for novel, sustainable food products.

1. Introduction

Although thousands of edible species exist worldwide, human nutrition and food production rely on only a small number of them. For instance, of the approximately 6000 plant species cultivated for food, fewer than 200 contribute substantially to global food production, with just nine accounting for over 66 % of total crop production (FAO, 2019). This limited biodiversity underscores the need to explore novel and underutilized food sources, including microbial-based ingredients, to enhance sustainability and resilience in global food systems. Similarly, in livestock production, the global food supply depends on around 40 animal species, of which only a handful provides most of the meat, milk, and eggs consumed (Food and Agriculture Organization of the United Nations, 2021). This heavy reliance on a narrow range of species increases the vulnerability of food systems to diseases, pests, and climate change while also restricting our capacity to meet future food demands and introducing socio-economic limitations (Tchonkouang et al., 2024). In a global context where food security, sustainability, and biodiversity

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are critical priorities, diversifying food sources emerges as an essential strategy (Varzakas and Smaoui, 2024).

In this regard, edible microorganisms, including yeasts, bacteria, microalgae, and filamentous fungi, have emerged as promising biotechnological resources for food production. These microorganisms are rich sources of proteins, vitamins, and beneficial lipids, positioning them as viable alternatives to conventional food sources. They offer a sustainable alternative to traditional food production methods, which are often resource-intensive and environmentally damaging (Linder et al., 2019). Unlike conventional agriculture, the cultivation of microbial biomass does not require arable land, making it an efficient option for regions with limited agricultural capacity. This feature aligns with the growing need for resilient food systems capable of adapting to climate change (Matassa et al., 2016). Furthermore, microbial growth can occur on simple organic substrates, enabling industrial-scale production that does not compete with traditional agriculture. By reducing dependence on conventional feed sources such as soy and fishmeal, microbial-based foods contribute to resource efficiency and the development of more sustainable food chains (Matassa et al., 2016; Linder et al., 2019; Kusmayadi et al., 2021).

Microorganisms contribute significantly to the development of new foods and flavors, the diversification of food matrices, and the reuse of food by-products. For instance, fermentation with selected microbial strains has been used to convert bread waste into beverages (Sharma et al., 2019; Massa et al., 2022). Similarly, the bio-valorization of okara, a soybean by-product, through microbial fermentation has been investigated to enhance its digestibility, reduce off-flavors, and promote its use as a functional food ingredient (Feng et al., 2021). More recently, advances in precision fermentation and synthetic biology have enabled the engineering of microorganisms to produce alternative proteins and high-value ingredients, enhancing nutritional quality and sustainability in food systems (Mirsalami and Mirsalami, 2025).

Among the most widely used microorganisms in food production are filamentous fungi, which have long been utilized in culinary traditions around the world. These fungi have enhanced the nutritional and sensory profiles of various foods, enabling the development of diverse products from a single substrate (Molfetta et al., 2022; Maini Rekdal et al., 2024a). A classic example is their role in cheese production, where filamentous fungi contribute to both flavor and texture (Metin 2018). Beyond traditional applications, these microorganisms have enabled the creation of entirely new foods from undervalued substrates. For instance, while tempeh traditionally uses soybeans, it has since been adapted to include fruits, grains, or nuts, demonstrating the fungi's flexibility in modern gastronomy (Cantabrana et al., 2015). Moreover, ongoing research continues to explore the applications and capabilities of filamentous fungi, like the development of a fungal burger made from *Aspergillus oryzae* (Rousta et al., 2021) or the production of a new ingredient made from acid-tolerant *Rhizopus oligosporus* (Massa et al. 2024). Despite the increasing use of filamentous fungi in diverse food systems, sensory characterizations have largely focused on their role within composite food matrices, such as their influence on the flavor or texture of products like tempeh or fungal-based meat analogs.

However, fermentation techniques are known to profoundly alter the physicochemical, nutritional, and sensory attributes of food materials, often enhancing digestibility and enriching the concentration of bioactive compounds. These transformations make fermentation a powerful tool for converting raw substrates into functional and palatable ingredients.

Rhizopus oligosporus, a filamentous fungus from the Mucoraceae family, has long been used in the preparation oftempeh, a fermented food primarily made primarily from soybeans, (Glycine max) (Ahnan-Winarno et al., 2021). Moreover, ongoing research continues to explore the applications and capabilities of filamentous fungi, like the development of a new ingredient made from acid-tolerant *Rhizopus oligosporus* (Massa et al., 2024). While tempeh has been extensively studied from a sensory perspective (Wikandari et al., 2021), recent studies have also compared fungal-based meat analogs to traditional proteins like chicken and soy. (Barkman et al., 2023). However, *R. oligosporus* biomass has not yet been characterized as an isolated ingredient, independent or further processing or added components.

To address this gap, a combined chemical and sensory characterization of *Rhizopus oligosporus* obtained through two biomass fermentation methods: SSF and SmF (submerged-state fermentation). In this regard, an untargeted GC–MS analysis was performed to elucidate volatile compounds profile of the standalone biomass. In parallel a consumer study using a hedonic scale and CATA (check-all-that-apply) methodology provided sensory insights, complemented by qualitative analysis of open-ended responses. This multidisciplinary approach enables a comprehensive assessment of sensory attributes and potential applications, drawing on a consumer panel with gastronomic training.

2. Materials and methods

2.1. Raw material

The *R. oligosporus* saito strain, sourced from TopCultures in Zoersel, Belgium, was genetically identified by the Westerdijk Fungal Biodiversity Institute in Amsterdam, Netherlands. DNA isolation was performed using the Qiagen DNeasy Ultraclean™ Microbial DNA Isolation Kit. The resulting sequences were compared to those in the GenBank database and the internal sequence database of the Westerdijk Fungal Biodiversity Institute.

Biomass production followed the methodology described by Massa et al. (2024), employing both SSF and SmF fermentation under acid-tolerant conditions (pH 3). The culture media included the following nutrients: diammonium phosphate (DAP) (Prayon, Belgium), yeast hull (ActimaxCorcell, Argovin, Spain), malt extract syrup (Finestra Cielo, Spain), lactic acid (Corbion, Spain), and agar-agar (Guthaus, Spain).

2.2. Analysis

2.2.1. GC–MS analysis

For GC–MS analysis, biomass was harvested from the cultivation medium and subsequently freeze-dried at −50 °C for 24 h using a Teslar, LyoQuest freeze-dryer (Spain). Two grams of freeze-dried biomass were mixed with 1 mL of methanol. Then, 0.25 μL of 1-dodecanol, diluted in methanol at a 1/100 ratio as the internal standard. The mixture was agitated on a thermoshaker (RET BASIC, IKA, Spain) for 30 min at 2.24 x g. Then, the mixture was transferred to an Eppendorf tube and centrifuged for 10 min at 32.3 x g (Minispin, Eppendorf, Germany). The resulting supernatant was carefully collected for subsequent GC–MS analysis. This entire procedure was performed in triplicate to ensure reproducibility. Samples were analyzed in a 7890A gas chromatograph coupled to a 5975C single quadrupole mass spectrometer, both from Agilent Technologies (Palo Alto, CA, USA). Chromatographic analyses were carried out on a Zebron ZB-5 capillary column (30 $m \times 0.25$ mm i. d., 0.2 μm film thickness; Phenomenex, CA, USA) using helium carrier gas at a flow rate of ~1 mL min $^{-1}$. Injections (1 μL) were performed in a split mode (1:15) at a temperature of 245 °C. The oven temperature was programmed from 50 to 300 °C at 10 °C min $^{-1}$ and held for 10 min.

