Food Bioscience 80 (2026) 108967

Contents lists available at ScienceDirect

Food Bioscience

journal homepage: www.elsevier.com/locate/fbio

Effect of cultivation conditions on *In vitro* protein bioaccessibility of edible filamentous fungi

Ricky Wang [a,*], Ophelie Lemaire [a,b], Amir Mahboubi [a], Mohammad J. Taherzadeh [c]

[a] Swedish Centre for Resource Recovery, University of Borås, 501 90 Borås, Sweden
[b] Université de Toulouse, IMT Mines Albi, Campus Jarlard, 81013, Albi Cedex 09, France
[c] Millow AB, Gothenburg, Sweden

ARTICLE INFO

Keywords:
Edible filamentous fungi
Protein
Cell wall
In vitro gastrointestinal digestion

ABSTRACT

Edible filamentous fungus or mycoprotein, is promising sustainable protein sources and its protein quality plays a crucial role in their nutritional value. It is hypothesized that prolonged cultivation of filamentous fungus would reduce protein content and increase cell wall thickness, thereby hampering its protein digestibility. Filaementous fungi *R. oryzae* was cultivated for 24, 48, and 72 h at two aeration rates (0.3 and 1 vvm) and three pH levels (4, 5.5, and 7). Biomass was analyzed for crude protein content, amino acid composition, cell wall content, and amino acid bioaccessibility using INFOGEST *in vitro* gastrointestinal digestion model. Prolonged cultivation significantly reduced protein content, while the amino acid profile remained stable across treatments and was notably rich in lysine (8.5–9.4%). In addition, cell wall content increased with cultivation time, accompanied by a reduction in amino acid bioaccessibility.

1. Introduction

Edible filamentous fungi, also known as mycoprotein, represent an emerging sustainable protein source with the potential to address two of the most pressing global challenges: food security and climate change. They are highly nutritious, containing a substantial amount of protein and being rich in dietary fiber (Finnigan et al., 2025). Filamentous fungi also have the remarkable ability to degrade complex substrates, allowing them to grow on a wide variety of raw materials, including food industry side-streams, thereby promoting circularity within the food system (Ferreira et al., 2016; Souza Filho et al., 2019). Well-known species such as *Aspergillus oryzae* have long been used in rice and soy fermentation, while *Rhizopus oligosporus* and *Rhizopus oryzae* are used to produce tempeh, a traditional fermented soy product (Martín-Miguélez et al., 2025).

For edible filamentous fungi to be fully integrated into modern diets, their nutritional quality must be thoroughly evaluated. A key component of this evaluation is protein quality, particularly amino acid composition and protein digestibility. Protein digestion involves several stages: proteins from the food matrix are hydrolyzed by digestive enzymes into soluble amino acids and smaller peptides, which are then taken up and transported by intestinal enterocytes into the bloodstream, and subsequently utilized by human tissues. The proportion of protein or amino acids released into and soluble in the intestinal fluid is referred to as bioaccessibility (Perez-Moral et al., 2023). *In vivo* studies have reported that the protein digestibility and net protein utilization of *Fusarium venenatum* are approximately 78% and 65%, respectively, compared with about 95% and 80% for milk protein (Udall et al., 1984). While *in vivo* assays provide valuable insights, they are both costly and time-consuming. In contrast, *in vitro* methods, such as the standardized INFOGEST gastrointestinal model, offer a reproducible and widely adopted framework for assessing *in vitro* protein digestibility in wide-range of food matrices (Brodkorb et al., 2019).

Several previous studies have applied the INFOGEST method to assess the *in vitro* protein digestibility of filamentous fungi. Filamentous fungi *Fusarium venenatum* has been evaluated for the protein bioaccessibility and in comparison with other dietary protein sources (Ariëns et al., 2021; Colosimo et al., 2020). Treatments aimed at disrupting mycelial integrity were shown to improve *in vitro* digestibility (Chen et al., 2025). In addition, four other species of edible filamentous fungi: *Aspergillus oryzae, Neurospora intermedia, Rhizopus oligosporus,* and *Rhizopus oryzae*, have *in vitro* protein digestibility comparable the animal-based protein, such as salmon, chicken breast, and beef (Wang et al., 2023). Furthermore, mycoprotein of strain *Paecilomyces variotii*

* Corresponding author.
E-mail address: ricky.wang@hb.se (R. Wang).

https://doi.org/10.1016/j.fbio.2026.108967
Received 19 January 2026; Received in revised form 27 March 2026; Accepted 22 April 2026
Available online 22 April 2026
2212-4292/© 2026 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

demonstrated to have *in vitro* protein digestibility up to 77% (Nordlund et al., 2024).

On the other hand, the production of edible filamentous fungi presents unique challenges in bioprocess engineering. Several cultivation parameters, including pH, temperature, aeration rate, fermentation time, and substrate type and concentration, play crucial roles in determining fungal growth, yield, and crude protein content (Sar et al., 2022; Svensson et al., 2021). Although previous studies have investigated how these cultivation factors influence the crude protein content of fungi, the extent to which these conditions affect protein quality remains largely unexplored.

There are reasons to believe that fungal cultivation conditions may influence its protein quality. Protein digestibility of fungi may be limited by the presence of rigid cell walls (Colosimo et al., 2021); while the cell wall thickness and structure can change considerably during fungal growth (Ferreira et al., 2012; Mohammadi et al., 2013). For example, during the stationary growth phase, yeast cell walls become thicker, less permeable, and more resistant to enzymatic hydrolysis (Klis et al., 2002). Anaerobic conditions can influence fungal growth, morphology, and cell wall composition, including the chitin and chitosan content (Lennartsson et al., 2009). Similarly, pH stress can induce significant transcriptional changes and affect fungal growth. Most fungal species, such as the yeasts *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, as well as the filamentous fungus *Rhizopus oryzae*, show better growth under acidic conditions compared to neutral or alkaline environments (Ribeiro et al., 2022; Sar et al., 2022).

Despite increasing interest in fungal-based foods, to the best of our knowledge, no studies have examined how cultivation conditions influence protein quality in edible filamentous fungi. This study aimed to investigate the effect of the cultivation parameters: duration, pH, and aeration rate, on the protein quality of filamentous fungus *Rhizopus oryzae*. We hypothesized that these parameters could affect fungal protein content, cell wall content, and protein bioaccessibility. Understanding how cultivation conditions influence protein bioaccessibility is critical for optimizing production processes to enhance both yield and nutritional value, thereby contributing to more sustainable and nutritionally rich food sources.

2. Material and methods

2.1. Cultivation of edible filamentous fungi

The edible filamentous fungal strain *R. oryzae var. Delemar* CBS 145940 was used for the study. The fungus was grown in a 4-liter bubble column bioreactor (Belach Bioteknik, Stockholm, Sweden) using a defined glucose-based medium. The composition of this medium was based on Rousta et al. (2021): 30 g/L glucose, 7.5 g/L $(NH_4)_2SO_4$, 3.5 g/L KH_2PO_4, 1 g/L $CaCl_2$, 0.8 g/L $MgSO_4{\cdot}7H_2O$, 1 mL/L vitamin solution and 10 mL/L trace element solution according to (Sues et al., 2005). The pH was maintained at 4.0, 5.5, or 7.0 using 2 M H_2SO_4 and 2 M NaOH. Inoculation was done by adding 35 mL of spore suspension (1.53×10^6 spores/mL) of *R. oryzae* into 3.5 L of liquid medium. The cultivation was carried out at 35 °C with an aeration rate of either 0.3 vvm or 1 vvm (volume of air per volume of medium per minute) for 24, 48, or 72 h. The fungi biomass was harvested using stainless steel kitchen sieve (1 mm^2 pore area) and kept frozen in −20 °C. For the analysis, the frozen fungal biomass was freeze-dried and milled (Retsch MM400). Fungal cultivation in bioreactor was performed in duplicate for all cultivation with aeration rate 1 vvm and single replicate for cultivation with aeration rate 0.3 vvm.

