

Enhancing the Sustainability of Fungal Proteins from *Fusarium venenatum* and *Saccharomyces cerevisiae* through Membrane Filtration Optimization

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ABSTRACT

Due to the growing global demand for sustainable protein sources, fungal proteins have gained significant attention because of their efficient nutritional value and potential health benefits. Large-scale production of fungal proteins is pivotal to meet elevating global demand for nutritious proteins while diminishing the reliance on resource-intensive protein production methods, such as animal agriculture. Moreover, industrial scale production will guarantee food security by providing high quality, valuable protein sources with lower water or land use. However, the efficiency of mycoprotein downstream processing remains a key obstacle in their large-scale production. Microfiltration (MF) and ultrafiltration (UF) are membrane-based separation techniques that demonstrate effective future methods for the recovery and purification of fungal proteins. This paper evaluates the use of MF and UF in mycoprotein processing, focusing on species like *Fusarium venenatum* and *Saccharomyces cerevisiae*, to improve protein quality, recovery, and feasibility. The findings demonstrate that optimization of membrane-based filtration parameters such as pH, membrane pore size, transmembrane pressure, cross-flow velocity and cleaning strategies enhance protein quality and yield. Compared to traditional methods, membrane-based filtration demonstrates improved performance and MF and UF represent a promising method for a large-scale mycoprotein production. The findings emphasize the significance of suitable parameters in case to avoid potential limitations like fouling, lower flux or cake layer formation and support the amelioration of scalable and sustainable alternative protein production systems. However, further pilot studies are recommended to confirm laboratory findings.

Keywords: fungal protein; mycoprotein; *Fusarium venenatum*; *Saccharomyces cerevisiae*; microfiltration; ultrafiltration; membrane separation.

INTRODUCTION

It is estimated that the global population will reach approximately 10 billion by 2050, which poses a significant issue in terms of ensuring enough nutritionally adequate protein sources (Hamad, 2025).

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Nowadays, meat and plants remain the main protein production chains. However, both engender environmental challenges that question their long-term scalability.

Animal products present an abundant source of proteins, but their production heavily relies on resource-intensive processes. Livestock farming takes up 77 % of worldwide agricultural land, yet only provides around 40 % of the world's protein supply, which makes the land use insufficient (Poore & Nemecek, 2018). In addition to the need for arable land, animal agriculture consumes large amounts of freshwater. For instance, around 15,000–20,000 liters of water is necessary for beef production, per kilogram (Mekonnen & Hoekstra, 2012). Additionally, livestock generation contributes to global climate change, as it produces around 14.5 % of greenhouse gas emissions due to methane release (Food and Agriculture Organization [FAO], 2013).

On the other hand, production of plant-based proteins demands comparably mitigated resources than animal-based proteins. Nevertheless, they still depend on arable land. However, due to an incomplete amino acid profile, mostly plant-based proteins are low in protein quality compared to meat-based proteins. Most of them have lower emulsifying activity and solubility, which diminishes their sustainability in the food industry and requires certain modifications, like pH adjustment, enzymatic hydrolysis, and heat treatment (Lima, 2023). Therefore, plant-based proteins cannot be the potential major protein source, as they are resource-intensive, requiring around 13 - 23 % of total agricultural land and contributing to climate change (Xu and Jain 2021).

The limitations of animal and plant-based protein generation coerce us to consider alternative sustainable protein resources, like fungal proteins derived from species such as *Fusarium venenatum* and *Saccharomyces cerevisiae*. It is known that fungal biomass contains high protein content, has an exponential growth rate, and can be cultivated without the use of arable land. Among fungal protein sources, *F.venenatum* has gained particular attention due to its ability to produce mycoprotein, a high-protein biomass with a fibrous texture resembling animal meat. This mycoprotein is commercially used in meat substitute products, including burgers, nuggets, sausages, and mince alternatives, demonstrating its feasibility as a sustainable alternative to conventional animal-derived proteins. Therefore, fungal proteins offer a viable alternative for conventional protein production.

Nonetheless, the large-scale generation of mycoproteins requires high-quality downstream processing methods to separate and purify proteins. Microfiltration (MF) and ultrafiltration (UF) are generally widely used in bioprocessing, but they are not utilized for mycoprotein production. Therefore, it is pivotal to analyze optimal filtration parameters including membrane pore size, pH, and temperature.

This review aims to synthesize information about *F. venenatum* and *S. cerevisiae*, their downstream processing, optimizing MF and UF methods for producing alternative, sustainable protein sources. By identifying the challenges and advantages, this paper will evaluate the potential of fungal proteins and their downstream methods, contributing to a significant impact on the food industry.

LITERATURE REVIEW

Nutritional traits of fungal proteins, their production and their applications

Nowadays, fungal proteins are gaining increasing attention as alternative protein sources. On the basis of their nutritional composition, they constitute high-quality sources of dietary proteins. Also referred to as mycoproteins, generally contain a superior amino acid profile, compared to plant-based proteins, rendering them remarkable and feasible alternative sources. *F. venenatum* and *S. cerevisiae* are both extensively investigated species, widely utilized in the food industry. *F. venenatum* contains 44% of protein, including all the important amino acids. Humans require nine essential amino acids, all of which can be found in mycoproteins: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine (Ritala *et al.*, 2017).

