# A Functionality Study of Mycoprotein

A master's thesis by

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# Abstract

The mycoprotein was obtained from the company, Mycorena. The mycoprotein is a potential source for alternative protein from the animal origin used as the functional ingredients in the food industry. In this project, mycoprotein concentrate (MC) and mycoprotein isolate (MI) were produced by freeze-drying the biomass and pH-shift processing together with freeze-drying, respectively. Moreover, the functionality study was focused on the foaming properties, emulsifying properties, and gelling ability of mycoprotein.

This project was primarily focused on three parts: the mycoprotein isolation, the functionality impacted by factors such as salt, pH, pre-thermal treatment, and protein powder concentration, and benchmarking of the MC and MI with other plant-based proteins. MI was isolated using the pH-shift process which allowed the protein to solubilize at pH 10 and recover (precipitated) at pH 4 to reach the overall extraction yield of 37% from biomass (dry matter basis). The soluble protein content of MC and MI did not show any significant difference at pH 7. It was found that the salt concentration could affect the foaming capacity of MC. After a pre-heat treatment at 70°C of MC solution, the foaming capacity was significantly improved while the foaming stability was significantly decreased. At pH 5, 7, and the original pH (5.7), the emulsifying properties (capacity and stability) did not show any significant difference. Different salt and pH treatments lead to the different gelling performance of MC. As for benchmarking the functionality of MC and MI to other plant-based proteins, the foaming stability and emulsifying capacity of MC displayed the best performance, whereas the MI observed a relatively poor functionality in all foaming, emulsifying, and gelling experiments. It was suggested that such poor functionality of MI could result from the proteolytic hydrolysis.

In conclusion, the pH-shift processing conducted in this project was not a promising approach to studying the functionality of mycoprotein. However, the native protein in MC observed a greater emulsifying capacity which has the potential for further application. Further research surrounding the optimal protein isolation method without compromising the functionality of mycoprotein is worth studying.



**Key words**: *mycoprotein*, *protein isolation*, *protein functionalities*.

# Popular Summary

Protein is an important macromolecule that works on building new structural and functional proteins for both humans and animals. With the increased global population, animal protein cannot meet the nutritional demand, therefore, more alternative protein sources need to be explored. Mycoprotein is a novel protein source gaining more interest currently due to its complete essential amino acids (EAAs) composition and favorable protein utilization (NPU) value which is comparable to those of milk. For food application, study on the functionality of mycoprotein plays an important role in developing food formulations.

As the freeze-dried biomass already has a high protein content, it is also called mycoprotein concentrate (MC) which can be used for the functionality study. However, it would offer a higher protein content and steady performance for functionality study and further application if the fermentation residue and fiber content are removed by extracting mycoprotein isolate (MI) with the pH-shift method. The pH-shift method was developed in the 1990s and it isolates proteins based on the difference in solubility that proteins in water exhibit at different pH conditions. The conformational changes of protein could occur during pH shift processing.

The ability of a protein to impart beneficial properties to food in addition to its nutritional value is protein functionalities. Some factors can influence the functionality performance of protein such as salt, pH, and preheat treatment of protein solution. Studying these factors could provide a wider food application in the future. Although the protein structure changes during the pH-shift process, it is of interest to know both the functionality performance of MC and MI compared with other plant-based proteins that can be found on the market today.

As an alternative protein source to functional proteins of animal origin, the functionality study of mycoprotein is worth investigating as it impacts the physical and chemical properties of food products. Understanding the functionality of mycoprotein provides an overall view of its future applications.

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# 1 Introduction

The worldwide population reached 7.7 billion in 2019, and it is expected to reach 9 billion in 2040 (Nations, 2019) (Bertasini et al., 2022). The increasing global population has a concomitant impact on the demand for food production to meet the population's nutrition needs (Tessari et al., 2016). Protein, as a source of nitrogen and essential amino acid, plays an important role in building new structural and functional proteins in humans and animals such as enzymes and hormones. Also, under extreme conditions protein can be used as the energy source (Wu, 2009). Animal proteins have been used as a conventional protein source in the past and still account for a large portion of food products that have been highly studied for many decades. However, the large-scale production of animal protein was reported to be a major driver of biodiversity loss, climate change, and freshwater depletion (Aiking, 2014). Consequently, the use of alternative protein sources as potential alternatives to animal-derived proteins has been explored in recent years (Maurya & Kushwaha, 2019). Using the alternative proteins needs to be characterized according to their functional and structural properties. Enhanced texture, foaming, emulsifying, and gelling ability are some of the functional properties that are vital to formulations (Alonso-Miravalles & O'Mahony, 2018).