The substrate sample was processed in the same way as the biomass. To determine whether the compounds originated from either the fungal biomass or the culture medium, the chromatograms were overlaid and compared to determine their source.

For data acquisition and analysis, MSD ChemStation software (Agilent Technologies, Palo Alto, CA) was used. Peak identifications were performed employing the Wiley library database (McLafferty, F. W., Stauffe, D. B., The Wiley/NBS Registry of Mass Spectral Data, Wiley, New York 1989).

2.2.2. Sensory analysis

Before sensory evaluation, the biomass was pasteurized in an autoclave at 120 °C for 20 min. After pasteurization, it was rapidly cooled in an ice bath for 15 min and then stored at 5 °C until the tasting session. Approximately 2 g of each sample dish was placed on a disposable plate, each labeled with a unique three-digit code. The biomass growth under SmF was coded as 667 (Fig. 1A), and the biomass obtained from SSF was coded as 407 (Fig. 1B). Sensory characterization was conducted using two objective and quantitative tests, CATA and 9-point hedonic scale as well as by using 2 objective and quantitative tests (CATA and Hedonic scale) as well as subjective and qualitative open-ended questions.

2.2.2.1. Participants. The protocol for the consumer study was approved by the Mondragon Unibertsitatea Research Ethics Committee (IEB-2024,021). The study complied with regulations on the protection of natural people regarding the processing of personal data. A total of 101 healthy adult participants were recruited from the Basque Culinary Center in Spain, ($n = 101$; F: 41 M: 60, age range 18–52 years; mean age 23.7 ± 5.9 years) and represent diverse national backgrounds. Individuals with allergies to gluten, fungi, or malt allergies were excluded. Tastings were conducted at Basque Culinary Center's sensorial analysis room, designed in compliance with ISO 8589:2010 standards.

2.2.2.2. Focus group. The focus group was designed following methods adapted from Rodrigues et al. (2022) and was employed as a targeted approach for descriptor in generation. Each session was guided by a trained moderator, and all comments and attributes identified during the discussion were transcribed by a designated note-taker. Each focus group lasted 60 min and was composed of six professionals from the gastronomy sector with experience in the production and consumption of fermented foods. Three sessions were conducted in total. The most relevant and frequently repeated attributes were used to develop the CATA questionnaire. To identify the most referenced sensory attributes, terms mentioned by participants were first grouped by consolidating synonyms and eliminating redundancies. The frequency of each attribute was then quantified based on the number of times it was mentioned across sessions. Additionally, agreement among participants was evaluated within each session to determine whether specific attributes were consistently recognized and shared by the majority. Focus group participants were assessed within the same session to determine whether a specific attribute was consistently recognized and shared by the majority. Attributes that were mentioned most frequently were included in the CATA questionnaire.

2.2.2.3. Check All That Apply. CATA method was used to evaluate the sensory attributes of the samples (Ares and Varelas 2018). The list of descriptors was based on the results of the focus group discussion. Participants were asked to select all applicable attributes from a predefined list divided into two categories, as shown in Table 1. A total of 27 attributes were included: 15 related to taste, and 12 related to texture. Additionally, an "Other" section was provided to allow participants to include any descriptors they felt were missing. The questionnaire was administered using a Google form (Google, 2024).

2.2.2.4. Hedonic characterization. To assess consumer acceptance of the sample, a 9-point hedonic scale was employed (Jones et al., 1955; Peryam and Haynes, 1957). Participants were asked to rate how much they liked a specific characteristic of the product on a scale from 1 to 9, where: 1= "Dislike extremely", 5="Neither like nor dislike", 9= "Like extremely". The scale was used to evaluate overall liking, as well as liking of specific attributes such as taste, acidity and texture. This allowed for assessing whether the low pH (pH3) of the cultivation medium had positive or negative influence on the sensory properties of biomass.

2.2.3. Open answer

At the end of the questionnaire, participants were asked to answer a set of open-ended questions to broaden the understanding of consumer perceptions regarding this type of ingredient (Schuman and Presser, 1979; Reja et al., 2003). These questions were grouped into three categories. The first category aimed to explore participants' attitudes toward fermented foods and their dietary preferences. The second focused on perceptions of the product's taste and its potential applications in new food developments. The third category assessed familiarity with mycelium-based products and invited participants to provide examples of such products they might know.

Table 1
Sensory attribute listed in the Check All That Apply.

Categories	Attribute
Taste	"Sweet", "Bitter", "Salty", "Umami", "High acidity", "Low acidity", "Good acidity", "Yeast flavor", "Irony", "Fruity", "Soy sauce", "Tasteless", "Meaty taste", "Vinegar-like" and "Mushroom"
Texture	"Meaty texture", "Chewy", "Watery", "Spongy", "Astringent", "Mouth coverage", "Hard", "Soft", "Creamy", "Slobbering", "Fibrous" and "Chewing"

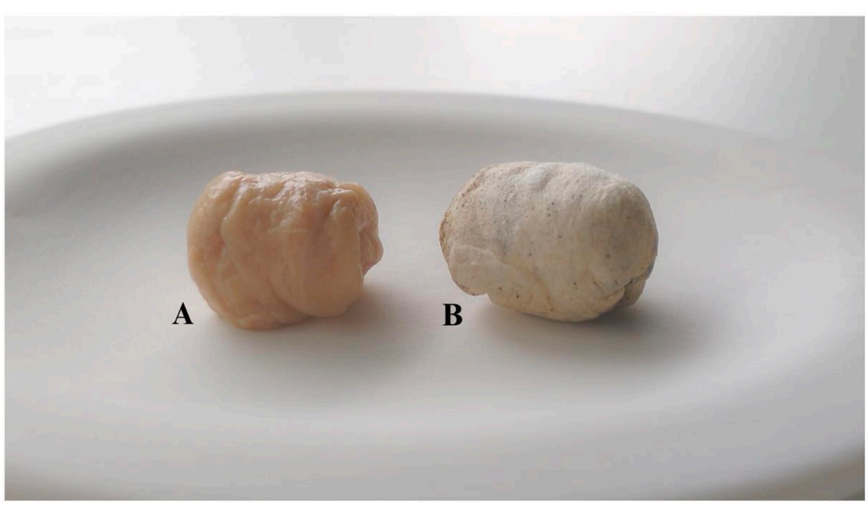

Fig. 1. Mycelium biomass obtained from SmF (A) and SSF (B), used for the sensory analysis.

2.3. Data analysis

2.3.1. Data analysis of quantitative test

The CATA data were analyzed to determine the frequency of each sensory attribute associated with the samples via the two fermentation techniques. Spider diagrams were created in Microsoft Excel (version 2019, Microsoft Corporation, Redmond, WA, USA) to visually representing the frequency distribution of sensory attributes. A one-way Analysis of Variance (ANOVA) was conducted using Jamovi software (version 2.3.1, The Jamovi Project, Australia) to evaluate differences in sensory attributes between the two fermentation techniques. Attributes that resulted as statistically significant differences ($p < 0.05$) are marked with an asterisk (*).