2.2. Static in vitro gastrointestinal digestion

The *in vitro* gastrointestinal digestion was conducted following the INFOGEST 2.0 protocol (Brodkorb et al., 2019), with slight modifications as described by Wang et al. (2023), including reductions in the concentrations of pancreatin enzymes and omission of salivary amylase. In brief, in a 50 ml centrifuge tube, 250 mg of freeze-dried fungal biomass, 2.5 ml of water and 2.5 ml of simulated salivary fluid were added. For the digestion blank, digestion was conducted without any fungal biomass sample. The digestion process included two phases: gastric digestion with added 5 ml of simulated gastric fluid and pepsin (2000 U/mL) at pH 3 for 2 h; and intestinal digestion with added 10 ml of simulated intestinal fluid containing pancreatin (10 U/mL) and bile (1 mM) at pH 7 for 2 h. The intestinal phase was stopped by adding 2 ml of Bowman-Birk inhibitor (0.05 g/L). All phases were carried out at 37 °C with gentle mixing (5 rpm). After digestion, samples were frozen at −80 °C until analysis, with all experiments performed in triplicate.

2.3. Analytical method

2.3.1. Glucose and ethanol analysis

Glucose and ethanol concentrations were measured using high-performance liquid chromatography (HPLC) (Waters 2695, Waters Corporation, Milford, USA) equipped with an ion exchange column (Aminex HPX-87H, BioRad, USA). The HPLC analysis was operated at column temperature 60 °C with 0.6 mL/min of 5 mM H_2SO_4 as eluent and the detection was carried out by a refractive index (RI) detector (Svensson et al., 2021).

2.3.2. Crude protein

Nitrogen content was analyzed using a FlashSmart Elemental Analyzer CHNS/O (Thermo Fisher Scientific, USA), as described in Krotz et al. (2016). Briefly, about 3 mg of samples in a tin capsule are combusted is 950 °C furnace with 140 ml/min helium as carrier. Protein content was estimated by multiplying the nitrogen content with a conversion factor of 6.25. The analyses were performed in duplicate.

2.3.3. Cell wall analysis

Fungal cell wall content was measured as alkali-insoluble material followed a method previously described by Zamani et al. (2008). One hundred milligram of freeze-dried biomass was placed in a glass test tube and added 10 mL of 0.5 M NaOH. The mixture was autoclaved at 121 °C for 20 min. After cooling, the entire content was transferred to a pre-weighed centrifuge tube and centrifuged at 3000×*g* for 10 min. The supernatant was carefully discarded, and the remaining pellet was washed by adding Milli-Q water, followed by another centrifugation. This washing step was repeated four times. The centrifuge tube containing the pellet was then freeze-dried and weighed. The cell wall content was determined by the difference in weight of the centrifuge tube before and after the procedure. The analyses were performed in duplicate.

Glucosamine content of fungal biomass was measured according to (Mohammadi et al., 2012). The previously freeze-dried cell wall in the glass centrifuge tube was added 0.3 mL of 72% sulfuric acid and incubated for 90 min. The sulfuric acid was then diluted to 2.48% (v/v) by adding 8.4 mL of water, followed by autoclaved at 121 °C for 1 h. An aliquot of 0.5 mL was then added 0.5 mL of 1 M $NaNO_2$ for about 12 h for complete deamination of glucosamine into 2,5-anhydromannose. A 0.5 mL aliquot of the solutions was mixed with 0.5 mL of 12% sodium sulfamate. The 2,5-anhydromannose was quantified by HPLC using a hydrogen-form ion-exchange column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA) operated at 60 °C, with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min. Measured glucosamine represents the total amount derived from both chitosan and chitin, since N-acetylglucosamine releases is converted to glucosamine during acid hydrolysis. Chitosan from shrimp shell (Merck, Sweden), were used as control samples. Glucosamine hydrochloride 0.125 – 1 g/L (Merck, Sweden) was subjected to demination using $NaNO_2$ and used as standards for calibration.

2.3.4. Amino acid content and accessibility analysis

The amino acid content of solid fungal biomass samples and intestinal digests was analyzed following the method described by Trigo et al. (2021). Briefly, solid samples (50 mg) in the test tube underwent acid hydrolysis with 6 M HCl (8 ml) for 24 h, with nitrogen gas purging. The entire contents of the tube were diluted to 10 ml. Subsequently, 0.5 ml aliquot was transferred to a new tube, evaporated in air, and reconstituted with 10 ml of 0.2 M acetic acid, and filtered through a 0.2 μm syringe filter prior to LC/MS analysis. Two microliters of each sample were analyzed using an LC/MS system (Agilent 1100 HPLC and 6120 B Single Quadrupole MS). Chromatographic data were processed using MassHunter Quantitative Analysis software (version B.09.00, Agilent Technologies). Due to acid hydrolysis, this method was not suitable for quantifying tryptophan. Additionally, cysteine was oxidized and therefore not quantified. Asparagine and glutamine were co-determined with aspartic acid and glutamic acid, respectively. The analyses were performed in triplicate.

The amino acid in intestinal digests, obtained after *in vitro* gastrointestinal digestion, was measured following filtration of the intestinal digests with a 0.2 μm syringe filter (Wang et al., 2024). Four milliliters of the filtered intestinal digests was added with 4 mL of 12 M HCl, followed by nitrogen gas purging and acid hydrolysis as described previously.

Amino acid accessibility was calculated using:

$$\text{Amino acid accessibility } (\%) = \frac{\text{Total amino acids of filtered digest} - \text{total amino acid of digestion blank}}{\text{added sample total amino acid}} x\,100$$

2.3.5. Statistical analysis

Statistical analysis was performed using Minitab 21 software. One way ANOVA was applied followed by Tukey's post hoc for pairwise comparison at a significance level of 95%. Different groups of superscripts are used in tables to indicate the statistically significant pairwise comparisons at a threshold p-value <0.05. Results in tables and figures are presented as mean values ± standard deviation.

3. Result and discussion

In this study, the effect of cultivating conditions of edible filamentous fungi on their protein bioaccessibility was investigated. We hypothesized that prolonged cultivation time could lower fungal protein content and has thicker cell wall, hampering their protein digestibility. Therefore, we cultivated filamentous fungus *R. oryzae* for 24, 48, and 72 h at two different aeration rates of 0.3 and 1 vvm (volume of air per volume of medium per minute), under three different pH levels: pH 4, pH 5.5, and pH 7. The filamentous fungus was then analyzed for the crude protein, cell wall content, amino acids profile, and followed by *in vitro* gastrointestinal digestion to investigate the amino acid bioaccessibility.

3.1. Effect of cultivation conditions on fungal yield and protein content

Aeration rate is one of the critical factors influencing fungal growth in submerged fermentation. In the present study, *R. oryzae* was cultivated in a defined glucose-based medium at two different aeration rates: 0.3 vvm and 1 vvm. At the lower aeration rate (0.3 vvm), glucose was fully consumed only after 48 h of cultivation, whereas at the higher aeration rate (1 vvm), complete glucose consumption was observed within 24 h (Fig. 1a). The corresponding biomass yield followed a similar trend, reaching 1.33 g/L in 24 h at the higher aeration rate, but only 0.25 g/L at the lower aeration rate, which eventually increased to 1.01 g/L after 48 h (Fig. 1c). A lower aeration rate produced the highest ethanol concentration, reaching about 3 g/L only after 48 h, whereas the highest concentration at 1 vvm was reached within 24 h (Fig. 1b). This indicates that low aeration delayed the fungal growth. These results underscore the importance of sufficient oxygen supply for achieving rapid substrate utilization and higher productivity in fungal fermentations.