Mycoproteins also contain high dietary fibers (chitin and β -glucans), which facilitate digestion, due to their porous and non-rigid cell wall. In contrast to fungi, plant cell walls are mechanically inflexible, slowing down enzyme access (Li *et al.*, 2023). Specifically, *F. venenatum* has a protein digestibility-corrected amino acid score (PDCAAS) of 0.96, which is superior to chicken and beef (0.92) (Edwards & Cummings, 2010). A study conducted by Dunlop *et al.* (2017) explored an ingestion comparison between milk and mycoproteins. Volunteers were instructed to consume a beverage containing either 20 g milk protein or a mass-matched bolus of mycoprotein. The experiment showed that ingestion of 20 g milk protein increased plasma leucine concentration of approximately 201 $\mu\text{mol/l}$ at 30 min postprandial, whereas the same amount of mycoprotein resulted in a lower peak of about 118 $\mu\text{mol/l}$ at 90 min. These results indicate that amino acids in milk reach highest concentration in the bloodstream, while fungal protein provides more sustained hyperaminoacidemia over the 4-hour postprandial period.

In addition, in a randomised controlled trial, study participants were assigned a diet in which lunch and dinner proteins were provided from mycoprotein instead of meat or fish for a week. The study concluded that no significant change in insulin sensitivity was noticed; however, it was observed that levels of low-density lipoprotein (LDL) cholesterol decreased, indicating some cardiovascular and metabolic benefits. Additionally, fiber level was higher in the mycoprotein group due to the chitin and β -glucans in the fungal cell wall (Coelho *et al.*, 2021). Moreover, intake of *F. venenatum* over 3 weeks resulted in the decrease of LDL cholesterol by 9 %, whereas high-density lipoprotein (HDL) cholesterol was increased by 12 % (Turnbull, Leeds, and Edwards 1990).

Mycoproteins also contribute to better gut health. A randomized test, which included 20 adult men, demonstrated that substituting red and overprocessed meat with *Fusarium*-containing meat substitutes diminished fecal genotoxicity and nitroso compounds, which are connected to colorectal cancer. It contributed to the increased number of beneficial gut bacteria like *Lactobacilli*, *Roseburia*, and *Akkermansia*, which are essential for supporting gut barrier and immunity (Farsi *et al.*, 2023).

Mycoproteins are also connected with satiety. High protein and fiber augment feelings of fullness and reduce overall calorie intake (Bottin *et al.*, 2016). Dietary fiber can suppress hunger for up to 10 hours after consumption, resulting in reduced food intake. Based on a study, overweight female participants were given meals containing mycoproteins. Subsequently, energy intake was dramatically reduced the day of study by 24 % and the next day by 16.5 % after consuming mycoproteins compared to chicken (Turnbull *et al.*, 1993). Therefore, dietary fiber has a significant influence on satiety and energy intake, which can address weight-related problems.

Additionally, mycoproteins can be a remedy for controlling blood sugar levels and preventing Type 2 diabetes. A study conducted by Turnbull and Ward (1995) investigated the effects of mycoprotein on blood sugar levels. It demonstrated that, when healthy subjects took their milkshakes containing 20g of mycoproteins, their postprandial glucose level diminished by 13% at 60 minutes, and insulin was significantly reduced by 36 %. Therefore, intake of fungal proteins is beneficial for managing blood glucose levels and Type 2 Diabetes.

Mycoproteins are a great protein source for muscle synthesis. They provide essential amino acids, like leucine, that are needed for muscle repair. Monteyne *et al.* (2020) conducted a blind trial, where participants consumed either 31 g of milk protein or 70 g of mycoprotein after performing physical exercise. Intake of milk resulted in augmented protein synthesis in both rested and exercised muscles. However, after consuming mycoprotein, the increase was even greater. In rested muscle, Fractional Synthetic Rate (FSR), reflecting the speed at which the new muscle proteins are synthesized, went from 0.025 to 0.057%/h with mycoprotein, compared with 0.036 to 0.052%/h with milk. In exercised muscle, FSR went from 0.024 to 0.072%/h with mycoprotein, compared with 0.035 to 0.056%/h with milk (Monteyne *et al.*, 2020).

Mycoproteins are widely used in the food industry. One of the most popular brands using mycoproteins in their product is Quorn. Quorn originated from the United Kingdom and is primarily sold across Europe. They offer meat substitutes and utilize mycoprotein, specifically *F. venenatum* (Quorn, n.d.). Quorn mycoproteins have a substantially less negative impact on the environment, compared to animal or plant-based proteins. Another similar company is The Better Meat Co, which originated in the United States. The company produces mycoproteins under the name of Rhiza. They produce commercial products, like steak, chicken, hot dogs, and fish (The Better Meat Co., n.d.).

S. cerevisiae is widely utilized in the food and beverage sector. Around 1 million tons are produced each year in the European yeast industry (Parapouli *et al.*, 2020). It is utilized in the production of fermented drinks, like wine or beer. Additionally, it plays a crucial role in the baking field. *S. cerevisiae* is directly used in food. As it presents an excellent source of protein and B-vitamins, it is widely consumed as a dietary supplement. Well-known brands like Bragg and Now Foods sell nutritional yeast powder made from inactive *S. cerevisiae* as a dietary supplement.

Despite the advantages of *F. venenatum* as a sustainable source of mycoprotein, several challenges must be addressed to ensure its safe and efficient application as a food ingredient. One of the primary concerns associated with filamentous fungi is the potential production of mycotoxins, which are toxic secondary metabolites produced by certain fungal species. Although *F. venenatum* is considered safe for food production, studies have reported the possible formation of fumonisins at low concentrations (approximately 8.6 µg/kg), with production potentially influenced by environmental conditions such as moisture content in the cultivation medium (D’Almeida *et al.*, 2025). Similarly, *Saccharomyces cerevisiae* represents a promising microorganism for SCP production due to its established safety profile, rapid growth, and ability to utilize various low-cost substrates. However, yeast-based SCP production also presents several limitations. A major challenge is the relatively high nucleic acid content of yeast biomass, which may restrict direct consumption due to potential health concerns associated with excessive nucleic acid intake, including an increased risk of gout. Therefore, additional processing steps are required to reduce nucleic acid levels before human or animal consumption (D’Almeida *et al.*, 2025).