Normalized frequencies of attribute selection were normalized and visualized using a heatmap, with color intensity indicating the relative frequency of each term. A Chi-square test to assess whether the fermentation technique significantly influenced attribute perception.

Descriptive analysis was used to calculate the mean scores for each liking attribute (overall taste, texture, and acidity) based on the 9-point hedonic scale. Paired *t*-tests were conducted to compare sensory acceptability between samples obtained via SSF and SmF. Statistical significance was set at $p < 0.05$. All analyses were conducted and visualized using GraphPad Prism (version 10.4.1, GraphPad Software, San Diego, CA, USA). Results are reported as the mean ± standard deviation for each evaluated attribute.

2.3.2. Data analysis qualitative test

The open-ended responses were analyzed using Natural Language Processing (NLP) methodologies (Kochmar, 2022). Text processing included tokenization, stopword removal, and lemmatization were conducted using the Natural Language Toolkit software library (Loper and Bird, 2022) to clean the data and remove irrelevant terms. The cleaned text was then labeled based on the presence of specific keywords or entities deemed important. Entity extraction was performed using Named Entity Recognition with spaCy ((Honnibal et al., 2024) and Zero Shot Classification with BART-Large-CNN model (Lewis et al., 2019). For topic Modeling a hybrid approach was applied combining Latent Dirichlet Allocation implemented in Gensim library (Řehůřek and Sojka, 2024) with reasoning outputs from the DeepSeek-R1 (DeepSeek-AI, 2025) to extract key semantic themes. This approach allowed for the patterns and facilitated the expression of extracted topics in a more interpretable semantic framework.

To explore response patterns and segment consumers, K-Means clustering was applied using the *scikit-learn* library (Pedregosa et al., 2025), grouping observations based on shared topic mentions. Prior to clustering, Principal Component Analysis (PCA) was performed to reduce data dimensionality and enhance cluster separation.

3. Results

3.1. GC–MS analysis

The semi-quantitative GC–MS analysis of volatile compounds presents in the biomass highlights differences between SSF and SmF (see Table 2). A total of fifteen compounds were identified in both biomasses. Out of these, six were classified as originated from the substrate and marked with an asterisk. In SmF biomass, nine compounds originating from the fungal biomass and six compounds from the substrate were detected. For SSF, eight compounds originated from the fungal biomass and seven compounds originated from the substrate.

These compounds can be classified into furfural derivatives, fatty acids, steroids, and miscellaneous compounds.

Furfural derivatives classified as originating from the substrate, such as 5-Hydroxymethyl-2-furfural and 5-methylfurfural were identified, with the former being the most abundant in SmF (18.81 ± 4.45 μg g^{-1}) and found at much lower concentrations in SSF biomass (0.58 ± 0.04 μg g^{-1}). Similarly, 5-methylfurfural was predominantly present in SmF (1.83 ± 0.21 μg g^{-1}) and undetected in SSF. Phenylethyl alcohol and pyranone exhibited a similar trend. Phenylethyl alcohol was more concentrated in SmF (2.41 ± 0.39 μg g^{-1}) compared to SSF (0.43 ± 0.06 μg g^{-1}). Similarly, pyranone was more abundant in SmF (2.38 ± 0.66 μg g^{-1}) than in SSF (0.36 ± 0.07 μg g^{-1}).

The fatty acid group, originating from the fungal biomass, was found in higher amounts in SmF than SSF. Palmitic acid was most abundant in SmF (0.96 ± 0.14 μg g^{-1}). Methyl linoleate, linoleic acid, and oleic acid followed the same trend, with higher levels in SmF (0.53 ± 0.26 μg g^{-1}, 0.59 ± 0.11 μg g^{-1}, and 0.59 ± 0.14 μg g^{-1}, respectively) than in SSF, where their concentrations were lower (0.21 ± 0.11 μg g^{-1}, 0.15 ± 0.11 μg g^{-1}, and 0.20 ± 0.25 μg g^{-1}, respectively). Steroids classified as originated from the fungal biomass, such as squalene and ergosterol were more abundant in SmF (3.36 ± 1.09 μg g^{-1} and 25.03 ± 2.39 μg g^{-1}, respectively) compared to SSF (0.94 ± 0.03 μg $g^{-1}$and 0.05 ± 0.05 μg g^{-1}, respectively). Squalene was detected at 3.36 ± 1.09 μg g^{-1} in SmF, while it was much lower in SSF (0.94 ± 0.03 μg/g). Ergosterol exhibited the most significant variation, being highly concentrated in SmF (25.03 ± 2.39 μg g^{-1}) compared to a minimal amount detected in SSF (0.05 ± 0.05 μg g^{-1}). Other compounds such as lauryldimethylamine and dimethylsiloxane cyclic trimer were identified and classified as originated from the fungal biomass. Lauryldimethylamine was exclusively present in SmF (0.23 ± 0.20 μg g^{-1}), whereas dimethylsiloxane cyclic trimer showed similar concentration in both methods (0.39 ± 0.19 μg g^{-1} in SmF and 0.44 ± 0.03 μg g^{-1} in SSF).

Table 2
Semi-quantitative composition of SmF and SSF biomass of *Rhizopus oligosporus* using GC–MS in *μg g*$^{-1}$. (SmF/SSF) represents the ratio of compounds concentration in SmF relative to SSF. Values are expressed as mean ± standard deviation ($n = 3$). Compounds classified as originated from the substrate have been marked with an asterisk (*).

Retention time	Compounds	SmF	SSF	SmF/SSF
		μg g^{-1}	*μg g*$^{-1}$	-
5.15	5-methylfurfural*	1.83 ± 0.21	-	-
7.34	Phenylethyl alcohol*	2.41 ± 0.39	0.43 ± 0.06	7.73
7.76	Pyranone*	2.38 ± 0.66	0.36 ± 0.07	6.54
8.06	Isopropylethylene oxide*	6.05 ± 0.33	–	–
8.92	5-Hydroxymethyl-2-furfural*	18.81 ± 4.45	0.58 ± 0.04	32.92
9.95	1,1-Dimethyl-2-sec-butylhydrazine*	0.78 ± 0.28	–	–
17.19	Palmitic acid	0.96 ± 0.14	0.60 ± 0.04	15.00
18.80	Methyl Linolenate	0.53 ± 0.26	0.21 ± 0.11	9.20
19.09	Linoleic acid	0.59 ± 0.11	0.15 ± 0.11	2.55
19.27	Oleic acid	0.59 ± 0.14	0.2 ± 0.25	3.76
20.77	Lauryldimethylamine	0.23 ± 0.20	–	–
24.70	Squalene	3.36 ± 1.09	0.94 ± 0.03	3.59
26.65	Glyceryl monooleate	0.47 ± 0.23	0.33 ± 0.01	0.09
27.58	Dimethylsiloxane cyclic trimer	0.39 ± 0.19	0.44 ± 0.03	0.93
28.14	Ergosterol	25.03 ± 2.39	0.05 ± 0.05	53.54

3.2. Check all that apply

The sensory profiles of the two biomass samples produced via SSF and SmF were compared using a spider plot (Fig. 2), illustrating the normalized selection frequencies of the CATA terms. Differences between the samples were evident across several attributes. The attributes most frequently selected for SSF were “Spongy" (selected 35 times), "Soy Sauce" (selected 28 times), and "Fibrous" (selected 30 times). In contrast, the attributes most frequently selected for SmF were "Meaty texture" (selected 32 times) and "Hard" (selected 27 times).