Currently, plant-based protein is the primary replacement for animal-based protein within meat analogues production. However, it still cannot satisfy the market demand. Thus, some other novel protein sources can be used to fill the gap resulting from growing demand (Ahmad et al., 2022). Since mycoprotein, a protein source derived from the fermentation of filamentous fungus, provides all the essential amino acids (EAAs) with better Net Protein Utilization (NPU) values than milk, it is a desirable alternative protein source to be used for the production of meat analogues (Sharif et al., 2021) (E. J. Derbyshire & Delange, 2021).

This master thesis collaborated with a company called Mycorena which aims to produce a lower environmental impact of a revolutionary source of edible vegan protein made from filamentous fungi. So far little knowledge is known about the functionalities of mycoprotein and how those functionalities were influenced by factors such as salt and pH. Therefore, this study will focus on isolating protein from fungi biomass and evaluate the functionality of fungi protein on foaming, emulsifying and gelling ability together with benchmarking.

## 1.1 Aim

The overall objective of this master thesis was to design a proper process to isolate mycoprotein from fungi biomass and investigate the functionality of mycoprotein isolate (MI) and mycoprotein concentrate (MC) comparing with other plant-based proteins. Also evaluate how some factors influence the functionality of MC such as salt, pH, and pre-heat treatment. The functionalities in this study include foaming properties, emulsifying properties, and gelling ability. The following questions were answered in this master thesis:

- What raw material, MC powder or biomass, is better to be used to isolate MI?
- Can different pH and salt treatment, MC powder concentrations, and pre-thermal treatment affect the foaming properties?

- Can different pH treatment affect emulsifying capacity?
- Can different pH and salt treatment affect gelling ability?
- How enzyme affect the soluble protein content?
- How does the functionality of MC and MI compare to other plant-based proteins?

## 1.2 Background

### 1.2.1 Mycoprotein

Mycoprotein is a type of protein that is derived from filamentous fungus fermentation which as its name implies is a mycelium (Souza Filho et al., 2019). Similar to Quorn-mycoprotein, mycoprotein produced at Mycorena has a higher percentage of protein content which is 52% (dry weight basis) than protein derived from plant and other fungal protein, but lower than meat (Coelho et al., 2020). All the essential amino acids (EAAs) comprised in mycoprotein and its net protein utilization (NPU) value is comparable to that of milk, and the EAAs composition of mycoprotein is higher than most commonly consumed plant-based protein (E. J. Derbyshire & Delange, 2021). In addition, the Protein Digestibility Corrected Amino Acid Score (PDCAAS) of mycoprotein is 0.996 which is not only very close to the maximum score of 1.0 achieved by milk and egg but also higher than chicken and beef (E. Derbyshire & Ayoob, 2019) (Finnigan et al., 2019). Apart from protein, fiber is another main component contained in the cell wall of mycoprotein and it is mostly insoluble, up to about 25%. The fiber in mycoprotein consists of one-third of chitin and two-thirds of *beta*-glucan which create a water-insoluble chitin-glucan complex (Bottin et al., 2016).

### 1.2.2 pH-shift method for protein isolation

pH-shift protein isolation is also called acid or alkaline solubilization and precipitation method, or isoelectric precipitation method which was invented and introduced in 1990s (Arfat & Benjakul, 2012) (Undeland et al., 2002) (Hultin & Kelleher, 2000) (Hultin & Kelleher, 1999). This method mainly includes two-part, protein solubilization and protein precipitation at isoelectric point (Zeng, 2021). At the protein solubilization stage, protein becomes more negatively charged with the addition of base and subsequent dehydrogenation. Meanwhile, charged amino acids on the protein repel each other which leads to individual proteins separating from each other, thereby attracting more water and becoming partly unfolded, thus more soluble (Kristinsson & Hultin, 2003). Turning to the protein precipitation step, the purified protein remains soluble until adjusting pH to the isoelectric point which was demonstrated at pH 4 (Zeng, 2021). During the second pH adjustment, unfolded protein at the solubilization step starts to refold, although not necessarily in their native conformation. And at the isoelectric point, the interaction between water and protein is minimal. Salt bridges are also thought to form between oppositely charged amino acids. Finally, the precipitated protein is collected from the solution via centrifugation (Marmon, 2012). A study suggests pH-shift could perform at cold temperature, with a maximum temperature of 10°C to avoid heat-induced denaturation and enzymatic degradation of protein (Hultin & Kelleher, 2000).