PRODUCTION OF MYCOPROTEINS FROM F. VENENATUM AND S. CEREVISIAE

There are two main types of mycoprotein fermentation: solid-state fermentation (SSF) and submerged fermentation (SmF). SSF is a bioprocess in which microorganisms grow on moist solid substrates (Majumder *et al.*, 2024). It has limited control of process parameters, uneven oxygen and moisture distribution, and increased risk of contamination.

Over the years, SmF has been actively utilized for mycoprotein production. SmF involves the growth of microorganisms in a nutrient-rich liquid under aerobic conditions. It employs bioreactors to optimize oxygen transfer and ensure long hyphal growth. This method maximizes protein content, biomass yield, and sustainability, as it allows precise control over pH, nutrients, and oxygen levels (Gnaim *et al.*, 2025). SmF is particularly convenient for *F. venenatum* due to its strict aerobic nature and the demand for high oxygen levels, which can be efficiently met in well oxygenated bioreactors. Additionally, SmF supports large-scale and continuous production, simplifies downstream processing, and facilitates compliance with food safety regulations, making it more practical than solid-state fermentation for industrial mycoprotein production.

The process generally commences with selecting a high-yield commercial strain, for example A3/5. They are cultivated in petite seed cultures to generate healthy inoculum, consisting of fungal hyphae. Once it reaches sufficient density, it is processed in industrial-scale stainless-steel bioreactors, where glucose, ammonia, essential minerals, and vitamins are added to support their optimal growth, while aerobic fermentation (Gnaim *et al.*, 2025).

After fermentation, the fungal biomass is suspended in the fermentation broth and must be recovered through downstream processing. Currently, the primary recovery step involves centrifugation, which separates the solid fungal biomass from the spent growth medium. The recovered biomass is then subjected to heat treatment to reduce nucleic acid content and ensure food safety. In commercial

mycoprotein production, the entire biomass is used as the final product, and extensive protein purification is not required. However, if isolated fungal proteins are desired, additional steps such as cell disruption, clarification, and protein concentration using membrane-based techniques may be applied (Finnigan *et al.*, 2019). There are, however, some limitations to these methods. Although mycoprotein is generally considered safe for consumption, the use of whole fungal biomass rather than purified protein raises certain concerns. After conducting heat treatment, residual nucleic acids may still remain and the presence of non-protein cellular components can have an immense influence on protein purity, digestibility, and nutritional standardization (Wiebe, 2002). These factors highlight the potential benefits of downstream protein purification strategies.

A further limitation of current downstream processing is the reliance on centrifugation for biomass recovery, which is an energy-intensive operation at an industrial scale. Although centrifugation effectively separates fungal biomass from the fermentation broth, it provides limited selectivity and does not enable protein purification. In contrast, membrane-based separation techniques such as MF and UF operate at lower energy parameters and offer the potential for continuous processing and improved separation efficiency (Bilad *et al.*, 2014).

MICROFILTRATION AND ULTRAFILTRATION

MF and UF are filtration methods, utilizing a semi-permeable membrane. They operate under low pressure and offer certain advantages over the centrifugation method. MF is a membrane-based separation process used to recover fungal biomass following fermentation. During this process, the fermentation broth is passed across a membrane with pores small enough to retain fungal cells but large enough to allow liquid and dissolved compounds to pass through. The stream that is retained by the membrane is referred to as the retentate, while the stream that passes through the membrane is known as the permeate. The pores in a microfiltration membrane typically range from 0.1 to 0.5 microns, while the cell size of *F. venenatum* ranges from 3-5 microns in diameter and 5-10 microns for *S. cerevisiae* cells (Wiebe, 2002). In the context of mycoprotein production, the retentate consists primarily of cells of *F. venenatum* and fungal biomass, whereas the permeate contains water, dissolved salts, residual sugars, and other low-molecular-weight metabolites.

As mentioned, currently, centrifugation is actively utilized in mycoprotein production. However, it has several disadvantages that need to be taken into account. While it is effective for separating solid fungal cells from liquid media, centrifugation presents several technical, economic, and sustainability-related limitations when applied at a large scale. One major problem is that it has high energy demands. A typical centrifuge operates at speeds ranging from a few thousand to over 150,000 revolutions per minute (Westlab Group Ltd., 2023). Therefore, economic and environmental challenges are encountered which prohibit mycoproteins from being considered an eco-friendly alternative protein source, as was intended (“Chemistry centrifugation,” n.d.). In contrast, MF and UF only require energy to power pumps needed to create transmembrane pressure. MF typically operates at low pressures, approximately 0.1–2 bar, while UF around 1-10 bar (Membrane technology for water applications, n.d.).

Another issue with centrifugation is the lack of selectivity, as it separates materials based on density differences and does not enable the purification of proteins. It cannot separate the molecules that are similar in density, even with the highest revolution (Centrifugation, n.d.). In contrast, MF and UF rely on size exclusion rather than density, enabling selective retention based on their physical dimensions relative to the membrane pore size. This ability gives us the chance to separate macromolecules and smaller solutes (Ultrafiltration, n.d.; 2025). The purpose of this separation is to efficiently concentrate the fungal biomass, while removing excess liquid and soluble fermentation components. This step enables effective biomass recovery, reduces downstream processing volume, and offers a lower-energy alternative to conventional centrifugation. Importantly, MF does not purify proteins, as proteins remain enclosed within intact fungal cells at this stage.