The frequency of sensory attributes associated with different fermentation techniques (SSF vs. SmF) was represented in a heatmap (Fig. 3). Attributes such as “Chewy”, “Mushroom”, “High Acidity”, and “Meaty texture” were more frequently associated with SmF, while “Tasteless”, “Low Acidity”, and “Soft” were predominant in SSF. A highly significant association between the fermentation method and sensory attribute perception was confirmed ($\chi^2 = 124.1$, df = 34, $p < 0.0001$).

3.3. Hedonic consumer test

The mean overall liking for the samples ranged between 4.26 ± 2.20 and 4.82 ± 2.05 on the nine-point hedonic scale (Fig. 4). These results indicate that the samples were rated between “dislike slightly” (4) and “neither like nor dislike” (5) on the scale. The SmF sample received slightly higher overall liking scores than the SSF sample, but the difference was not statistically significant ($p > 0.05$).

Similarly, no significant differences were observed in participants’ liking of texture, with scores ranging from 3.96 ± 1.80 to 4.18 ± 1.94 ($p > 0.05$), indicating a slight dislike for the texture of both samples. In contrast, acidity scores differed significantly between fermentation methods: SmF received a mean score of 5.70 ± 1.99, while SSF scored 4.48 ± 1.90 ($p < 0.05$). This suggests that the acidity of the SmF sample was more appreciated, with ratings falling between “neither like nor dislike” and “like slightly,” whereas SSF ranged between “dislike slightly” and “neither like nor dislike.”

3.4. Open answer

Among the partecipants, 7.92 % reported having dietary requirements, such as insulin resistance, a high-protein diet, a low-fat and complex sugar diet, not consuming red meat, following a vegan, or being gluten-free. 35.64 % of the respondents answered that they usually consume fermented foods with fungi, such as bread, soy sauce, or miso. Additionally, 29.70 % responded that they are familiar with or know about mycelium-based products, citing examples such as tempeh, meat substitutes or analogs, and koji ferments.

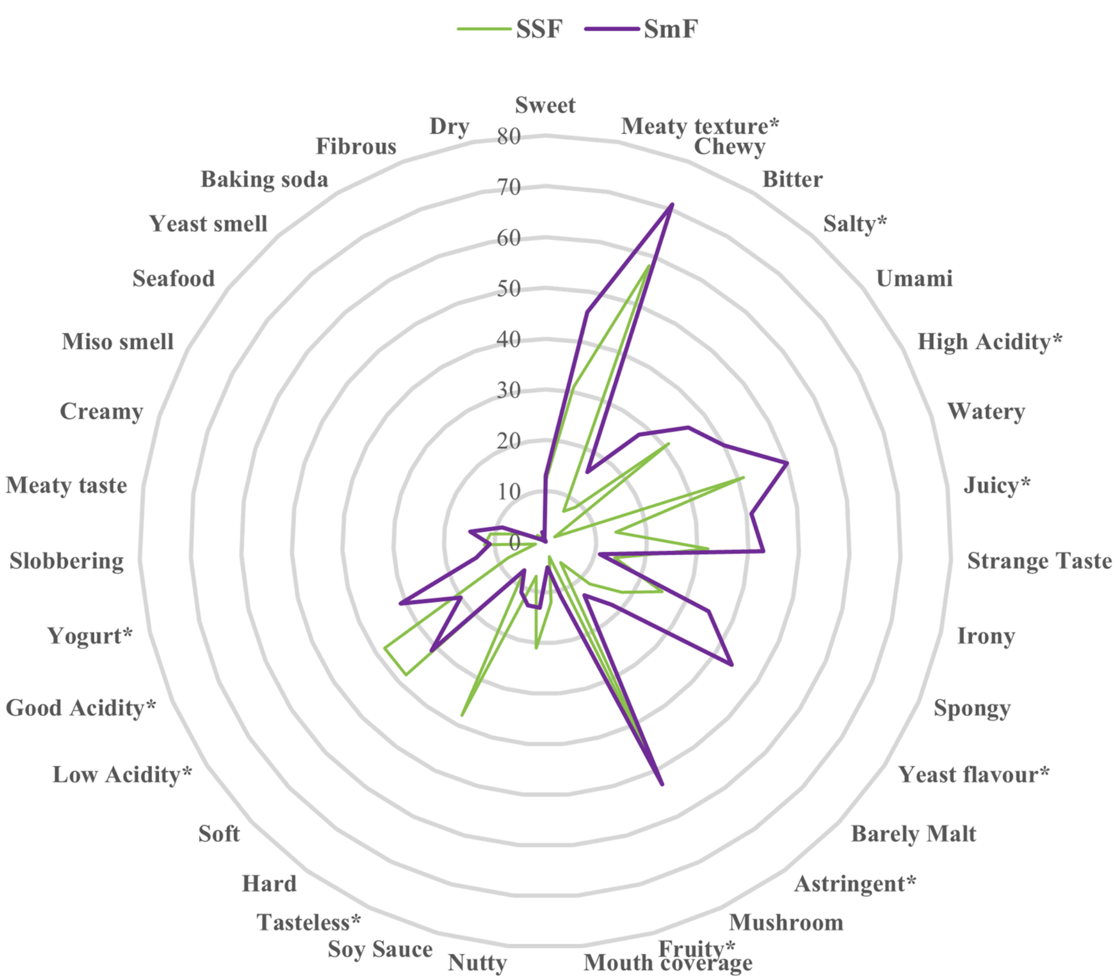

Fig. 2. Attribute frequency of Check-All-That Apply questionnaire of biomass produced under SSF (green) and SmF (purple). Attributes resulted as statistically significant ($p < 0.05$) are represented with (*). Overlapping regions highlighted similarities in attributes, while non-overlapping regions emphasized distinguishing features between the samples.

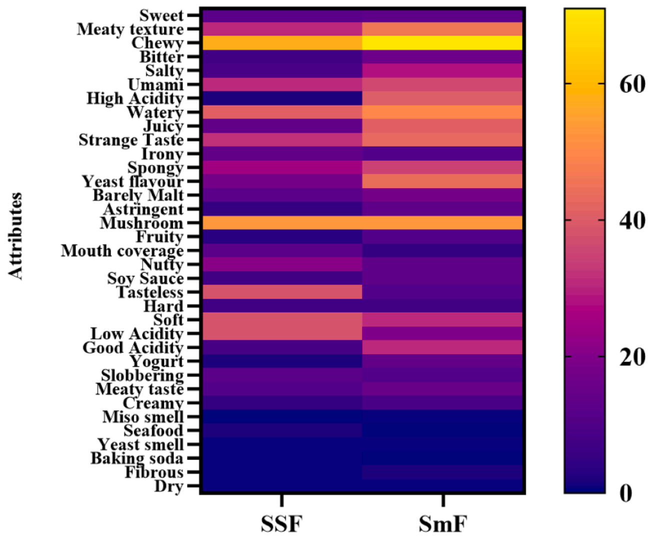

Fig. 3. Heatmap of sensory attributes of *Rhizopus oligosporus* biomass obtained through SSF and SmF. The color intensity corresponds to the frequency of selection for each attribute, with yellow shades indicating higher frequency and purple shades indicating lower frequency. A statistically significant association was found between fermentation method and attribute perception ($\chi^2 = 124.1$, df = 34, $p < 0.0001$).