### 1.2.3 Protein isolation yield

Protein isolation yield considers one of the most important factors to evaluate the pH-shift process. Generally, the isolation yield of 55%-80% is common. Many factors can affect the isolated yield during the pH-shift process, including water addition, and pH value used for solubilization and precipitation (Nolsøe & Undeland, 2009). To be more specific, a higher water addition at the protein solubilization step could lead to s higher protein yield, while a large amount of water could result in more water waste at the end of the process (Hultin et al., 2005). More harsh pH conduction contributes to the higher solubility of the protein, however, it could also cause the structural changes of protein and thereby change the solubility of protein (Tadpitchayangkoon et al., 2010).

#### 1.2.3.1 Protein functionality

Generally, the food protein functionality is viewed in terms of how individual molecules or protein components function in solution and form simple colloidal structures (Foegeding & Davis, 2011). In food applications, the three-dimensional structure is rarely as same as the native protein whose function can be revealed by biological structure-function relationships, and the Functionality of protein is closely associated with secondary and tertiary structure changes. A model for protein denaturation can be expressed as:

$$Native\,structure \rightleftharpoons intermediate\,state \rightarrow denatured\,state \tag{1}$$

The native structure of a protein from many sources of proteins is known, but the fine structure of its denatured structure is still unknown (Foegeding & Davis, 2011).

### 1.2.4 Protein functionalities in food system

The solution or dispersion of a single protein is the simplest way to understand the protein functionality. However, the protein ingredients in the food system are always a mixture of proteins together with some other molecules such as sugars and fibers. Since proteins are one of several ingredients in food, they must "function" in a complex food system (Foegeding & Davis, 2011).

### 1.2.5 Foaming properties

Foam is defined as gas in liquid dispersions or gas in solid materials. In its simplest form, foam is a colloidal system where air bubbles are surrounded by a continuous aqueous phase (Campbell & Mougeot, 1999). Proteins are dispersed in a continuous phase and at the interface. The difference between protein and small molecular weight surfactants is that they can interact with other adsorbed proteins to form films with some properties including thickness, gas permeability, etc (Dickinson, 1992) (Dickinson, 1999) (Wilde et al., 2004). When the liquid is agitated, the air is introduced under the stirring part, causing air bubbles to be trapped by the liquid. The presence of high or low molecular weight of surface-active constituents contributes to air bubbles formation and stabilization by influencing the dynamic surface properties of the foam films (Prins, 1988). The foaming properties of proteins involve two aspects, including the ability to generate large interfacial areas for the incorporation of a large amount of gas into liquids, and the ability to form tenacious interfacial films than can withstand internal and external forces (Damodaran, 1997).

### 1.2.6 Emulsifying properties

Proteins work as surface-active molecules with good emulsifying and emulsion stability due to the presence of hydrophobic and hydrophilic amino acids (Lam & Nickerson, 2013). Food emulsions are defined at the molecular level as complex colloidal systems consisting of two immiscible phases. It could be more complicated to study food emulsions due to multiple components being present compared with a dilute solution. Generally, the emulsifying properties of proteins depend on two effects including the interfacial tension which is reduced while the protein is adsorbed at the oil-water interface, as well as the electrostatic which is a structural and mechanical energy barrier caused by interfacial layers opposing the destabilization process (Méx, 2008). When protein molecules are present at oil-water emulsions, they preferentially migrate to the oil-water interface and rearrange themselves, allowing hydrophobic groups to penetrate the oil droplets and hydrophilic groups to extend into the water phase. Owing to this configuration, proteins could produce loops structure which effectively slows down the flocculation and coalescence of oil droplets (Hasenhuettl, Hartel, et al., 2008).

### 1.2.7 Heat-induced gel

Protein gelation and stable network formation are prerequisites for many applications of food products. The gelation properties of proteins can be categorized by some mechanism of gelation including acid, heat, additional salts, etc, or by the morphological properties of a gel including fine-standard, mixed, or particulate gel (Foegeding et al., 1995). Heat-induced gel networks composed of globular proteins primarily follow the change in protein structure, by unfolding in their native state exposed to the solution, hydrophobic patches are often hidden within protein matrices, which in turn facilitate new protein-protein interactions, thus aggregation and cross-linking occur (Nicolai & Durand, 2013) (He et al., 2013) (X. Li et al., 2007) (Sun & Arntfield, 2011). For aggregate formation, hydrogen bonds, hydrophobic interactions, and disulfide bonds were proved to play critical roles in aggregation (Yamagishi et al., 1983). In addition, many studies, recently, introduced phase separation and it received considerable attention which can be seen as the last stage of the protein gelling mechanism (Clark et al., 2001).

The morphological properties of the gel are depending on the mechanisms of binding between proteins, such as hydrogen bonds hydrophobic interactions, covalent bonds, and ionic interactions. And these binding between proteins also influences the microstructure, texture, viscoelasticity, and stability (Mäkinen et al., 2016). High temperature-induced gels show higher stability compared with low temperature due to disulfide bonds dominating the protein-protein interactions caused by high temperature (Avanza et al., 2005).