Current mycoprotein production methods include centrifugation, heat treatment, dewatering, texturisation, and food formulation. However, the application of MF and UF would enable a fundamentally different processing strategy. Firstly, MF could be used to recover and concentrate fungal biomass following fermentation. Afterwards, the process of cell disruption, the physical process where the cell wall of fungi and membranes are broken down, is pivotal as it releases intracellular contents like proteins. After this it is possible to conduct UF. UF is a pressure-driven membrane process that uses membranes with much smaller pores, typically defined by a molecular weight cut-off (MWCO) in the range of 1–100 kDa, enabling retention of fungal proteins while allowing smaller molecules such as salts, sugars, and nucleic acids to permeate (Mulder, 1996). This approach would reduce the need for extensive food-structuring steps and provide greater control over protein purity and composition. Consequently, MF and UF offer a more effective downstream processing when the objective is the production of fungal protein ingredients rather than whole-biomass food products.

It is important to mention that MF and UF cannot be utilized independently, they need to be operated one after another. After fermentation, fungal broth contains fungal cells and intracellular proteins. MF is used first because it separates fungal cells from liquid broth and the concentration of cell biomass is higher. Depending on the intended food application, a heat treatment step may then be applied to reduce the naturally high ribonucleic acid (RNA) content of the fungal biomass, as excessive dietary RNA can increase uric acid levels and elevate the risk of hyperuricemia and gout in susceptible individuals. Following cell disruption, UF is employed to separate proteins from other molecules like metabolites, sugars, salts. This sequential MF–UF strategy enables a transition from bulk biomass recovery to targeted protein fractionation, which cannot be achieved using either technique independently. The integration of MF and UF thus provides a rational and sustainable downstream processing route when the objective is the production of fungal protein ingredients rather than whole-biomass food products.

LIMITATIONS OF MICROFILTRATION AND ULTRAFILTRATION

On the other hand, it is essential to realize the potential disadvantages of MF and UF, which need to be taken into consideration. Membrane performance is strongly influenced by the accumulation and interaction of macromolecules such as proteins and polysaccharides. They widely contribute to membrane

fouling, which later results in lower flux and lower protein retention. UF membranes typically have a petite membrane pore size, similar or even smaller than protein sizes, so they cannot pass through the membrane, which is known as protein retention. These proteins accumulate near the membrane and build up a block that is called concentration polarization, later resulting in protein fouling (Tanudjaja *et al.*, 2022). Proteins are extremely prone to fouling due to their amphiphilic structure, containing both hydrophilic and hydrophobic parts. Hydrophobic parts of proteins tend to attract to the hydrophobic regions of the membrane, eventually leading to fouling and facilitating the attachment of additional foulants (Gupta, Bhattacharjee, & Hussain, 2005).

Electrostatic interactions play a crucial role in protein fouling or decreasing flux. Proteins do not have one fixed charge, which is why they are extremely dependent on the pH of the solution. When pH is close to the protein's isoelectric point, the net charge on the proteins becomes smaller (AlSawaftah *et al.*, 2021). As the electrical repulsion diminishes, proteins tend to come closer and congregate, causing an increase in hydraulic resistance and a drop in permeate flux (Sioutopoulos, Karabelas, & Mappas, 2019).

Another important limitation of MF and UF is cake layer formation. All retained materials, including proteins, polysaccharides, cell fragments, lipids, and colloidal particles commence to accumulate on the membrane surface and create a heterogeneous layer, known as the cake layer (AlSawaftah *et al.*, 2021). Additionally, polysaccharides form a hydrated gel matrix that traps proteins and cell debris, creating a compact fouling layer with high hydraulic resistance. This multi-component matrix produces a dense atmosphere and is more resistant to the flow, hindering the increase of the flux.

Overall, cake layer formation, sensitivity to operating conditions, and polysaccharide–protein interactions represent major challenges for MF and UF applications in fungal protein recovery. These phenomena can significantly reduce filtration efficiency and process stability, highlighting the need for careful control of operating parameters and targeted optimization strategies in membrane-based downstream processing.

Table 1. Summary of key findings from literature on fungal proteins and membrane-based downstream processing.

Topic	Key findings from literature	Implications for fungal protein production
<i>Fusarium venenatum</i> mycoprotein	Contains approximately 44% protein and all essential amino acids; PDCAAS of 0.96; provides dietary fibers such as chitin and β -glucans	High nutritional quality and suitable for meat substitute applications
<i>Saccharomyces cerevisiae</i>	Widely used in food production; high protein content and GRAS status; utilized in fermentation	Promising protein source, although nucleic acid reduction is required before consumption

	and nutritional supplements	
Health benefits of mycoproteins	Studies report improved satiety, reduced LDL cholesterol, improved gut microbiota composition, and potential benefits for glucose regulation	Supports the nutritional value and consumer acceptance of fungal proteins
Commercial applications	<i>F. venenatum</i> is actively utilized in Quorn products and other meat alternatives such as burgers, nuggets, sausages.	Demonstrates industrial feasibility of fungal protein-based foods
Current downstream processing	Industrial mycoprotein production mainly relies on centrifugation, heat treatment.	Effective for whole biomass products but provides limited protein purification
Microfiltration (MF)	Membrane pore size (0.1–0.5 µm) allows fungal cell retention while removing soluble compounds; lower energy requirement compared with centrifugation	Suitable for fungal biomass recovery after fermentation
Ultrafiltration (UF)	MWCO range of approximately 1–100 kDa enables protein concentration and removal of small molecules	Enables production of more purified fungal protein ingredients
MF–UF integration	MF can recover biomass, followed by heat treatment, cell disruption and UF for protein purification	Provides a more selective downstream strategy compared with conventional centrifugation
Challenges of MF and UF	Membrane fouling, concentration polarization, cake layer formation, and protein–polysaccharide interactions reduce filtration efficiency	Optimization of pressure, pH, cross-flow velocity, and cleaning strategies is required