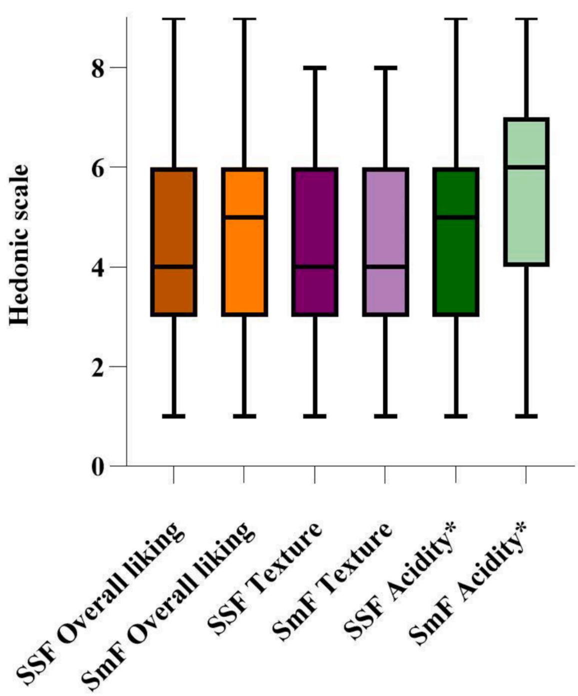

Fig. 4. Consumer acceptability scores (mean ± standard deviation) for overall liking, texture, and acidity of *Rhizopus oligosporus* biomass produced under SSF and SmF. Horizontal bars represent the mean; vertical bars represent standard deviation. (*) indicates a statistically significant difference between SSF and SmF ($p < 0.05$).

3.4.1. *What product does this food remind you of?*

A total of 110 keywords were identified for the SmF sample, and 118 for the SSF sample. . For SmF sample, the most frequently mentioned were "Mushroom" (38 mentions), "Meat" (18), "Texture" (14), and "Yeast" (10). In the SSF sample, the most frequent words keywords were "Mushroom" (42), "Meat" (16), "Texture" (11), and "Chicken" (8).

3.4.2. *What product would you develop using this food ingredient?*

Based on the responses collected the SmF sample yielded 104 keywords, with the most frequent being "Meat" (31 mentions), "Sauce" (18), and "Vegan" (14). The SSF sample generated a total of 106 keywords, also highlighting "Meat" (31), "Sauce" (17) and "Vegan" (15).

A frequency box chart was created based on the results of the Named Entity Recognition analysis, highlighting the most frequently mentioned entities related to potential product development using the ingredient, separately for biomass obtained through SmF (Fig. 5) and SSF (Fig. 6). A total of 188 entities were identified: 96 for SmF and 92 for SSF. The most common entity categories included beverage ingredients (29 in SmF, 33 in SSF), snack component (24 in SmF, 25 in SSF), and fermented product development (15 in both).

To analyze open-ended consumer responses, a topic modeling and clustering approach was conducted in three main steps, as shown in Fig. 7.

First, the optimal number of topics was determined using Latent Dirichlet Allocation (LDA), which was iteratively run with varying topic numbers. For each model, coherence scores were calculated based on word co-occurrence patterns. The analysis revealed that a four-topic model yielded the highest coherence, indicating optimal interpretability.

Second, the prevalence of each topic was quantified across the dataset by assigning probability distributions to each response. A large language model (DeepSeek-R1) was then used to generate descriptive titles for the topics, based on the most representative terms in each cluster. The resulting topics were labeled as "Texture", "Umami", "Meatiness" and "Fermentation".

In the third step, a two-dimensional representation of topic relationships was generated using Principal Component Analysis (PCA). This technique reduces the dimensionality of the semantic data by identifying the principal components, directions of maximum variance, through eigenvalue decomposition of the covariance matrix. Principal Component 1 (PC1), accounting for 48.11 % of the variance, captured the main differences in topic prevalence and contextual use across responses. Principal Component 2 (PC2), explaining 28.87 % of the variance, reflected the lexical diversity within individual responses.

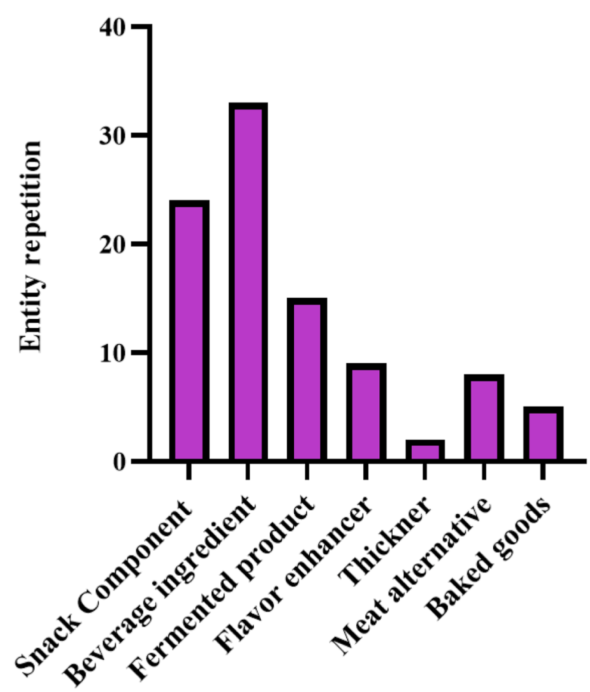

Fig. 5. Frequency of product-related entities identified through NER based on open-ended responses about SmF biomass.

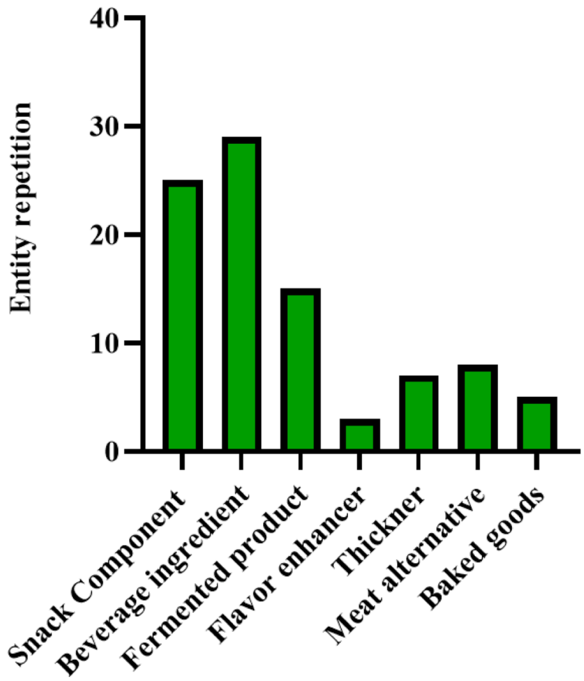

Fig. 6. Frequency of product-related entities identified through NER based on open-ended responses about SSF biomass.