### 1.2.8 Functionality of mycoprotein

The fermentation biomass contains a large number of cell components including nucleotides, the dense network of mycelium debris aggregations, and proteinaceous biomass. The functional profile plays an important role in converting viscoelastic biomass into meat-like texture (Lonchamp et al., 2019). Many studies have shown that mycoprotein is a good source of emulsifying and foaming agents in food application (Murray, 2020). A study conducted by Lonchamp et al (2019) isolated a range of protein fractions from mycoprotein biomass and displayed good foaming, emulsifying and rheological properties

by using 100 kDa ultrafiltration. Besides, the mycoprotein fraction produced by gas-sparging had a lower surface tension compared with the whey protein fraction favoring the emulsifying and foaming properties. (Lonchamp et al., 2019). A recent study performed by Lonchamp et al (2020) indicates that the mycoprotein emulsion displayed a smaller droplet size than the whey protein emulsion (Lonchamp et al., 2020).

### 1.2.9 Emzymes in mycoprotein

Filamentous fungi were exploited for the production of industrial enzymes due to they can produce a high capacity of extracellular enzymes in high abundance, and also they can offer several advantages including low cost of the raw materials, high productivity, rapid production, easy enzyme modification, and easy enzyme recovery characteristics (Vishwanatha et al., 2010) (Troiano et al., 2020). Such enzymes secreted by filamentous fungi include cellulases, xylanase, lignin peroxidase, manganese peroxidase, laccase, protease, *alpha*-amylase, *beta*-xylosidase, amyloglucosidase, and glucoamylase (Troiano et al., 2020). Proteases, which account for approximately 65% of the global enzyme market, can be isolated by fungi biomass (Vishwanatha et al., 2010).

# 2 Materials & Methods

## 2.1 Mycoprotein isolation and yield

### 2.1.1 Non heat-treated biomass preparation

The filamentous fungi were pre-cultured and incubated into the controlled bioreactor together with substrate and water fermenting for 24h to get a big amount of biomass. The biomass used in this functionality study was harvested from a bioreactor without heat treatment. After harvesting the biomass was washed with water to remove the fermentation residual media to get purified fresh biomass. Finally, the fresh biomass was stored at a freezer (-18°C) until further analysis.

### 2.1.2 Mycoprotein concentrate production

After the non-heat treatment of frozen biomass was received, a freeze-drying process was conducted to produce mycoprotein concentrate (MC). MC was collected and stored at the dry ambient temperature for further experiments. The process of mycoprotein production is shown in the dashed box in Figure 1.

### 2.1.3 Mycoprotein isolation

To isolate the mycoprotein, a pH-shift method was used which refers to protein solubilization and isoelectric precipitation. Based on the previous study investigated by Baohong Zeng (Zeng, 2021), the isoelectric point of mycoprotein extracted from fungi is around pH 4, and it was shown a higher solubility from pH 10 to pH 12. In this project, two raw materials were considered to be used to isolate mycoprotein: (1) MC, and (2) raw biomass (frozen). The mycoprotein isolate (MI) was obtained from these two origins and the isolation yield was compared.

#### 2.1.3.1 Mycoprotein isolate production

To isolate protein from frozen biomass, the frozen biomass was defrosted at room temperature for 2 hours before isolation. To ensure biomass dispersed into the water properly and to remove residual fermentation liquid, the defrosted biomass was squeezed out of the water and blended into fine dry pieces. Some dry pieces were collected for dry matter determination for further yield calculation. Then five times of water was added while mixing. Meanwhile, the 1M sodium hydroxide (NaOH) was added to adjust the pH to 10. The pH was measured using Benchtop pH Meter. The extraction process from another origin, MC, was carried out as same as the extraction process from frozen biomass after 25% w/w protein concentrate slurry. The supernatant was collected after protein solubilization together with the supernatant of frozen biomass sample using a BCA assay kit to evaluate the protein content.

After the first pH adjustment, considering a study show that the protein isolation yield enhances by a longer alkaline extraction time (Shen et al., 2008), the slurry was stood at room temperature to incubate for 1 hour to ensure enough protein can dissolve in water. The pH of the slurry was checked within 1 hour. If the pH was dropped below 9 add more sodium hydroxide (NaOH). And then the samples were transferred into 50 ml falcon tubes

and centrifugated at 4000 RPM for 30 min to separate the protein-riched supernatant.

All the supernatant was collected into beakers to conduct the second pH adjustment by addition of 1 M hydrochloric acid (HCl) to pH 4 which was the isoelectric point of mycoprotein produced at Mycorena (Zeng, 2021). All the solutions went to centrifugation and the sediment was collected.