METHODS

This literature-based analytical review focuses on analyzing fungal proteins derived from species such as *F. venenatum* and *S. cerevisiae*, as an alternative future protein source. Its goal is to discuss optimization of membrane-based filtration, MF and UF, in fungal protein separation and purification processes. Relevant studies were identified through searches of peer-reviewed scientific databases, including Web of Science, PubMed, and ScienceDirect. The search strategy used combinations of keywords such as “*Fusarium venenatum*,” “*Saccharomyces cerevisiae*,” “mycoprotein,” “single-cell protein,” “fungal protein,” “microfiltration,” “ultrafiltration,” “membrane filtration,” “protein purification,” “downstream processing,” and “sustainable protein production.” Articles were selected based on the following criteria: 1- studies published in English-language peer-reviewed journals; 2- research focused on fungal protein production, extraction, purification, or downstream processing; 3- articles providing information on nutritional composition, functional properties, or industrial feasibility of fungal proteins. The selected articles were analyzed by comparing reported fermentation conditions, downstream processing approaches, membrane operational parameters, nutritional characteristics, and limitations associated with fungal protein production. The relevance of each article was determined based on its contribution to understanding the challenges and optimization strategies involved in fungal protein separation and purification. The collected information was synthesized to identify current trends, technological limitations, and future opportunities for improving MF and UF applications in fungal protein processing. Tables and figures were used to summarize key operational parameters, experimental outcomes, and comparative findings from the selected studies.

SOLUTIONS

In order to overcome the previously discussed limitations, the operation parameters for MF and UF must be optimized. The transmembrane pressure (TMP) directly encourages permeates to flow, but also influences fouling. Conducted studies concluded that operating at lower TMP or below the critical flux is crucial to prevent proteins from fouling (Mulder, 1996). Tanudjaja *et al.* (2022) demonstrated that excessive TMP leads proteins into membrane pores and accelerates cake layer formation, leading to irreversible fouling and sharp flux decline. Maintaining TMP below the critical value allows a stable permeate flow and diminishes fouling over long filtration duration, especially in protein-rich feeds.

MF and UF can be operated with dead-end or cross-flow configurations. In dead-end filtration, molecules pass through the membrane perpendicularly, leading to rapid accumulation of proteins, polysaccharides, and cell debris on the membrane surface and eventually causing severe fouling and flux decline (Sioutopoulos, Karabelas, & Mappas, 2019). However, cross-flow, also called tangential filtration, produces a shear layer and at the membrane surface, which reduces concentration polarization, slows cake layer formation, and helps maintain higher permeate flux. Therefore, tangential filtration is an appropriate and sustainable way for UF to recover concentrated mycoproteins in the retentate (Tanudjaja *et al.*, 2022).

Another important point is cross-flow velocity (CFV), which has an immense impact on flux and fouling. Higher CFV augments shear at the membrane surface and slows down cake buildup. Krishnan *et al.* (2022) discovered that increased CFV helps to maintain higher flux by reducing foulant accumulation. One of the important parameters that needs to be taken into consideration is pH and ionic strength. Proteins are likely to gain or lose their charge based on the pH level in the solution. Studies show that utilizing pH levels away from the protein isoelectric point (pI) augments electrostatic repulsion that mitigates the cake buildup and increases flux (Ding *et al.*, 2019).

Effective cleaning strategies must be used in order to prevent membrane fouling and flux decline and hence preserve the long-term filtration performance. Cleaning strategies can be classified into two categories: physical and chemical. Physical cleaning includes hydraulic, forward, and reverse flushing, backwashing, membrane relaxation, and air flushing. Flushing is utilized for dislodging colloidal particles from the membrane, as at high pressure, pollutants are coerced to leave the membrane surface (Gul, Hruza, & Yalcinkaya, 2021). Air flushing is the process of using air to separate the membrane and pollutants; this can be simultaneously utilized during the filtration process. However, backwashing is defined as the reverse filtration process in which the filtered material flows from the membrane to the concentrated side.

On the other hand, chemical cleaning is commonly applied to address irreversible fouling, particularly when organic matter, such as proteins and polysaccharides, forms compact cake layers or penetrates membrane pores. Suitable cleaning agents should be chosen wisely as they have different characteristics, and each is used for different pollutants. Agents can be classified into the following: caustics, alkalis, acids, enzymes, surfactants, sequestrants, and disinfectants. Caustic is effective for removing organic compounds from the membrane, like polysaccharides. Alkaline cleaning agents are effective in dissolving protein-based fouling, while acidic solutions are typically used to remove inorganic scaling (Gul, Hruza, & Yalcinkaya, 2021). Mycoproteins contain a high fraction of proteins and polysaccharides, which can form dense and adhesive fouling layers. Therefore, combining physical cleaning with periodic chemical cleaning is considered an effective strategy to maintain stable flux and extend membrane performance used for mycoprotein separation and concentration.

RESULTS

The comparative analysis of nutritional and physiological outcomes associated with mycoprotein consumption is summarized in Table 2. The data collected from controlled human intervention studies indicate that mycoproteins derived from *F. venenatum* have significant effects on lipid metabolism, energy intake, and muscle protein synthesis when compared with traditional animal-based proteins.

Physiological outcome	Mycoprotein (F. venenatum)	Animal protein (meat/ milk)
LDL cholesterol	↓ 9% after 3 weeks	No significant reduction
HDL cholesterol	↑ 12 % after 3 weeks	No increase
MPS (Rested), FSR %/h	0.057	0.052
MPS (Exercised), FSR %/h	0.072	0.056

Table 2. Comparison of physiological outcomes, LDL/HDL cholesterol and FSR values, in participants after consuming mycoprotein versus animal- based proteins over a three-week intervention (Turnbull et al., 1990, Monteyne et al.,2020)

As shown in the comparative dataset, regular intake of mycoprotein was associated with a reduction in LDL cholesterol of approximately 9%, alongside an increase in HDL cholesterol of 12% following a three-week intervention period (Turnbull *et al.*, 1990). In contrast, diets based on animal-derived proteins did not demonstrate comparable improvements in lipid profiles over similar timeframes. These findings suggest a favorable impact of mycoproteins on cardiovascular risk markers.