After the identification of 1 vvm as the favorable aeration rate at pH 5.5, we further investigated whether cultivation pH affected glucose consumption. No significant differences in glucose utilization were observed across pH values 4, 5.5, and 7 (Fig. 1). This suggests that *R. oryzae* is capable of adapting to a wide pH range with respect to primary carbon metabolism when glucose is used as the sole carbon source.

The observations in this study are consistent with previous reports on fungal cultivation. For example, Cultivation of *Mucor indicus* using xylose showed that increasing the aeration rate did not significantly change the final biomass yield but increased the rate of sugar utilization (Millati et al., 2008). Ethanol formation under aerobic conditions followed by its later consumption has also been reported in several fungal species and can be explained by the Crabtree effect, where at high glucose concentrations glycolysis is favored over the tricarboxylic acid cycle, leading to temporary fermentative metabolism even in the presence of oxygen (Lübbehüsen et al., 2004; Millati et al., 2008). In terms of pH, similar findings were reported by Gmoser et al. (2018) for *Neurospora intermedia*, where pH had little effect on biomass yield when glucose was used as the carbon source, although it influenced pigment production as a secondary metabolite. However, fungal cultivation using dairy side streams showed that neutral pH improved substrate utilization due to the optimal activity of lipase enzymes required for complex substrate utilization (Mahboubi et al., 2017).

In this study, crude protein content, total amino acids, and cell wall content of *R. oryzae* was measured over time at both aeration rates (Fig. 2). After 24 h of cultivation, the crude protein content reached approximately 64% of dry weight for both 0.3 vvm and 1 vvm aeration rates. However, when the cultivation was prolonged to 72 h, the crude protein content dropped to about 47%. This indicates that while the early growth phase is associated with high protein accumulation, extended cultivation under carbon-limited conditions leads to a reduction in protein content. Importantly, the pH of cultivation did not significantly affect crude protein levels, suggesting that protein content in *R. oryzae* is more closely linked to the nutrient availability than to external pH within the tested range (Fig. 2d).

The observed decline in crude protein content during prolonged cultivation is consistent with previous reports. Ferreira et al. (2012) described a similar trend in glucose medium, noting that fungal crude protein content decreases as cultivation progresses into the stationary growth phase. This decrease in protein content may be attributed to autophagy, a cellular process that maintains energy homeostasis under nutrient-limited conditions. During autophagy, some proteins are degraded to recycle amino acids for the synthesis of essential proteins and to generate energy through intermediates such as pyruvate and acetyl-CoA (He, 2022).

Total amino acid content was also analyzed to provide a more accurate estimation of the fungal biomass protein content (Fig. 2). There are significant discrepancies between the crude protein content and the total amino acid content of the fungal biomass. The highest total amino

acids was at about 40% after 24 h of cultivation, whereas crude protein was measured at 60%. After 72 h, the total amino acid dropped to only 28%, further supporting the observation that prolonged cultivation reduces fungal protein content. A previous study by Karimi et al. (2021) reported similar findings, showing that *R. oryzae* with a crude protein content of 50% had a total amino acid content of only 30%. The discrepancy between crude protein and total amino acid can be attributed to the presence of non-protein nitrogen in filamentous fungi, such as nucleic acids. In Zygomycetes such as *R. oryzae*, a considerable portion of nitrogen is incorporated into fungal cell wall, including chitin and chitosan (Mohammadi et al., 2012), which do not contribute to the amino acid pool. Because crude protein measurements rely on total nitrogen content (N × 6.25), it overestimate the true protein content of fungal biomass.

Previous studies also support the trend observed in this work. For example, Wang et al. (2023) reported amino acid levels of approximately 38% after 24 h of cultivation of *Rhizopus oryzae* in a glucose-based medium containing 30 g/L glucose and yeast extract. In contrast, another study using a lower glucose concentration (15 g/L) reported only about 22% total amino acids after 48 h of cultivation (Hoxha et al., 2025). This difference is likely related to the lower glucose concentration and prolonged cultivation duration. A similar pattern has also been reported for *Aspergillus oryzae* cultivated on glucose media: approximately 37% total amino acids was obtained after 24 h of cultivation with 30 g/L glucose (Wang et al., 2023), whereas another study reported significantly lower total amino acids levels (around 25%) after 48 h of cultivation (Rousta et al., 2022). Together, these results are consistent with the findings of the present study, indicating that fungal protein content tends to decrease significantly as the culture transitions into the stationary growth phase.

The reduction in protein content during prolonged cultivation was accompanied by an increase in fungal biomass yield. To better understand this relationship, the protein yield was estimated based on biomass yield and total amino acid content. At an aeration rate of 0.3 vvm, the protein yield increased from 0.10 g protein/L to 0.65 g protein/L after 72 h of cultivation. Similarly, at an aeration rate of 1 vvm, the protein yield increased from 0.53 g protein/L to 0.68 g protein/L. These results indicate that although the protein content of the biomass decreases over time, the overall protein yield can still increase due to higher biomass production. From a processing perspective, higher biomass yield may therefore be advantageous if the fungal biomass is intended for downstream protein extraction. However, if the fungal biomass itself is used directly as a food product, the reduced protein content may lower its overall nutritional protein value.

Producing mycoprotein for food applications using a defined glucose medium is neither sustainable nor economically viable on an industrial scale; therefore, the use of complex media derived from industrial sidestreams is generally preferred. However, several studies have reported lower protein contents in fungal biomass cultivated on such complex substrates or sidestreams. For example, fungi cultivated on oats had a total amino acid content of only up to 23.5 % (Rousta et al., 2022; Wang et al., 2024), while submerged cultivation of several species of fungi on grape marc liquor yielded total amino acids up to 16% (Svensson et al., 2021). Two factors may explain the lower protein content observed in complex media. First, solid particles can become physically entrapped within the fungal mycelial network, diluting the protein fraction on a dry-weight basis. Second, the carbon-to-nitrogen (C/N) ratio of the medium plays a critical role, with media having higher C/N ratios generally favoring higher protein accumulation in the biomass (Cao et al., 2025).