Each point on the PCA plot represents a single consumer response, color-coded according to its assigned topic cluster. Responses positioned closer together indicate greater semantic similarity, while those farther apart show greater divergence in language or content. The centering of the data ensures that the origin of the plot reflects the meaning of all responses, with values approximately ranging from −0.6 to 0.6 along both axes.

The distribution of responses across clusters highlights key patterns. "Texture" responses (red) were clearly separated from the rest, forming a distinct cluster in the upper-left quadrant of the plot. This indicates a consistent and unique semantic profile for participants who emphasize textural attributes. Similarly, "Meatiness" responses (green) were tightly grouped on the far right of PC1, suggesting a homogeneous and strongly defined perception among those participants.

In contrast, responses associated with "Umami" (blue) and "Fermentation" (purple) appeared more dispersed and partially overlapping near the plot center and lower-left quadrant. This distribution suggests greater lexical and conceptual variability in how these themes were described. The overlap between the "Umami" and "Fermentation" clusters indicates that consumer perceptions of these concepts may be interrelated or commonly co-expressed using similar vocabulary.

Overall, the PCA projection reveals that "Texture" and "Meatiness" emerged as clearly distinguishable and consistently articulated sensory themes, while "Umami" and "Fermentation" reflect more complex or context-dependent interpretations. These findings suggest that certain sensory attributes are more readily recognized and described by consumers, whereas others may require more nuanced language or may vary more across individuals.

The figure displays the distribution of consumer responses across the four identified topics: "Texture", "Umami," "Meatiness", and "Fermentation." Each point represents one individual response, positioned according to the two principal components (PC1 and PC2), and colored by topic group.

4. Discussion

This study aimed to characterize *Rhizopus oligosporus* biomass obtained by SSF and SmF using chemical and consumer sensory analyses.

In the GC–MS analysis, distinct sensory attributes were observed between the two fermentation methods. These differences lay the foundation for tailored food applications, where specific organoleptic profiles can be selected according to product requirements. These results underscore the strong impact that fermentation conditions exert on the chemical composition of fungal biomass. In particular, SmF was associated with a higher concentration of certain key compounds, such as ergosterol, 5-hydroxymethyl-2-furfural, and squalene while SSF favored different metabolic pathways, resulting in lower concentrations of these volatiles. Such compositional differences may directly influence sensory perception. For instance, 5-hydroxymethyl-2-furfural is associated with roasted and caramel-like notes, while squalene and ergosterol contribute to fatty and more complex aromas. The elevated presence of these

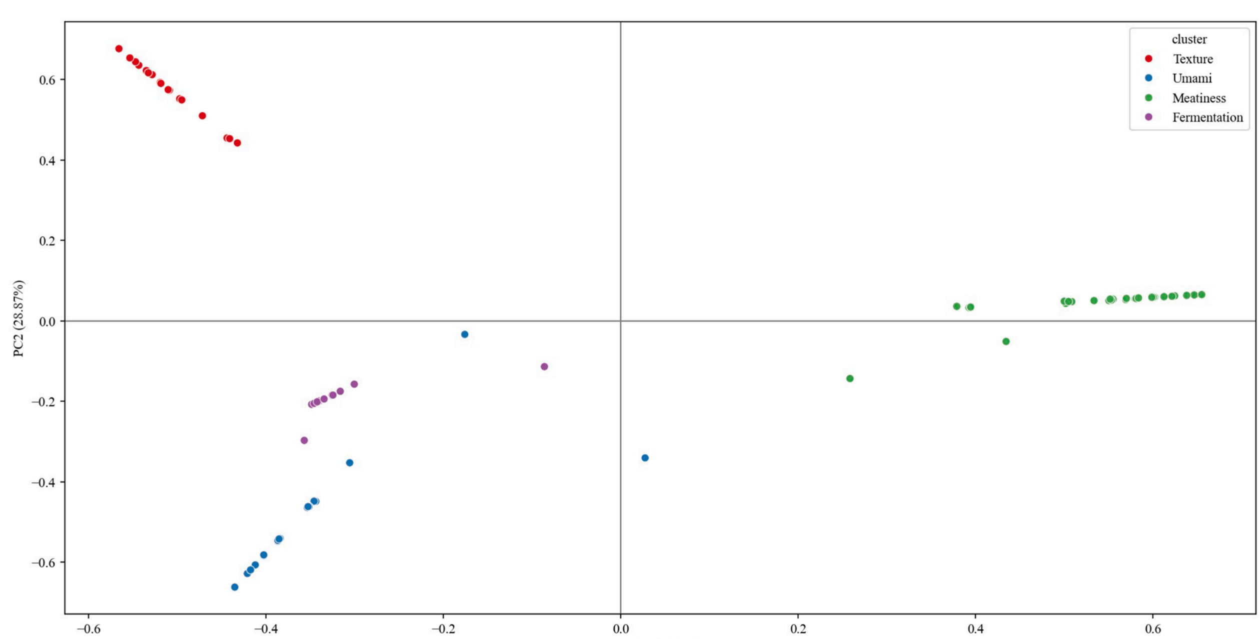

Fig. 7. Consumer segmentation based on topic distribution in a two-dimensional PCA representation.

compounds in SmF biomass could explain why consumers more frequently described it using terms related to umami and meaty flavors. In contrast, the lower concentration of volatiles in the SSF biomass might account for its milder aroma and the emphasis on texture-related descriptors such as “spongy” or “fibrous.”

This evidence suggests that the fermentation method influences not only the nutritional and biochemical composition of the biomass, but also its sensory perception, thus opening opportunities for selecting the fermentation strategy based on desired product outcomes.

Furfural derivatives absorbed by the biomass from the substrate, such as 5-hydroxymethyl-2-furfural, were predominantly found in the SmF biomass, with significantly higher concentrations compared to SSF. These compounds are associated with caramel-like, fatty, and waxy sensory notes and are commonly found in thermally processed foods such as coffee, baked goods, and honey (Petisca et al., 2013; Shapla et al., 2018;). Their presence in SmF biomass suggests a more pronounced toasted and caramelized sensory profile, potentially enhancing its suitability for food formulations that benefit from greater depth of flavor.

In SmF, among the volatiles detected, phenylethyl alcohol, also classified as substrate-derived, stood out due to its characteristic floral and rose-like aroma. This compound is frequently associated with wines and fermented beverages (Cordente et al., 2018; Chen et al., 2020), as well as foods like roasted peas and dark chocolate (Deuscher et al., 2020). It was more abundant in SmF biomass and may contribute to a subtle yet distinctive aromatic complexity in food applications. Similarly, pyranone compounds, which impart toasty and slightly sweet notes, were also found in higher concentrations in SmF biomass, further supporting its potential to deliver a more complex and layered aroma profile.

Although these compounds were originally transferred from the substrate to the biomass, they ultimately become part of the final organoleptic profile of the ingredient contributing to additional aromatic and flavor qualities to the overall food matrix.