Differences were also observed in satiety-related results. Participants consuming mycoprotein-based meals exhibited a marked reduction in total energy intake, with a 24% decrease recorded on the day of consumption and a sustained 16.5% reduction on the following day. No equivalent reduction was reported in groups consuming chicken or other animal protein sources (Turnbull, Leeds, and Edwards 1990).

In addition, mycoprotein intake resulted in greater stimulation of muscle protein synthesis compared with animal protein. Measurements of FSR revealed higher values in both rested and exercised muscle following mycoprotein ingestion, with the most pronounced difference observed in exercised muscle tissue (Monteyne *et al.*, 2020). These results indicate that mycoproteins provide an effective amino acid supply capable of supporting post-exercise muscle anabolism.

Overall, the results presented in Table 2 demonstrate that mycoproteins have a positive influence on multiple physiological parameters, including lipid regulation, appetite control, and muscle protein synthesis.

Feature	Microfiltration	Ultrafiltration
Typical pore radius (nm)	50-10,000 nm (0.05- 10 µm)	1-100 nm
Transport Mechanism	Sieving	Sieving based on MWCO

Table 3. Description of typical pore radius for microfiltration and ultrafiltration and their transport mechanism (Kujawski et al., 1989).

Table 3 illustrates the pore size characteristic of MF and UF membranes. According to membrane characterization studies, MF membranes typically possess a pore radius of around tens to thousands of nanometers (0.05 – 10 µm), whereas UF membranes are characterized by much smaller pore radius from 1 to 100 nm. Such differences in pore structure explain the functional separation characteristics observed in downstream processing. Larger MF pores retain whole fungal cells, while the finer UF pores enable selective retention of proteins and macromolecules. These structural differences also influence fouling behavior and operational optimization strategies (Kujawski et al., 1989).

Parameter	Microfiltration	Ultrafiltration
Operating pressure	01.2 bar	1-10 bar
Energy consumption	Low	Moderate
Fouling tendency	Moderate	High
Applications	Cell removal	Protein concentration

Table 4. Description of operating parameters including pressure, energy consumption, fouling tendency and applications in MF and UF (Mulder, 2012; Tanudjaja et al., 2022; AlSawafthah et al., 2021; Ding et al., 2019).

The comparison between MF and UF membranes highlights the trade-offs between pore size, operating conditions, and protein recovery efficiency. The table shows that while UF achieves higher protein recovery due to its selective filtration, it requires stricter control of operational parameters such as TMP, pH, and ionic strength to maintain stable flux (Ding et al., 2019). MF, in contrast, demonstrates more stable flux with minimal maintenance but cannot achieve the same protein purity as UF. It is important that these membrane-based filtrations do not consume tremendous amounts of energy for cell removal and protein concentration. However, fouling rate in MF is comparatively lower than in UF. This comparison indicates that a combined approach, where MF is used for primary cell removal and UF for final protein purification, may offer the most efficient downstream processing for fungal proteins, balancing energy use, flux stability, and product quality (Mulder, 2012; Tanudjaja et al., 2022; AlSawafthah et al., 2021; Ding et al., 2019)

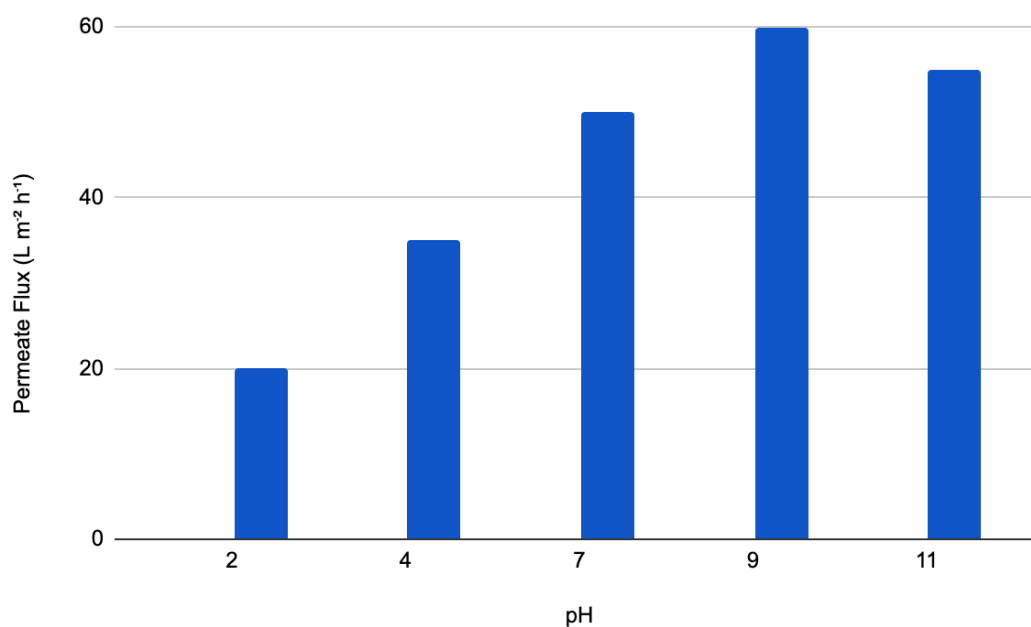


Figure 1. Relationship between pH levels and permeate flux (Md Saleh et al., 2021)

Operational parameters are important as they impact the downstream processing of fungal proteins. Figure 1 shows how pH affects the membrane flux in UF. Experiments measured permeate flux at pH 2, 4, 7, 9, and 11. The results indicate that flux increases with pH, reaching about $20 \text{ L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$ with the highest value at pH 9. However, the flux decreases slightly at pH 11. Overall, the chart shows that flux in ultrafiltration depends strongly on pH, and higher flux helps reduce the risk of membrane fouling and cake layer formation.