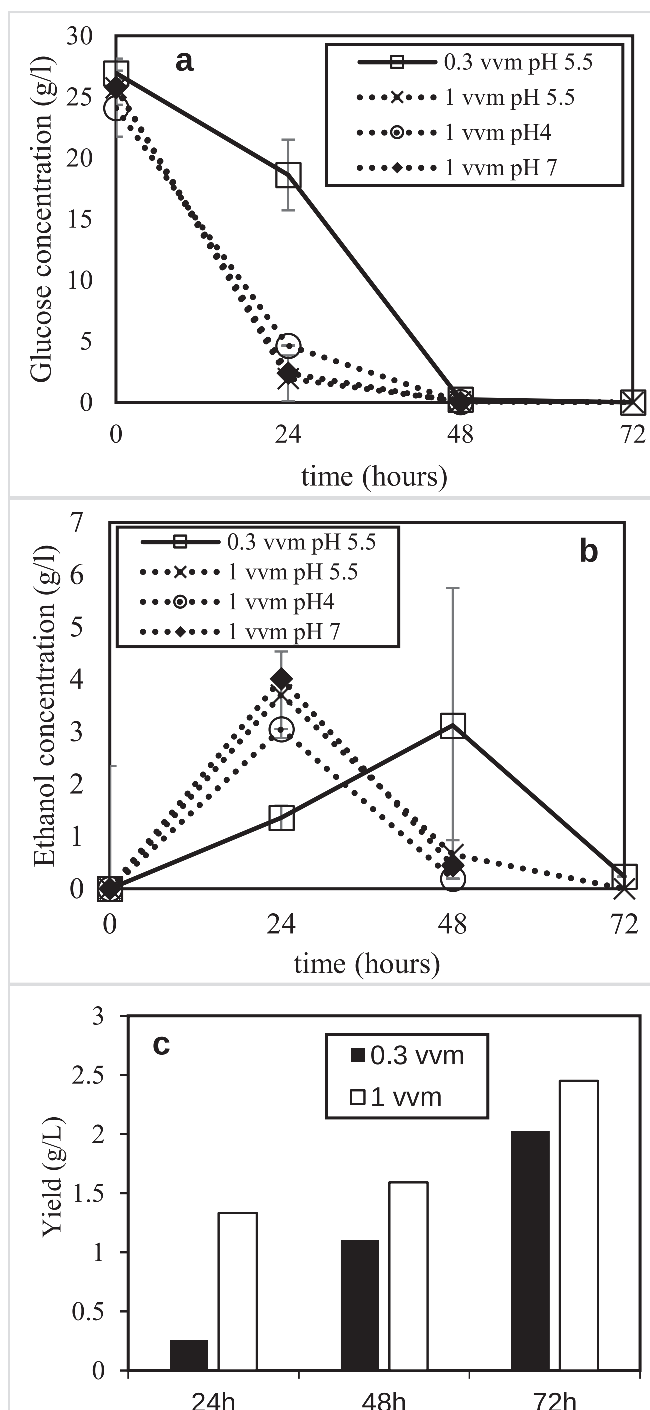

Fig. 1. Glucose (a) and ethanol (b) concentration in the cultivation medium (c) fungal biomass yield (g dry weight/L) of *R. oryzae* grown in defined glucose medium over 72 h at 0.3 vvm (pH 5.5) and 1 vvm at pH 4, 5.5, and 7. Fungal biomass yield at 1 vvm 48 h cultivation was cultivated only at pH 5.5. Error bars of glucose and ethanol concentration represent mean ± standard deviation; n = 3 for 0 h and 24 h, n = 2 for 48 h, n = 1 for 72 h, n = 1 for fungal biomass yield.

3.2. Effect of cultivation conditions on fungal cell wall

A noteworthy observation was the increase in cell wall content during prolonged cultivation (Fig. 3). Cell wall content of fungal biomass was estimated as alkali-insoluble material (AIM). As the carbon source was depleted, *R. oryzae* exhibited a significant rise in AIM content, increasing from 16% to 22% between 24 and 72 h of cultivation. Interestingly, pH also appeared to influence the AIM content of the fungal biomass (Fig. 3b). At pH 7, a significant increase in cell wall content at 30% was observed compared to pH 4 or 5.5 at about 21%.

The carbohydrate cell wall composition of *R. oryzae* is primarily composed of chitin and chitosan (34–57%), β-glucans (3–12%), fucose

(approximately 20%), glucuronic acid (3–23%), and galactose (12%) (Cheng et al., 2024). Therefore, to obtain a more accurate estimation of fungal cell wall content, the total glucosamine content of the biomass (estimating the total of chitin and chitosan) was measured. The glucosamine content increased significantly at an aeration rate of 1 vvm from 7.4%dw of fungal biomass to 10.14% after 72 h of cultivation (Fig. 3c). In contrast to the AIM content, cultivation pH did not have a major effect on the glucosamine content of the biomass (Fig. 3d).

In the present study, glucosamine, representing chitin and chitosan, in the fungal cell wall accounted for 45–70% of the cell wall across different cultivation conditions. In general, prolonged cultivation of filamentous fungi is associated with an increase in cell wall content, which can rise from approximately 20% to 30% of the biomass (Ferreira et al., 2012). In addition, the content of chitin and chitosan within the alkali-insoluble material can also change during cultivation, increasing from around 50% to approximately 80% of AIM as the culture ages (Ferreira et al., 2012).

3.3. Effect of cultivation conditions on fungal protein quality

Protein content alone does not determine nutritional value; protein quality is equally important. Protein quality is characterized by both the essential amino acid profile and the protein digestibility. In this study, protein digestibility is estimated as amino acid accessibility, which quantifies the fraction of amino acids released from proteins into the aqueous fraction of the intestinal digests, separated using a 0.22 μm filter (Trigo et al., 2021). To account for background autolysis, a digested water blank was used to normalize the total amino acids released in the intestinal digests.

The filamentous fungus *Rhizopus oryzae* exhibited a balanced and

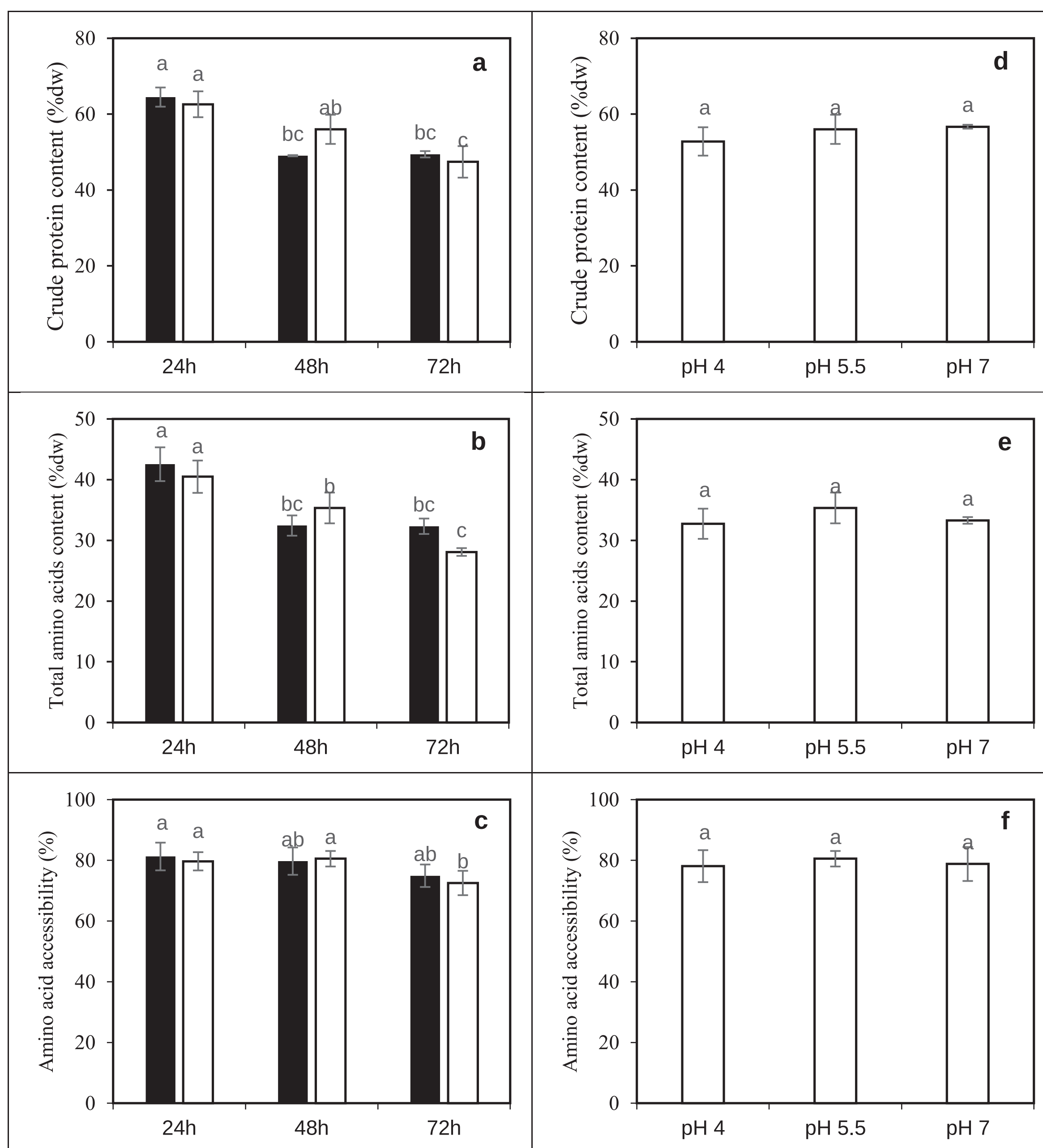

Fig. 2. Effect of (a,b,c) cultivation duration at pH 5.5 and aeration rates of 0.3 vvm (■) and 1 vvm (□), (d,e,f) cultivation pH on *R. oryzae* fungal biomass (a,d) crude protein content (%dry weight, dw), (b,e) total amino acid content (%dw), and (c,f) amino acid accessibility (%). Data are expressed as average values ± standard deviation depicted as error bars (n = 3). Values that do not share the same subscripts denote statistically significant differences ($p < 0.05$) among samples.