Fatty acids, produced by the fungal biomass, such as palmitic acid, linoleic acid, and oleic acid, play a key role in determining food texture and mouthfeel. These compounds are widely present in oils, dairy products, and nuts, where they contribute to creamy, fatty, and waxy textures (Li et al., 2021). The higher concentrations of these fatty acids in SmF biomass suggest a richer mouthfeel, making it more suitable for applications requiring fuller and more indulgent sensory experience. The presence of these fatty acids is particularly relevant for food formulations that aim to enhance the perception of smoothness and richness, attributes often associated with traditional animal-based products.

Among the sterols, ergosterol was significantly more abundant in the SmF biomass than in the SSF. Although it does not directly influence the sensory properties of mushrooms, ergosterol is the predominant fungal sterol and a precursor of vitamin D_2. Upon exposure to ultraviolet light or heat, it undergoes photochemical and thermal conversion into vitamin D_2, in a process analogous to vitamin D_3 synthesis in human skin. This transformation is influenced by fungal physiological conditions, including hyphal development and sporulation (Sharma et al., 2024).

This suggests that SmF biomass may exhibit sensory similarities with fungal-based foods, potentially making it attractive for umami-rich culinary applications. Additionally, squalene, also present at higher concentrations in SmF biomass, is involved in the biosynthetic pathways of terpenoids. Squalene is commonly found in honeysuckle flowers (*Lonicera japonica)*, where it contributes to overall flavor quality (Li et al., 2022).

The SmF method appears to facilitate a greater absorption of aromatic and flavor-active compounds originating from the substrate, resulting in a more complex and structured sensory profile. While fungal biomass has traditionally been used in savory or protein-rich applications (Finnigan et al., 2019; Rousta et al., 2021; Gamarra Castillo et al., 2022), these findings open new avenues for its potential use in sweet and pastry-style formulations. Conversely, the SSF-derived biomass, characterized by lower absorption of substrate-derived compounds and a reduced concentration of volatiles, may offer a more neutral sensory base. This can be advantageous for applications where subtle flavor is required, such as in textural enhancers or in formulations focused on protein fortification.

Regarding the sensory tests, this approach allowed for the evaluation of how consumers perceive the fungal biomass as a standalone ingredient and how such insights could guide its future integration into food formulations.

The use of the CATA questionnaire enabled a comparative analysis of the sensory attributes influenced by each fermentation technique. In the case of biomass derived from SSF, specific descriptors such as "spongy," "soy sauce," and "fibrous" were frequently selected. This indicates that the SSF method may enhance distinct textural and aromatic characteristics. On the other hand, the SmF-derived biomass presented a different sensory profile, where "meaty texture" and "hard" were the most frequently reported attributes.

It is particularly interesting to note that "sweet" was the least selected descriptor across both samples, appearing fewer than ten times in total. This suggests that the high concentration of carbon source used during fermentation (malt 95 g/L, as reported by Massa et al., 2024) does not significantly contribute to a perceived sweet flavor in the final biomass. The most frequently mentioned attribute for both samples was "meaty texture." This observation reinforces the potential application of fungal biomass in developing protein analogs, which aligns with the growing demand for meat alternatives (Finnigan et al., 2019).

The substantial Chi-square value and highly significant p-value obtained from the CATA data analysis confirm that the fermentation technique significantly affects the sensory profile of the biomass. The analysis encompassed 35 sensory attributes under two fermentation conditions, revealing clear and meaningful differences in sensory perception.

Although production techniques can vary depending on the intended use, this study represents the first direct comparison of the sensory perception of fungal biomass grown under SSF and SmF when tested as an isolated, raw ingredient.

Previous research has explored fungal biomass applications, as in the case of Rousta et al. (2021), who used submerged cultivation of *Aspergillus oryzae* to develop fungal-based burgers. Similarly, Barkman (2023) investigated the use of *Rhizopus oligosporus* cultivated in submerged conditions for chicken nuggets. In contrast, SSF is typically associated with traditional products like tempeh, where the substrate often a legume such as soybean remains physically integrated into the final food matrix (Erkan et al., 2020).

What distinguishes the present work is the fact that the fungal biomass obtained from both SSF and SmF processes were separated from the substrate before undergoing sensory analysis and consumer testing. As a result, unlike traditional SSF-derived products, the final ingredient does not include the substrate and thus offers a clearer picture of the intrinsic sensory and chemical properties of the fungal biomass itself.

This distinction is particularly relevant when interpreting the sensory evaluation results, as it ensures that the hedonic ratings, ranging from 4.26 for the SSF sample to 4.82 for the SmF sample on a 9-point scale, reflect the characteristics of the fungal biomass alone, independent of any substrate-related influence.

The 9-point hedonic scale indicated that the product was rated between “dislike slightly” (4) and “neither like nor dislike” (5), with mean scores ranging from 4.26 for the SSF sample to 4.82 for the SmF sample. No statistically significant differences were found between the two fermentation techniques, suggesting that the general acceptability of the ingredient does not appear to be influenced by the fermentation method used.

However, since the sensory evaluation was performed on the raw material in its pasteurized form, without incorporation into a final food matrix, its unrefined shape and presentation might have negatively influenced consumer acceptance. Additionally, this type of sensory questionnaire has not been previously developed for fungal biomass in raw form. The lower hedonic scores observed may also be partially explained by food neophobia, a phenomenon referring to the reluctance to try unfamiliar foods. Food neophobia can reduce product acceptance by increasing sensitivity to unusual textures, flavors, or aromas, especially when the product is not embedded within a familiar food context (Prescott et al., 2022). Integrating the biomass into a finished product or modifying its original format could help mitigate this effect and enhance acceptability (S.A. Siddiqui et al., 2022).

The hedonic scale scores for overall texture liking were also low, with a mean rating of 4.18 for the SmF sample and 3.96 for the SSF sample, both falling within the "dislike slightly" range. These results suggest that texture is an important factor requiring improvement to boost the ingredient's appeal. If the biomass is to be considered as a standalone product, optimizing its texture would be essential. Nevertheless, this ingredient has shown promising techno-functional properties relevant for food formulation, such as emulsifying activity and water-holding capacity (Massa et al., 2024). This indicates that, despite limited acceptability in its pure form, the biomass holds potential for incorporation into developed food products, where these properties can be leveraged to improve the final product quality.

Given that texture plays a central role in consumer perception of food quality, it is likely that the sensory scores for texture directly influenced the overall hedonic ratings (Nederkoorn et al., 2018). Texture is widely recognized as one of the most important factors in food acceptance, particularly for novel products. Recent research has further emphasized its role not only in driving consumer preferences but also in shaping emotional responses, purchase decisions, and perceived product quality (Baingana, 2024).

Since the samples were evaluated in a raw and pasteurized format, it is also plausible that their texture would improve with heat treatment or when combined with other ingredients during culinary preparation.