Eventually, the precipitated protein sediment was washed 3 times with distilled water, resuspended into the water, and adjusted pH to 7. A magnetic stirrer was used to suspend the protein. Therefore, the protein was recovered, and freeze-dried. The process of protein isolation is shown in Figure 1.

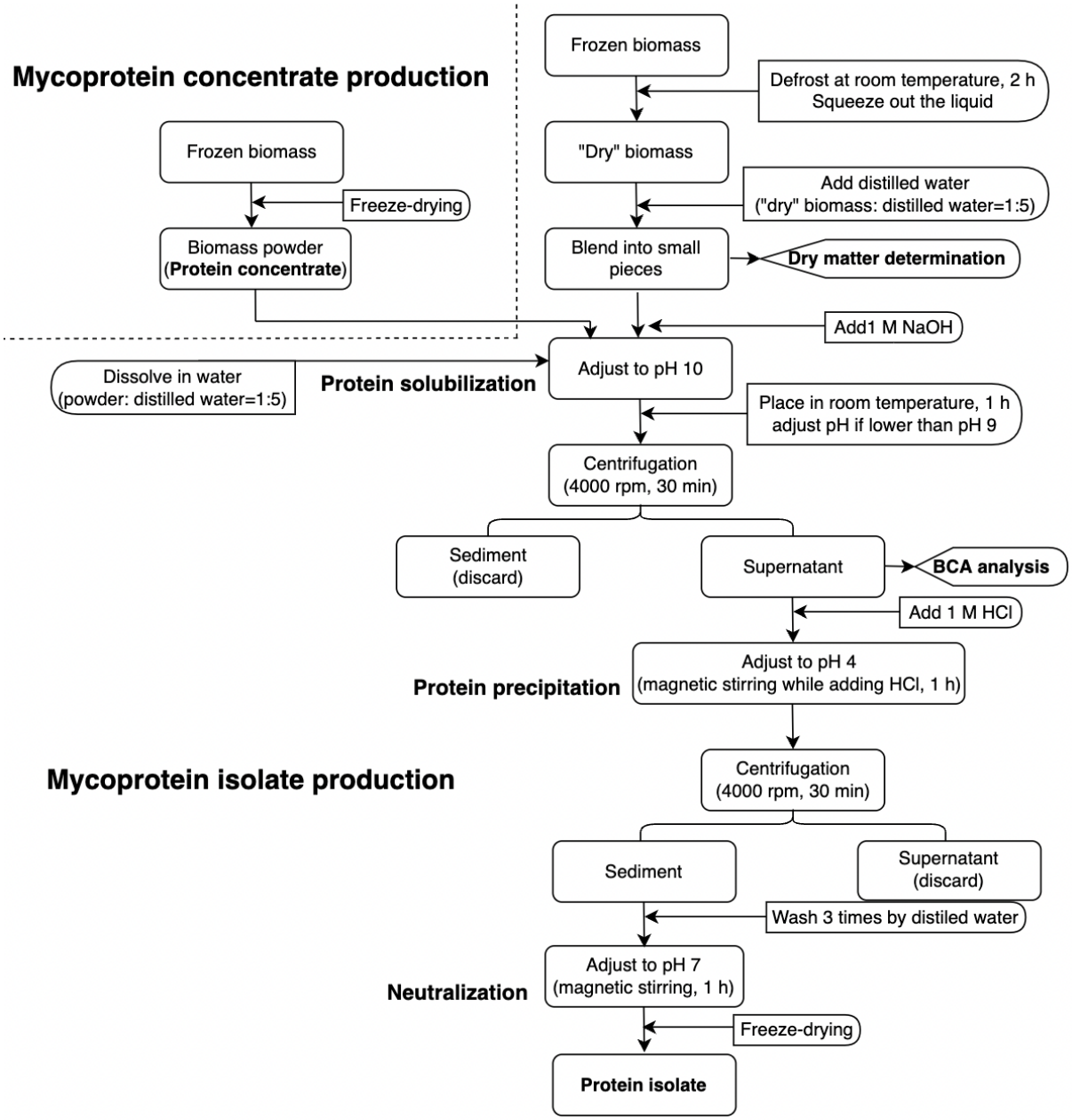



**Figure 1:** The mycoprotein isolation process

## 2.2 Protein isolation yield

The protein isolation yield was calculated based on how much protein can be extracted from a total protein of biomass (dry weight). To compare the isolation yield of two raw materials, BCA analysis was conducted after the first pH adjustment where the protein was solubilized in the supernatant. The supernatant from raw biomass (frozen) and MC were collected at the same time after centrifugation.