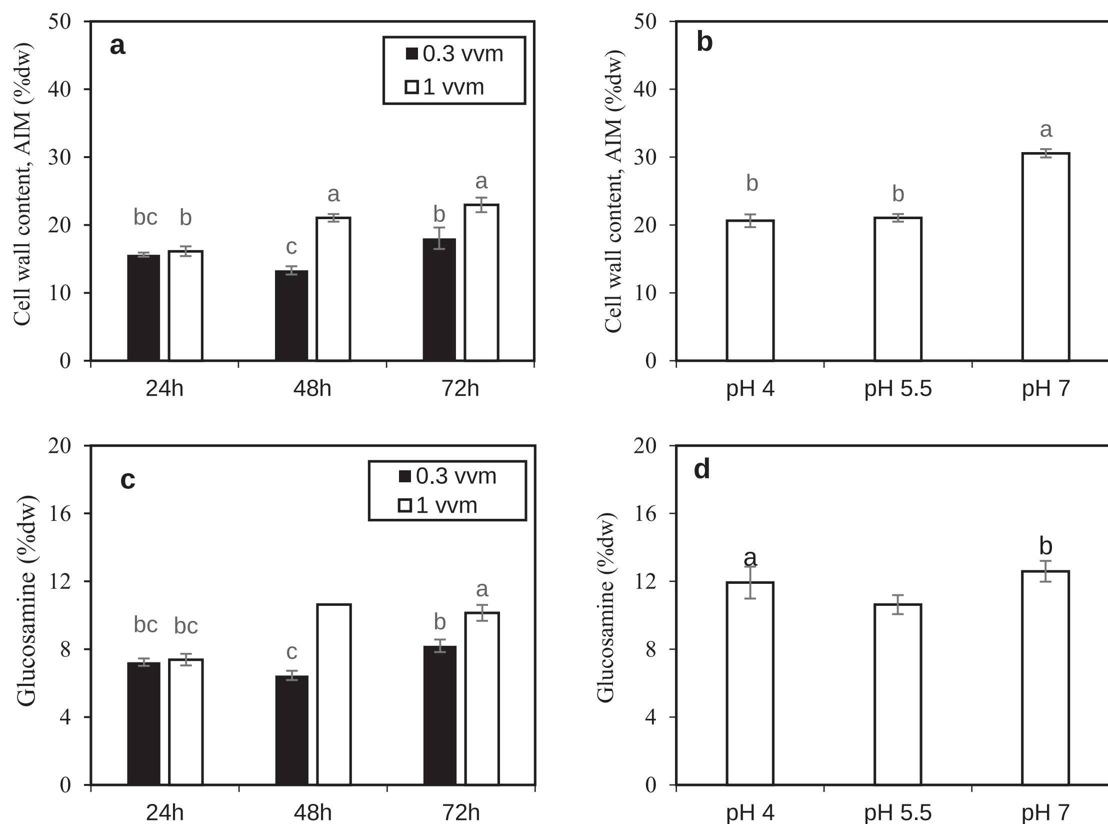

Fig. 3. Effect of cultivation duration and aeration rates (a,c) and pH (b,d) on *R. oryzae* fungal biomass cell wall content, represented by alkali insoluble material (AIM) (a,b) and fungal biomass glucosamine content (c, d). Data are expressed as average values ± standard deviation depicted as error bars (n = 3), except for glucosamine content of fungal biomass at 1 vvm, 48 h, and pH 5.5 (n = 1). Values that do not share the same subscripts denote statistically significant differences ($p < 0.05$) among samples.

nutritionally valuable amino acid profile (Table 1), with essential amino acids comprising 42.6–46.7% of total amino acids. The detailed profile was as follows (%t total amino acids): lysine (8.5–9.4%), histidine (2.5–3.0%), threonine (5.7–5.9%), methionine (1.5–2.1%), valine (5.8–6.8%), isoleucine (5.0–5.8%), leucine (7.7–8.8%), and phenylalanine (4.3–5.1%). Notably, fungus grown at 0.3 vvm for 24 h showed a higher content of essential amino acids. In all other cases, however, the amino acid profile remained relatively stable across different cultivation durations and pH values, even though the total amino acid content decreased during prolonged cultivation. This suggests that the amino acid profile in *R. oryzae* is robust to changes in environmental conditions, even if the absolute protein content is reduced under carbon-limited or late stationary-phase conditions (Fig. 2).

Based on the recommended daily protein intake of 0.66 g per kilogram of body weight, the WHO/FAO guidelines (FAO, 2007) specify the following essential amino acid (EAA) requirements for healthy adults:

Table 1
Amino acid profile of essential amino acids (% total amino acids) of *R.oryzae* fungal biomass cultivated under different condition. Data are expressed as average values ± standard deviation (n = 3) depicted as error bars. Values that do not share the same subscripts denote statistically significant differences ($p < 0.05$) among samples.

Aeration rate (vvm)	pH	Duration (hours)	Lys	His	Thr	Met	Val	Isoleu	Leu	Phe	Essential Amino acid
0.3	5.5	24	9.47 ± 0.09^{a}	2.91 ± 0.45^{a}	5.91 ± 0.06^{ab}	1.78 ± 0.3^{a}	6.84 ± 0.05^{a}	5.82 ± 0.16^{a}	8.89 ± 0.18^{a}	5.14 ± 0.21^{a}	46.73 ± 0.44^{a}
0.3	5.5	48	8.9 ± 0.36^{ab}	2.77 ± 0.66^{a}	5.71 ± 0.13^{b}	1.7 ± 0.21^{a}	6.26 ± 0.04^{b}	5.25 ± 0.16^{bc}	8 ± 0.3^{cd}	4.61 ± 0.07^{bcd}	43.16 ± 0.33^{cd}
0.3	5.5	72	8.76 ± 0.18^{ab}	2.75 ± 0.5^{a}	5.92 ± 0.13^{ab}	1.75 ± 0.41^{a}	5.97 ± 0.16^{cd}	5.22 ± 0.05^{bc}	7.72 ± 0.06^{d}	4.37 ± 0.11^{d}	42.43 ± 0.24^{c}
1	5.5	24	9.07 ± 0.27^{ab}	2.79 ± 0.35^{a}	5.87 ± 0.12^{ab}	1.78 ± 0.48^{a}	$6.1 \pm 0.1b^{c}$	5.18 ± 0.14^{c}	8.46 ± 0.13^{ab}	4.75 ± 0.14^{bc}	43.96 ± 0.54^{bc}
1	5.5	48	9.25 ± 0.55^{ab}	3.03 ± 0.22^{a}	5.82 ± 0.13^{ab}	1.87 ± 0.34^{a}	5.85 ± 0.1^{d}	5.14 ± 0.24^{c}	8.26 ± 0.26^{bc}	4.73 ± 0.18^{bc}	43.91 ± 0.99^{bc}
1	5.5	72	8.91 ± 0.12^{ab}	2.5 ± 0.47^{a}	5.98 ± 0.09^{a}	2.11 ± 0.4^{a}	6.17 ± 0.08^{bc}	5.59 ± 0.18^{ab}	8.57 ± 0.13^{ab}	4.92 ± 0.14^{ab}	44.71 ± 0.2^{b}
1	4	48	8.52 ± 0.36^{b}	3.09 ± 0.32^{a}	5.89 ± 0.13^{ab}	1.54 ± 0.83^{a}	6.04 ± 0.03^{bcd}	5.18 ± 0.17^{c}	7.96 ± 0.2^{cd}	4.53 ± 0.06^{cd}	42.72 ± 0.38^{cd}
1	7	48	8.5 ± 0.4^{b}	2.85 ± 0.27^{a}	5.7 ± 0.13^{b}	1.84 ± 0.35^{a}	5.8 ± 0.13^{d}	5.07 ± 0.17^{c}	8.28 ± 0.21^{bc}	4.66 ± 0.24^{bcd}	42.66 ± 0.47^{cd}