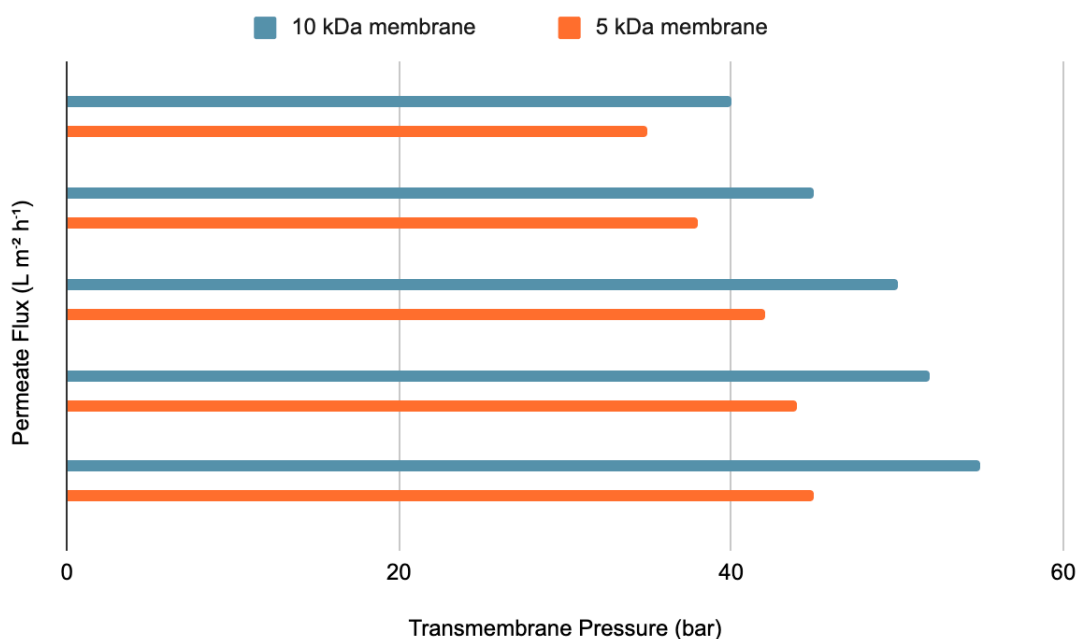


Figure 2. Relationship between permeate flux and TMP for 5kDa & 10 kDa for UF membranes (Md Saleh et al., 2021)

Another crucial point to consider is TMP. Figure 2 shows the relationship between permeate flux and TMP for 5 kilodalton (kDa) and 10 kDa UF membranes. It is evident that both trends are related, meaning that as one increases, the other tends to increase as well. For the 5 kDa membrane, a TMP increase of 0.3 bar results in a small increase in flux from 44 to 45 $\text{L} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$. In contrast, the 10 kDa membrane shows a more pronounced increase in flux over the same TMP range. Overall, the 10 kDa membrane consistently achieves higher flux than the 5 kDa membrane, indicating a lower likelihood of protein accumulation, cake layer formation, and membrane fouling.

Cleaning method	Fouling type	Target
Forward/ Reverse flushing	Reversible	Loosely attached colloids
Backwashing	Reversible	Cake layer removal
Alkaline cleaning	Irreversible	Protein, polysaccharide-rich fouling
Acidic cleaning	Irreversible	Inorganic deposits

Table 5. Comparison between cleaning methods for different fouling types and goals (Md Saleh et al.2021)

Despite the abovementioned operational parameters, cleaning strategies are pivotal to avoid cake layer formation, fouling and other complications. Table 5 summarizes the main cleaning strategies used to maintain long-term performance of MF and UF membranes. It shows that for removing loosely attached colloids physical cleaning methods are efficient. In contrast, chemical cleaning strategies are used for complex, protein or polysaccharide fouling. Furthermore, alkaline agents are effective at dissolving protein-based deposits, while acidic solutions target mineral or inorganic scales. In practice, a combination of physical and periodic chemical cleaning is recommended, especially when processing high-protein materials like mycoproteins. This approach ensures stable flux, reduces the risk of permanent membrane damage, and prolongs membrane lifespan, making it a critical factor in optimizing downstream processing.

DISCUSSION

This study investigated mycoproteins, *F. venenatum*, *S. cerevisiae*, as sustainable alternative protein sources while assessing the strengths of MF and UF as downstream processing techniques. The findings provide insight into both the nutritional value of mycoproteins and the technological limitations associated with their recovery.

It is clear that consumption of mycoproteins obtains a significant amount of health benefits. Mycoproteins cause a positive modulation of lipid metabolism as they diminish LDL cholesterol and increase HDL cholesterol. While, it also enhances satiety effects. Suggesting that mycoproteins may contribute to ameliorated cardiovascular and digestive system health, due to their elevated dietary fiber content and unique fungal cell wall structure. In contrast, animal-derived protein diets did not demonstrate comparable

lipid profile improvements, emphasizing a potential nutritional advantage of mycoproteins over traditional protein sources (Turnbull *et al.*, 1990). A key strength of these studies is the use of controlled dietary interventions in human subjects, which explains the positive impact of mycoprotein on human physiology. However, their primary limitation represents the short duration of intervention, which limits conclusions about long-term cardiovascular results. Monteyne *et al.* (2020) further reported enhanced muscle protein synthesis following mycoprotein ingestion, particularly post-exercise. This finding challenges earlier assumptions that fungal proteins are inferior to animal proteins in supporting muscle anabolism. Although their work provided valuable mechanistic insight, it did not address how downstream processing might influence protein integrity. The present study fulfills this gap by linking protein quality to biotechnology.