After deciding which raw material was used for protein extraction, the supernatant was collected immediately and conducted BCA analysis to determine the protein isolate yield.

### 2.2.1 Quantification of protein content by BCA analysis

After the first pH adjustment, the supernatant was collected and soluble protein content was determined by the BCA protein assay kit (Sigma-Aldrich, Germany) aiming for obtaining the efficacy protein worked for later functionality study.

Firstly, The Bovine Serum Albumin (BSA) solutions were prepared by diluting 2 mg/ml BSA solution to obtain the different concentrations of BSA solutions in 5 ml test tubes. The preparation of the BSA standard is shown in Table A.1 (Appendix). The dilutions of the standard assay are shown in Table A.1 (Appendix). And BCA working reagent was prepared by mixing 50 parts of BCA solution with 1 part of 4% Cupric Sulfate.

And then a hundred-fold dilution of supernatant was prepared. And then pipetted 50 $\mu$l each standard and sample solution into 5 ml test tubes adding 1ml BCA working agent and vortexed gently. All the tubes were incubated at 37°C for 30 min and cooled down at room temperature. 1 ml distilled water was added to a clean cuvette and adjusted the absorbance reading at 562 nm to zero was blank.

Finally, measuring all the samples at the absorbance of 562 nm and the protein concentrations were calculated based on the equation from the standard curve.

### 2.2.2 Determination of protein isolation yield

To determine which raw material, raw biomass (frozen) or MC, can be used to extract MI, the dry matter of the squeezed biomass ("dry" biomass) was needed to calculate the total protein content. The squeezed biomass sample was torn into small pieces and put into a dry matter determination machine (Precisa Gravimetrics AG, Switzerland)). The dry matter, DM(%), of the sample was read on the screen. And protein isolation yield(%) was calculated as:

$$Y_{protein}\,(\%) = \frac{m_{extracted\ protein\ content}}{m_{total\ protein\ content}} \times 100\% \tag{2}$$

## 2.3 Protease activity measurement

The protease activity was investigated by measuring the protein content changes with time. BCA analyses were conducted after the mycoprotein concentrate was dispersed into the water. The supernatant was collected and placed at room temperature, in the fridge (4°C), and freezer (-18°C). To obtain the protease activity curve, multiple measurements of protein content were performed.

## 2.4 Solubility

To quantify the soluble protein content in MC and MI powders, 0.1 g of MI and MC powders were dispersed in 10 ml water, and then the suspensions were centrifuged at 4000 rpm for 10 min. After collecting the supernatant, hundred-fold dilutions were prepared to measure the soluble protein content by BCA protein assay kit as described before. The protein solubility was calculated as follows:

$$Protein\ solubility\,(\%) = \frac{Protein\ content\ of\ supernatant\,(mg/ml)}{Total\ protein\ content\,(mg/ml)} \times 100\% \quad (3)$$

## 2.5 Water holding capacity

The water hold capacity (WHC) of MC was measured according to AACC (2000c) method 56-30 (AACC, 2022). 3g MC powder was dispersed into 25 ml distilled water in a 50 ml falcon tube and centrifuged at 3000× g for 25 min. WHC was calculated as follows:

$$WHC\,(\%) = \frac{W_1 - W_2}{W_1} \times 100\% \quad (4)$$

Where W1 is the the weight of dry matter of sample (g), W2 is the weight of supernatant after centrifugation (g)

## 2.6 Functionality of Mycoprotein

The functionality of protein was investigated including foaming properties, emulsifying properties, and gelling ability. The protein solution preparation and functionality determination methods were present in this section.

### 2.6.1 Foaming properties

The foaming properties were determined based on measuring foaming capacity (FC) and foaming stability (FS). MC, MI and other benchmarking plant-based proteins were measured in triplicate.

#### 2.6.1.1 Protein solution preparation

In order to investigate the foaming properties, the foaming capacity and stability were measured according to the frothing test described by Tsutsui and Tomoni (1988)(Tsutsui, 1988) with some modifications. To determine how salt and pH influence the FC and FS of MC, 0%, 0.1%,0.3%, and 0.5% w/w salt were added to pH 5 and pH 7 of 2% w/w protein solution. The pH of MC solutions was adjusted by adding 1 M hydrochloric acid (HCl) and 1M sodium hydroxide (NaOH). Based on the foaming properties performance, the best condition of salt and pH was selected to determine how protein concentration (1%, 2%, 4%, and 8% w/w) and preheated temperature (20 °C, 50°C, and 70°C) influence the foaming properties of MC. The water bath (FALC Instruments S.r.l., Italy) was used to warm up the prepared protein solution for 10 min and cool down for 20 min at room temperature before experiments. For benchmarking, 2% w/w of protein solutions were made without adjusting pH and addition of salt.