histidine (15 mg/g protein), isoleucine (30 mg/g), leucine (59 mg/g), lysine (45 mg/g), methionine (16 mg/g), phenylalanine plus tyrosine (38 mg/g), threonine (23 mg/g), tryptophan (6 mg/g), and valine (39 mg/g). When compared to these reference values, *R. oryzae* biomass consistently meets or exceeds the recommended levels for all major essential amino acids, confirming that its protein is of high nutritional quality. Although the total amino acid content of *R. oryzae* (28–40% of dry weight) is lower than that of conventional animal-based proteins such as meat, fish, and eggs (50–90% of dry weight) (Wang et al., 2023), its essential amino acid profile is comparable and complementary to many animal-based proteins.

Among these, lysine content was particularly high, ranging from 8.5 to 9.4% of total amino acids. This is noteworthy because lysine is considered the first limiting amino acid in most cereal grains, which typically contain less than 5% lysine per unit of protein (Young & Pellett, 1990). The elevated lysine content in *R. oryzae* is consistent with previous reports on filamentous fungi (Karimi et al., 2021; Wang et al., 2023). This is most likely due to fungi lysine biosynthetic pathway differ from plant and bacteria that use the diaminopimelate pathway. In fungi, lysine is synthesized through the α-aminoadipate pathway, which requires precursor from the tricarboxylicacid cycle intermediate α-ketoglutarate. In contrast, organisms that use the diaminopimelate pathway synthesize lysine from aspartate, which is derived from oxaloacetate and is shared among several amino-acid biosynthetic routes, including those for methionine, threonine, and isoleucine (Fazius et al., 2013; Xu et al., 2006).

The total amino acid bioaccessibility of *R. oryzae* in this study ranged from 72% to 81%, indicating that a substantial proportion of its protein becomes soluble in intestinal digests after simulated gastrointestinal digestion. At the lower aeration rate (0.3 vvm), extending the cultivation duration from 24 to 72 h did not significantly affect overall amino acid bioaccessibility. However, at the higher aeration rate (1 vvm), a slight but statistically significant decline was observed, with bioaccessibility decreasing from approximately 80.5% at 48 h to 72.5% at 72 hFigs. 2c and 2f. The high bioaccessibility of *R. oryzae* observed in this study is consistent with previous reported values of around 84.7% (Wang et al., 2023).

When individual essential amino acids were examined, their bioaccessibility remained relatively stable between 24 and 72 h at 0.3 vvm (Table 2). In contrast, cultivation at 1 vvm resulted in small but notable drop after 72 h. For example, methionine bioaccessibility decreased from 107.4% to 77.9%, while histidine increased from 59.6% to 77.8%. Similarly, isoleucine, leucine, and valine decreased from 85%, 83%, and 84.4% to 72.1%, 71.3%, and 69.1%, respectively. No significant drop in lysine accessibility was observed after prolonged fungi cultivation.

The decrease in amino acid accessibility was consistent with a significant increase in glucosamine content in the fungal biomass after 72 h of cultivation. Although higher pH conditions resulted in a greater amount of AIM, no significant differences were observed in glucosamine content. Similarly, no significant differences were detected in amino acid accessibility. These findings suggest that structural components of the fungal cell wall, particularly chitin and chitosan, may contribute to the slight reduction in amino acid accessibility. All essential amino acids showed either reduced accessibility or no significant change, with the exception of histidine, which exhibited increased accessibility. The reason for the increased histidine accessibility in fungal biomass after prolonged cultivation remains unclear.

Evidence from microalgae supports this concept, as prolonged cultivation leads to cell wall thickening and a marked reduction in protein bioaccessibility, decreasing from approximately 79% during the exponential phase to 46% after 48 h in the stationary (Abiusi et al., 2024). However, in the present study, the reduction in amino acid accessibility was relatively minor compared to that observed in microalgae. One possible explanation is that the stationary phase duration in this study was not sufficiently long to induce more pronounced structural changes in the cell wall. Colosimo et al. (2021) highlighted key differences between plant and fungal cell walls, noting that fungal cell walls are relatively porous. This porosity allows digestive enzymes to diffuse through intact cell walls, hydrolyze intracellular substrates, and release the resulting products into the extracellular environment. Consequently, the fungal cell wall may primarily slow down the rate of digestion rather than limit the overall extent of protein digestion. Therefore, the increase in fungal chitin content during prolonged cultivation may contribute to a slight decrease in protein digestibility. However, further studies, particularly those employing microscopy techniques, are required to better elucidate the underlying mechanisms.

We demonstrated that neither pH nor aeration rate significantly affected the amino acid profile or bioaccessibility. From an industrial perspective, this is an encouraging finding, as fluctuations in pH and aeration are often unavoidable in large-scale processes. However, prolonged cultivation time may contribute to reduce protein content and a slight decrease in amino acid accessibility, highlighting the importance of understanding fungal growth kinetics. To maximize protein quality, cultivation should ideally avoid extended stationary phase conditions. In addition, fungal biomass harvested during the logarithmic growth phase contains higher protein levels. Therefore, continuous production systems, such as chemostats, in which the culture is maintained in the exponential growth phase, may be more favorable for maximizing both

Table 2
Bioaccessibility of each essential amino acid after *in vitro* gastrointestinal digestion of *R.oryzae* fungal biomass cultivated under different condition. Data are expressed as average values ± standard deviation (n = 3) depicted as error bars. Values that do not share the same subscripts denote statistically significant differences ($p < 0.05$) among samples.

Aeration rate (vvm)	pH	Cultivation duration (hours)	Lys	His	Met	Thr	Isoleu	Leu	Phe	Val
0.3	5.5	24	91.6 ± 10.54^{a}	51.9 ± 1.83^{b}	103.6 ± 5.09^{ab}	80.1 ± 5.21^{a}	83.8 ± 6.23^{ab}	82.7 ± 5.46^{ab}	94.3 ± 6.46^{a}	82 ± 3.85^{ab}
0.3	5.5	48	90.8 ± 4.82^{a}	54.7 ± 11.71^{b}	104.2 ± 9.16^{ab}	78.5 ± 5.92^{ab}	81.4 ± 6.03^{ab}	84.3 ± 6.83^{a}	98.9 ± 6.75^{a}	79.5 ± 4.26^{abc}
0.3	5.5	72	86.9 ± 4.1^{a}	60.6 ± 2.78^{b}	86.5 ± 6.54^{ab}	69.2 ± 4.75^{ab}	71.1 ± 7.7^{ab}	77.5 ± 4.73^{ab}	91.4 ± 7.29^{a}	71.4 ± 5.6^{bc}
1	5.5	24	89.2 ± 5.38^{a}	59.6 ± 8.42^{b}	107.4 ± 22.07^{a}	74.8 ± 2.82^{ab}	81.9 ± 5.22^{ab}	80 ± 4.42^{ab}	95 ± 3.22^{a}	84.4 ± 3.7^{a}
1	5.5	48	90 ± 5.55^{a}	54.5 ± 6.08^{b}	98 ± 12.37^{ab}	77.5 ± 2.39^{a}	85.5 ± 6.42^{a}	83.2 ± 4.53^{a}	95.7 ± 4.45^{a}	83.3 ± 4.47^{a}
1	5.5	72	90.9 ± 5.68^{a}	77.8 ± 7.67^{a}	77.9 ± 14.41^{b}	68.7 ± 5.83^{b}	72.1 ± 6.54^{b}	71.3 ± 5.18^{b}	83.5 ± 10.34^{a}	69.1 ± 4.43^{c}
1	4	48	95.2 ± 7.96^{a}	54.9 ± 3.11^{b}	113.6 ± 10.21^{a}	72.5 ± 2.77^{ab}	79.8 ± 7.2^{ab}	83.7 ± 6.09^{ab}	99 ± 4.26^{a}	76.8 ± 3.46^{abc}
1	7	48	92.1 ± 6.87^{a}	53.8 ± 1.11^{b}	96.5 ± 11.46^{ab}	72.2 ± 4.25^{ab}	82.9 ± 6.71^{ab}	80.2 ± 6.65^{ab}	96.4 ± 6.75^{a}	80.5 ± 7.61^{ab}