Another sensory aspect that plays a critical role in food acceptance is acidity, as it contributes to the perceived tartness and overall flavor profile. In the current study, the acidity of the samples was attributed to the acidic cultivation conditions under which the biomass was produced. When consumers evaluated acidity using the hedonic scale, the SmF sample received significantly higher scores (mean of 5.70) compared to the SSF sample (mean of 4.48). The SmF sample fell between "neither like nor dislike" and "like slightly," while the SSF sample ranged from "dislike slightly" to "neither like nor dislike". This finding suggests that the level of acidity generated during SmF cultivation may enhance flavor perception, contributing positively to consumer response.

The open-ended responses provided by consumers offered valuable insights into their perceptions and associations with the fungal biomass samples. These data revealed both prior knowledge of fermented and mycelium-based products among respondents, as well as key sensory attributes that shaped their experiences with the SmF and SSF samples.

The low percentage of participants with dietary restrictions, along with their limited familiarity with fermented or mycelium-based foods, suggests that most respondents had minimal prior exposure to such ingredients. While this may have reduced potential bias stemming from preconceived expectations, it also indicates that many consumers lacked sensory familiarity with the distinct flavor profiles of fermented foods. This unfamiliarity could have influenced their perception and acceptance of the samples, as previous studies have demonstrated that product familiarity plays a critical role in shaping consumer attitudes toward alternative proteins (S.A. Siddiqui et al., 2022).

Responses to the question "What product does this food remind you of?" provided further insights into consumer sensory perception and potential market positioning. The strong association with mushrooms across both fermentation methods is consistent with earlier studies that link fungal-based ingredients to flavor profiles rich in umami and earthy notes.

This association may be explained by the presence of key volatile compounds, such as furfural derivatives, alcohols, and fatty acids, which contribute to the savory and slightly fermented character of the samples (Cordente et al., 2018; Chen et al., 2020). The frequent mention of "meat" further supports the idea that fungal biomass presents both aromatic and textural qualities that resemble those of animal-derived proteins.

Differences in descriptors such as "yeast" and "chicken" between the SmF and SSF samples may be linked to variations in the concentration of volatile compounds identified through GC–MS analysis. For example, the higher levels of furfural derivatives in SmF biomass, particularly 5-hydroxymethyl-2-furfural, may contribute to the meaty notes typically found in thermally processed foods (Petisca et al., 2013). This could explain the stronger meat-like perception in SmF samples. In contrast, the "chicken" association reported in SSF samples may relate to their milder aroma and specific volatile signatures.

The elevated presence of phenylethyl alcohol in SmF, a compound frequently associated with yeast-fermented products (Cordente et al., 2018), could also explain the "yeast" descriptor selected by participants.

These findings emphasize the sensory complexity of fungal biomass and reinforce its potential as an ingredient in the formulation of alternative protein products.

The recognition of fungal biomass through terms such as meat, mushrooms, and chicken suggests considerable potential for its incorporation into alternative protein food products. Additionally, responses to the open-ended question "What product would you develop using this food ingredient?" provided further insight into consumer perceptions and possible applications of fungal biomass produced by both SmF and SSF.

The word cloud analysis showed that, for both samples, the most frequently mentioned terms were "meat," "sauce," and "vegan." This indicates a strong consumer inclination to associate fungal biomass with protein-rich food applications, plant-based sauces, and vegan formulations. These findings are consistent with previous research showing that fungal-derived ingredients possess textural and aromatic properties like those of meat substitutes, making them a promising option for the development of sustainable protein products (Michel et al., 2021).

The Named Entity Recognition analysis further supported this perception. The most frequently identified categories were beverage ingredients, snack component, and fermented product development. These results suggest that fungal biomass is seen as a versatile ingredient with potential applications that go beyond traditional protein analogs. This includes its use in functional fermented beverages (Di Renzo et al., 2025) and innovative fermented food products (Maini Rekdal et al., 2024b).

Topic modeling analysis combined with principal component analysis (PCA) provided a deeper understanding of consumer responses. The strong presence of texture-related terms is in line with other studies that emphasize the importance of texture in consumer acceptance of alternative proteins, especially meat substitutes. This aligns with findings from S.A. Siddiqui et al. (2022), which identify texture as a central factor in consumer satisfaction. Replicating the textural characteristics that consumers expect from meat can significantly enhance the acceptance and adoption of alternative protein sources.

Moreover, the identification of snack and beverage components as potential applications broadens the scope of fungal biomass beyond solid food products. This opens the door to the development of novel formulations in the functional food sector, where texture, flavor, and nutritional functionality play a key role.

5. Conclusion

The chemical and sensory profiles of *Rhizopus oligosporus* biomass produced under SmF and SSF were characterized. SmF biomass exhibited higher concentrations of furfural derivatives (2-furaldehyde, 1.24 μg g^{-1}), phenylethyl alcohol (3.17 μg g^{-1}), and fatty acids, contributing to a caramelized, floral, and creamy profile. In contrast, SSF biomass had a milder sensory profile, making it more suitable for neutral formulations. Descriptive analysis confirmed that both fermentation methods produced a characteristic meaty texture, supporting the potential use of fungal biomass in protein analogs In addition, umami meatiness, and fermentation were the most f requently perceived and preferred attributes, with SmF samples receiving higher average scores for meaty texture and umami intensity ($p < 0.05$, ANOVA). Consumer feedback suggested potential applications not only in savory products, such as snacks and plant-based meats, but also in innovative fermented foods and sweet or pastry-style formulations, reflecting the versatility of the biomass across a broader flavor spectrum. Fermentation was found to significantly modulate the volatile compound composition, as evidenced by the distinct chemical fingerprints between SmF and SSF ($p < 0.001$, multivariate analysis), directly influencing sensory perception. Given its sustainable production, rich nutritional profile, and favorable sensory properties, *R. oligosporus* biomass emerges as a promising alternative protein ingredient for hybrid and plant-based formulations. To further advance the application of *R. oligosporus* biomass as a food ingredient, future studies should explore several key areas. These include flavor optimization and masking strategies, particularly for SmF biomass, to enhance consumer acceptance in sweet and mild-flavored formulations. Moreover, exploring the use of biomass as a primary ingredient in product development, followed by comparative sensorial and rheological analyses with other protein sources, would be valuable to better understand its potential and limitations. In addition, long-term storage and stability studies are essential to ensure the preservation of both sensory and functional properties across various product categories, thereby supporting its broader commercial application.

Ethical statement

Ethical approval for the involvement of human subjects in this study was granted by the Research Ethics Committee of Mondragon Unibertsitatea (internal code: IEB-20,240,214).

The approval was granted on February 14th, 2024, after evaluation of the project proposal, participant information and consent forms, and the confidentiality agreement signed by the project lead.

Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

All participants gave informed consent in accordance with institutional and national ethical standards. Participants were informed that the data they provided would remain anonymous and would be used for scientific publication as part of the articles authored by the researcher in the context of her doctoral thesis.

CRediT authorship contribution statement

A. Massa: Writing – original draft, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M. Delgado Placeres:** Methodology, Investigation, Formal analysis, Data curation. **E. Axpe:** Writing – review & editing, Supervision. **L.J. Rothschild:** Writing – review & editing, Supervision. **M.L. Sanz:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. **M.J. Ricatti:** Writing – review & editing, Validation, Supervision. **C. Carrero-Carralero:** Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of competing interest

We declare no conflicts of interest and confirm that this research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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Data availability

Data will be made available on request.

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