After all the solutions were prepared in 50 ml falcon tubes, the dispersions were whipped

for 2 minutes by using a high shear homogenizer (Ingenieurbüro CAT M.Zipperer, Germany) at 10,000 rpm. The first minute placed the probe of high shear homogenizer in the bottom of dispersions and then moved the probe to the air-solution interface for whipping for another minute.

#### 2.6.1.2 Foaming ability and stability measurement

After agitation, the total volume was read off after 30 seconds. The foaming capacity was calculated as:

$$Foam\,capacity\,(\%) = \frac{Volume\,after\;agitiation - Volume\,before\,agitation}{Volume\,before\,agitation} \times 100\% \quad (5)$$

The foaming stability was determined by allowing samples to stand for 30 min at room temperature and the residual foam volumes were measured. The foaming stability was calculated as:

$$Foam\,stability\,(\%) = \frac{Residual\,foam\,volume}{Total\;foam\,volume} \times 100\% \quad (6)$$

### 2.6.2 Emulsifying properties

The emulsifying properties were determined based on measuring emulsifying capacity (EC) and emulsifying stability (FS). MC, MI, and other benchmarking plant-based proteins were measured in triplicate, except for FS measurement which was conducted in duplicate.

#### 2.6.2.1 Emulsifying capacity measurement

The protein powders were dissolved in water to make 2ml 10% w/w protein solutions. 1 M hydrochloric acid (HCl) and 1M sodium hydroxide (NaOH) was used to adjust the pH of MC to 5 and 7. To each protein solution, different aliquots of rapeseed oil (Menigo Foodservice AB, Sweden) were added. The oil-protein solution mixtures were homogenized using a high shear homogenizer (Ingenieurbüro CAT M.Zipperer, Germany) at a speed of 16,000 rpm for 1 min. The probe of the homogenizer was placed at the solution-oil interface while mixing. For benchmarking, 10% w/w of protein solutions were made without adjusting pH and addition of salt.

The emulsifying capacity was determined according to the method of Guo et al (2021)(Guo & Xiong, 2021) with some modifications. Different amount of oil was added to protein solutions and the oil/powder ratio was calculated. The variation of oil addition was every 1 g oil/ g of powder. The electrical conductivity of freshly prepared emulsion was measured immediately using a handheld conductivity meter(VWR International AB, USA). The phase inversion point occurred when the electrical conductivity ($\mu$S/cm) of the emulsions reached zero. Once the phase inversion point was measured, the oil/powder ratio was calculated and the nearby concentrations of oil were added to new protein solutions until the phase inversion point was measured three times. The EC was calculated as follows:

$$Emulsifying\,capacity\,(ml\,oil/g\,of\,powder) = \frac{V_{oil}}{m} = \frac{V_{oil}}{c\,V} \times 1000 \quad (7)$$

Where Voil is the amount of oil in emulsion (ml), V is the initial volume of solution (ml), m is the weight of powder (g), and c is the initial powder concentration in solution (mg/ml).

#### 2.6.2.2 Emulsifying stability measurement

The emulsifying stability (ES) was determined according to the method of Capacidade et al (2015) (Capacidade et al., 2015) with some modifications. The protein suspensions were prepared in 50 falcon tubes by dissolving 3 g of protein powder in 15 ml of water, and 15 ml of rapeseed oil (Menigo Foodservice AB, Sweden) was added to each suspension. The mixtures were stirred using a high shear homogenizer (Ingenieurbüro CAT M.Zipperer, Germany) at speed of 10,000 rpm for 1 min. And then the emulsions were centrifuged at 4000 rpm for 6 minutes followed by heat treatment in the water bath at 60°C for 30 minutes. Finally, all samples were cooled at room temperature for 1 hour before being centrifugated again at 4000 rpm for 6 minutes. The ES was calculated as follows:

$$ES\,(\%) = \frac{The\,height\,of\,emulsified\,layer\,after\,heating}{The\,height\,of\,total\,content\,in\,the\,tube} \times 100\% \tag{8}$$

### 2.6.3 Gelling ability

The gelling ability was determined by the penetration test analyzing the force (N)-distance (mm) curve. Three parameters were summarized from the curve and all the measurements were conducted in duplicate.

#### 2.6.3.1 Heat-induced gels preparation

To investigate the influence of pH and salt of MC. 0%, 0.5%, and 1% w/w salt were added into 15% w/w of MC dispersion and adjusted pH to 5 and 7. The pH of protein solutions was adjusted by adding 1 M hydrochloric acid (HCl) and 1M sodium hydroxide (NaOH). The samples were prepared into 50 ml beakers. For benchmarking, suspensions of 25% w/w protein dispersion were prepared in 50 ml beakers from protein powders by adding water with stirring, and then the suspensions were hydrated for 1 hour at room temperature, except for MC and MI. The thermal treatment was carried out at 90 °C water bath for 20 minutes and cooled down at room temperature for 3 hours before further texture analysis.