productivity and protein quality.

One limitation of the present study is that the protein quality of the mycoprotein was evaluated using biomass that was directly harvested after fermentation and subsequently freeze-dried without any heat treatment. For industrial food applications, however, mycoprotein typically undergoes heat treatment to reduce RNA content (Finnigan et al., 2025). Such processing may significantly affect protein quality. For instance, heat treatment may induce protein cross-linking that can decrease fungal protein digestibility (Nordlund et al., 2024). It is also important to note that the result presented is an approximation of protein bioaccessibility with INFOGEST *in vitro* gastrointestinal model, rather than an absolute measure. Amino acid bioaccessibility only provides an indication that the filtered digests are available for intestinal uptake, rather than representing the complete digestibility of the fungi protein. Therefore, further evaluation is necessary: using models like the Caco-2 cell system to fully validate these findings.

4. Conclusion

In this study, the effects of cultivation conditions on the protein content and quality of the edible filamentous fungus *Rhizopus oryzae* were investigated. Prolonged cultivation, but not pH or aeration rate, led to a reduction in protein content in *R. oryzae* biomass, along with an increase in fungal cell wall components and a decrease in amino acid bioaccessibility. The amino acid profile of the fungal biomass remained relatively stable. Overall, these findings suggest that the fungal cell wall plays a role in limiting protein digestibility in *R. oryzae* biomass.

CRediT authorship contribution statement

Ricky Wang: Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation, Conceptualization. **Ophelie Lemaire:** Methodology, Investigation, Data curation, Conceptualization. **Amir Mahboubi:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Mohammad J. Taherzadeh:** Writing – review & editing, Supervision, Conceptualization.

Ethical approval

Not applicable.

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.

Acknowledgment

We would like to acknowledge University of Borås for the technical and financial support.

Data availability

Data will be made available on request.

References

Abiusi, F., Tumulero, B., Neutsch, L., & Mathys, A. (2024). Productivity, amino acid profile, and protein bioaccessibility in heterotrophic batch cultivation of *Galdieria sulphuraria*. *Bioresource Technology, 399*, Article 130628. https://doi.org/10.1016/j.biortech.2024.130628

Ariëns, R. M. C., Bastiaan-Net, S., van de Berg-Somhorst, D. B. P. M., Karim, El B., Anouk, B., van den Dool, R. T. M., de Jong, G. A. H., Wichers, H. J., & Mes, J. J. (2021). Comparing nutritional and digestibility aspects of sustainable proteins using the INFOGEST digestion protocol. *Journal of Functional Foods, 87*, Article 104748. https://doi.org/10.1016/j.jff.2021.104748

Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal, C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I. (2019). INFOGEST static in vitro simulation of gastrointestinal food digestion. *Nature Protocols, 14*, 991–1014. https://doi.org/10.1038/s41596-018-0119-1

Cao, L., El Mashad, H. M., Pan, Z., & Zhang, R. (2025). Production of filamentous fungal biomass (Aspergillus awamori) in pellet form from Almond hull extract. *Food and Bioprocess Technology, 18*, 8735–8750. https://doi.org/10.1007/s11947-025-03955-x

Chen, C., Song, Z., Muhedaner, M., Tao, Y., Zhou, G., & Ye, K. (2025). Influence of mycelial integrity damaged by ultrasonic treatment on product textural properties and *in vitro* digestibility. *Food Chemistry, 463*, Article 141536. https://doi.org/10.1016/j.foodchem.2024.141536

Cheng, Q., Dickwella Widanage, M. C., Yarava, J. R., Ankur, A., Latgé, J.-P., Wang, P., & Wang, T. (2024). Molecular architecture of chitin and chitosan-dominated cell walls in zygomycetous fungal pathogens by solid-state NMR. *Nature Communications, 15*, 8295. https://doi.org/10.1038/s41467-024-52759-8

Colosimo, R., Warren, F. J., Edwards, C. H., Ryden, P., Dyer, P. S., Finnigan, T. J. A., & Wilde, P. J. (2021). Comparison of the behavior of fungal and plant cell wall during gastrointestinal digestion and resulting health effects: A review. *Trends in Food Science & Technology, 110*, 132–141. https://doi.org/10.1016/j.tifs.2021.02.001

Colosimo, R., Warren, F. J., Finnigan, T. J. A., & Wilde, P. J. (2020). Protein bioaccessibility from mycoprotein hyphal structure: In vitro investigation of underlying mechanisms. *Food Chemistry, 330*, Article 127252. https://doi.org/10.1016/j.foodchem.2020.127252

FAO. (2007). *Protein and amino acid requirements in human nutrition : Report of a joint FAO/WHO/UNU expert consultation*. World Health Organization.

Fazius, F., Zaehle, C., & Brock, M. (2013). Lysine biosynthesis in microbes: Relevance as drug target and prospects for β-lactam antibiotics production. *Applied Microbiology and Biotechnology, 97*, 3763–3772. https://doi.org/10.1007/s00253-013-4805-1

Ferreira, J. A., Lennartsson, P. R., Niklasson, C., Lundin, M., Edebo, L., & Taherzadeha, M. J. (2012). Spent sulphite liquor for cultivation of an edible Rhizopus sp. *Bioresources, 7*, 173–188.

Ferreira, J. A., Mahboubi, A., Lennartsson, P. R., & Taherzadeh, M. J. (2016). Waste biorefineries using filamentous ascomycetes fungi: Present status and future prospects. *Bioresource Technology, 215*, 334–345. https://doi.org/10.1016/j.biortech.2016.03.018

Finnigan, T. J. A., Theobald, H. E., & Bajka, B. (2025). Mycoprotein: A healthy and sustainable source of alternative protein-based foods. *Annual Review of Food Science and Technology, 16*, 105–125. https://doi.org/10.1146/annurev-food-111523-121802

Gmoser, R., Ferreira, J. A., Lundin, M., Taherzadeh, M. J., & Lennartsson, P. R. (2018). Pigment production by the edible filamentous fungus Neurospora Intermedia. *Fermentation, 4*, 11. https://doi.org/10.3390/fermentation4010011

He, C. (2022). Balancing nutrient and energy demand and supply via autophagy. *Current Biology, 32*, R684–R696. https://doi.org/10.1016/j.cub.2022.04.071

Hoxha, L., Wang, R., Taherzadeh, M. J., & Undeland, I. (2025). *In vitro* protein digestion and mineral accessibility of edible filamentous fungi cultivated on winery and distillery by-products. *Food Bioscience, 73*, Article 107711. https://doi.org/10.1016/j.fbio.2025.107711

Karimi, S., Mahboobi Soofiani, N., Mahboubi, A., Ferreira, J. A., Lundh, T., Kiessling, A., & Taherzadeh, M. J. (2021). Evaluation of nutritional composition of pure filamentous fungal biomass as a novel ingredient for fish feed. *Fermentation, 7*, 152. https://doi.org/10.3390/fermentation7030152

Klis, F. M., Mol, P., Hellingwerf, K., & Brul, S. (2002). Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiology Reviews, 26*, 239–256. https://doi.org/10.1016/S0168-6445(02)00087-6

Krotz, L., Leone, F., & Giazzi, G. (2016). *High accuracy of Nitrogen, carbon and sulfur analysis for agronomy applications using the thermo scientific FlashSmart elemental analyzer (application note)*. Thermo Fisher Scientific.