The comparison between centrifugation and membrane-based filtration indicates that MF and UF may be less resource-intensive and more efficient in purification of mycoproteins. Mulder (2012), identifies centrifugation as an effective method for bulk fungal biomass separation. Its primary advantages represent rapid separation. However, high energy consumption and poor protein fractionation are main limitations. Membrane-based processes, as explored by Ding *et al.* (2019) and AlSawaftah *et al.* (2021) concentrate on improved selectivity. MF membranes tend to eliminate fungal cells with minimal fouling due to their larger pore size, resulting in more stable permeate flux. UF membranes, in contrast, enable selective protein retention but are more susceptible to fouling due to intensified protein–membrane interactions. However, many of these investigations evaluated MF or UF independently, while the present study builds upon this literature by demonstrating that neither MF nor UF alone represents an optimal solution for mycoprotein production. Instead, a sequential MF–UF configuration is shown to balance flux stability, energy efficiency, and protein purity. This combined approach addresses a key weakness in earlier studies that focused on single-stage filtration without considering cumulative fouling and process efficiency.

The strong dependence of permeate flux on pH highlights the importance of electrostatic interactions in protein fouling behavior. Md Saleh *et al.* (2021) systematically investigated the influence of pH and TMP on UF performance. It demonstrated a flux increase, while pH value is totally different from pI, enhancing electrostatic repulsion between protein molecules and the membrane surface, diminishing cake layer formation. The decline in flux at very high pH indicates potential protein denaturation, which may expose hydrophobic regions and encourage fouling. These results emphasize the necessity for attentive pH control to balance fouling mitigation and protein stability. A major strength of their work was the detailed quantitative analysis of flux behavior. However, their study did not assess protein structural stability at extreme pH or long-term fouling behavior under sustained operation. This literature-based analytical review concludes that flux increase does not depend on only pH level, but the merge of different operational parameters (Mulder, 2012).

Similarly, the relationship between permeate flux and TMP demonstrates that higher TMP does not necessarily ameliorate long-term performance. While increased TMP initially enhances flux, operation beyond the critical flux accelerates cake layer formation and pore blocking, leading to flux decline. The higher flux observed for the 10 kDa UF membrane compared with the 5 kDa membrane suggests reduced

resistance to flow and lower protein accumulation (Md Saleh *et al.*, 2021). However, excessive pressure remains detrimental regardless of membrane cut-off, reinforcing the importance of operating below critical TMP values.

Additionally, cross-flow velocity represents a highly pivotal point in this process. Higher CFV is beneficial for preventing protein from fouling and cake layer formation as it augments shear stress when more kinetic energy is on the surface and easily breaks weak proteins and their interactions (Baker, 2012). However, lower CFV can lead to immediate complications, as proteins have more duration of time to interact chemically with the surface, which later becomes the reason for fouling. On the other hand, excessive CFV can be detrimental for downstream processing. It is associated with prohibitive costs as it requires more energy. In addition, it can even destroy the protein content and value, creating a very strong shear force that can denature proteins as it physically stresses their structure. Denatured proteins may reveal unfolded hydrophobic regions that can stick even more to the hydrophobic surface membrane and form larger foulant clusters (Gupta, Bhattacharjee, & Hussain, 2005)

Cleaning strategies highlight the importance of membrane-filtration performance. A study by Gul *et al.* (2021) shows that physical cleaning methods are effective in restoring flux when fouling is caused by loosely attached cake layers, while chemical cleaning is essential for removing protein-polysaccharide complexes and inorganic scaling. Their study provided a clear classification of fouling types but did not address strategies to reduce cleaning frequency. Current findings suggest that fouling may be inevitable during membrane-based filtration, but fouling control should prioritize operational optimization to minimize irreversible fouling formation, using physical and chemical cleaning methods for the best outcomes. This approach improves membrane lifespan and performance, contributing to greater process sustainability.

Compared with centrifugation, membrane-based separation offers advantages in energy efficiency, selectivity, and continuous operation. While centrifugation remains effective for bulk solid-liquid separation, it lacks the capability to selectively recover protein fractions and may induce shear-related protein damage (Mulder, 2012). Optimized MF and UF processes therefore represent a more suitable long-term solution for high-quality mycoprotein production.

LIMITATIONS

A key strength of this study is its integrated analysis of nutritional benefits and downstream processing performance, linking protein quality with process feasibility. However, limitations include reliance on short-term nutritional studies and the absence of pilot-scale membrane operation data. Future research should focus on long-term intervention studies, scale-up validation, and economic and life-cycle assessments. Nevertheless, limitations still remain. This study mostly relies on data collected from laboratory-scale studies. Overall, the findings demonstrate that mycoproteins possess substantial nutritional advantages and that efficient downstream recovery depends on carefully optimized membrane

operation (Monteyne *et al.*, 2020) By interpreting how operating conditions influence fouling, flux stability, and protein integrity, this study contributes to the development of scalable and sustainable protein production systems capable of meeting growing global protein demands (Ding *et al.*, 2019).

CONCLUSION

This study summarizes data about fungal proteins derived from *F. venenatum* and *S. cerevisiae*, their nutrition value, health benefits, production methods and their efficiency of downstream processing via membrane-based filtration methods, including MF and UF. It shows that protein purification techniques rely on different parameters rather than a single component. Operational parameters like pH, TMP, CFV, cleaning methods should be optimized during MF and UF in aim to maximize the efficiency of their performance. Compared to conventional methods, membrane based filtration does not require much energy or resource, while providing improved protein selectivity. However, fouling remains the most important limitation that should be controlled through the operational parameters. Overall, integrating natural, valuable and viable resources with biotechnology principles can contribute to the solution of future world global problems and supplying people with proteins, via mycoprotein sources.

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