Lennartsson, P. R., Karimi, K., Edebo, L., & Taherzadeh, M. J. (2009). Effects of different growth forms of *Mucor indicus* on cultivation on dilute-acid lignocellulosic hydrolyzate, inhibitor tolerance, and cell wall composition. *Journal of Biotechnology, 143*, 255–261. https://doi.org/10.1016/j.jbiotec.2009.07.011

Lübbehüsen, T. L., Nielsen, J., & McIntyre, M. (2004). Aerobic and anaerobic ethanol production by Mucor circinelloides during submerged growth. *Applied Microbiology and Biotechnology, 63*, 543–548. https://doi.org/10.1007/s00253-003-1394-4

Mahboubi, A., Ferreira, J. A., Taherzadeh, M. J., & Lennartsson, P. R. (2017). Production of fungal biomass for feed, fatty acids, and glycerol by Aspergillus oryzae from fat-rich dairy substrates. *Fermentation, 3*, 48. https://doi.org/10.3390/fermentation3040048

Martín-Miguélez, J. M., Bross, J., Prado, D., Merino, E., Perisé Moré, R., Otero, J., Aduriz, A. L., & Delgado, J. (2025). Review: *Rhizopus* sp. beyond tempeh. An Occidental approach to mold-based fermentations. *International Journal of Gastronomy and Food Science, 39*, Article 101090. https://doi.org/10.1016/j.ijgfs.2024.101090

Millati, R., Karimi, K., Edebo, L., Niklasson, C., & Taherzadeh, M. J. (2008). Ethanol production from xylose and wood hydrolyzate by mucor indicus at different aeration rates. *BioResour, 3*, 1020–1029. https://doi.org/10.15376/biores.3.4.1020-1029

Mohammadi, M., Zamani, A., & Karimi, K. (2012). Determination of glucosamine in fungal cell walls by high-performance liquid chromatography (HPLC). *Journal of Agricultural and Food Chemistry, 60*. https://doi.org/10.1021/jf303488w

Mohammadi, M., Zamani, A., & Karimi, K. (2013). Effect of phosphate on glucosamine production by ethanolic Fungus Mucor indicus. *Applied Biochemistry and Biotechnology, 171*, 1465–1472. https://doi.org/10.1007/s12010-013-0440-7

Nordlund, E., Silventoinen-Veijalainen, P., Hyytiäinen-Pabst, T., Nyyssölä, A., Valtonen, A., Ritala, A., Lienemann, M., & Rosa-Sibakov, N. (2024). In vitro protein digestion and carbohydrate colon fermentation of microbial biomass samples from bacterial, filamentous fungus and yeast sources. *Food Research International, 182*, Article 114146. https://doi.org/10.1016/j.foodres.2024.114146

Perez-Moral, N., Saha, S., Pinto, A. M., Bajka, B. H., & Edwards, C. H. (2023). *In vitro* protein bioaccessibility and human serum amino acid responses to white bread enriched with intact plant cells. *Food Chemistry, 404*, Article 134538. https://doi.org/10.1016/j.foodchem.2022.134538

Ribeiro, R. A., Bourbon-Melo, N., & Sá-Correia, I. (2022). The cell wall and the response and tolerance to stresses of biotechnological relevance in yeasts. *Frontiers in Microbiology, 13*. https://doi.org/10.3389/fmicb.2022.953479

Rousta, N., Larsson, K., Fristedt, R., Undeland, I., Agnihotri, S., & Taherzadeh, M. J. (2022). Production of fungal biomass from oat flour for the use as a nutritious food source. *NFS Journal, 29*, 8–15. https://doi.org/10.1016/j.nfs.2022.09.001

Sar, T., Larsson, K., Fristedt, R., Undeland, I., & Taherzadeh, M. J. (2022). Demo-scale production of protein-rich fungal biomass from potato protein liquor for use as innovative food and feed products. *Food Bioscience, 47*, Article 101637. https://doi.org/10.1016/j.fbio.2022.101637

Souza Filho, P. F., Zamani, A., & Taherzadeh, M. J. (2019). Edible protein production by filamentous fungi using starch plant wastewater. *Waste and Biomass Valorization, 10*, 2487–2496. https://doi.org/10.1007/s12649-018-0265-2

Sues, A., Millati, R., Edebo, L., & Taherzadeh, M. J. (2005). Ethanol production from hexoses, pentoses, and dilute-acid hydrolyzate by Mucor indicus. *FEMS Yeast Research, 5*, 669–676. https://doi.org/10.1016/j.femsyr.2004.10.013

Svensson, S. E., Bucuricova, L., Ferreira, J. A., Souza Filho, P. F., Taherzadeh, M. J., & Zamani, A. (2021). Valorization of bread waste to a Fiber- and protein-rich fungal biomass. *Fermentation, 7*. https://doi.org/10.3390/fermentation7020091

Trigo, J. P., Engström, N., Steinhagen, S., Juul, L., Harrysson, H., Toth, G. B., Pavia, H., Scheers, N., & Undeland, I. (2021). In vitro digestibility and Caco-2 cell bioavailability of sea lettuce (Ulva fenestrata) proteins extracted using pH-shift processing. *Food Chemistry, 356*, Article 129683. https://doi.org/10.1016/j.foodchem.2021.129683

Udall, J. N., Lo, C. W., Young, V. R., & Scrimshaw, N. S. (1984). The tolerance and nutritional value of two microfungal foods in human subjects. *The American Journal of Clinical Nutrition, 40*, 285–292. https://doi.org/10.1093/ajcn/40.2.285

Wang, R., Rousta, N., Mahboubi, A., Fristedt, R., Undeland, I., Sandberg, A.-S., & Taherzadeh, M. J. (2024). *In vitro* protein digestibility and mineral accessibility of edible filamentous Fungi cultivated in oat flour. *NFS Journal, 36*, Article 100189. https://doi.org/10.1016/j.nfs.2024.100189

Wang, R., Sar, T., Mahboubi, A., Fristedt, R., Taherzadeh, M. J., & Undeland, I. (2023). In vitro protein digestibility of edible filamentous fungi compared to common food protein sources. *Food Bioscience, 54*, Article 102862. https://doi.org/10.1016/j.fbio.2023.102862

Xu, H., Andi, B., Qian, J., West, A. H., & Cook, P. F. (2006). The α-aminoadipate pathway for lysine biosynthesis in fungi. *Cell Biochemistry and Biophysics, 46*, 43–64. https://doi.org/10.1385/CBB:46:1:43

Young, V. R., & Pellett, P. L. (1990). Current concepts concerning indispensable amino acid needs in adults and their implications for international nutrition planning. *Food and Nutrition Bulletin, 12*, 1–13. https://doi.org/10.1177/156482659001200414

Zamani, A., Jeihanipour, A., Edebo, L., Niklasson, C., & Taherzadeh, M. J. (2008). Determination of glucosamine and N-Acetyl glucosamine in fungal cell walls. *Journal of Agricultural and Food Chemistry, 56*, 8314–8318. https://doi.org/10.1021/jf801478j