#### 2.6.3.2 Texture analysis

The penetration measurements were performed using a texture analyzer (Stable Microsystems, UK) with a 2 mm diameter flat cylinder probe. Force (N) -distance (mm) curves were generated by collecting 500 data points per second with the test speed of 2 mm/second, post text speed of 10 mm/second, and force trigger of 5 N. Three parameters were obtained from force-displacement curves: (a) breaking force (gel strength) (N), (b) deformation (N), and (c) firmness (N · mm). The breaking force is defined as the first significant break shown in the curve, the deformation is defined as the distance that the probe penetrate into gel at the initial slope of the curve, and the firmness was described as the initial slop of the penetration curve within the first 2mm during measurement. (Galante et al., 2017)

## 2.7 Benchmarking

To evaluate the functionality of mycoprotein, several plant-based proteins concentrates and isolates were compared including pea protein isolate 1 (Rawfoodshop, Sweden), pea

protein isolate 2 (Nutris, Sweden), mung bean protein isolate (Rawfoodshop, Sweden), hemp protein concentrate (Rawfoodshop, Sweden), pumpkin protein isolate (Rawfoodshop, Sweden), fava bean protein concentrate (Atura Proteins, UK), and rice protein isolate (Kebelco AB, Sweden). The protein content are shown as table 1 below:

| **Name** | **Protein content (%)** |
|---|---|
| Mycoprotein concentrate | 52 |
| Pea protein isolate 1 | 82 |
| Pea protein isolate 2 | 85 |
| Mung bean protein isolate | 71 |
| Hemp protein concentrate | 50 |
| Pumpkin protein isolate | 63 |
| Fava bean protein concentrate | 55 |
| Rice protein isolate | 80 |

**Table 1:** Protein content

## 2.8 Statistical analyses

All the experiments were conducted in triplicate if no additional description. The Statistical software SPSS (IBM, USA) software was used to analyze one-way of variance (ANOVA) statistically verifying the difference between means with Tukey post hoc. Results were presented as mean ± standard deviation. Significant differences between means were done using a confidence interval of 95%.

# 3 Results & Discussion

## 3.1 Mycoprotein isolation

### 3.1.1 Comparison of extraction yield from different raw material

Freeze-drying is one of the most expensive and energy-consuming steps in processing food products. So significant efforts within food processes were applied to reduce the freeze-drying time and decrease the energy consumption (Rybak et al., 2021). In this section, to investigate an efficient way to extract protein, the isolation yield of using MC and raw biomass (frozen) was compared by measuring the soluble protein content conducted by BCA analysis. The isolation yield (%) of two raw materials was calculated by equation2, and the results are shown in Table 2. The results show that the yield of the isolation process from frozen biomass was significantly higher ($p<0.05$) than that of MC.

| **Raw material** | **Dry matter (%)** | **Protein content in supernatant (mg/ml)** | **Isolation yield (%)** |
|---|---|---|---|
| Raw biomass (frozen) | 52.68 | 18.37±0.50 | $20.81\pm0.57^{a}$ |
| Mycoprotein concentrate | 100 | 45.81±0.33 | $18.25\pm0.10^{b}$ |

$^{a-b}$ within a column with different lowercase superscript letters are significant different ($p<0.05$). The value is present in mean±standard deviation. Standard deviation is displayed (n=2)

**Table 2:** Comparison of protein isolation yield from two types of raw materials

The intention of using MC to extract MI was because of the convenience of storage, and the hypothesis of higher yield result from well grind MC powder could disperse better than blended biomass. But as the results are shown in the table A.2, show a higher yield of isolation from raw biomass (frozen). Therefore, from efficiency and sustainability considerations, one freeze-drying process was omitted. Extraction MI from raw biomass (frozen) was applied to further isolation production. After isolation, the MI powder was collected and stored in a dry and ambient temperature environment for further functionality study.

### 3.1.2 Determination of protein isolation yield

Due to the hypothesis of protease presence, BCA analysis was conducted immediately after protein recovered and the supernatant was collected. The isolation yield was calculated by equation2. As the results in the table 2, 36.70% of total protein can be isolated from biomass by the pH-shift method.

| Raw material | Dry matter (%) | Protein content in supernatant (mg/ml) | Isolation yield (%) |
|---|---|---|---|
| Raw biomass (frozen) | 44.28 | 22.10±0.53 | 36.70±0.88 |

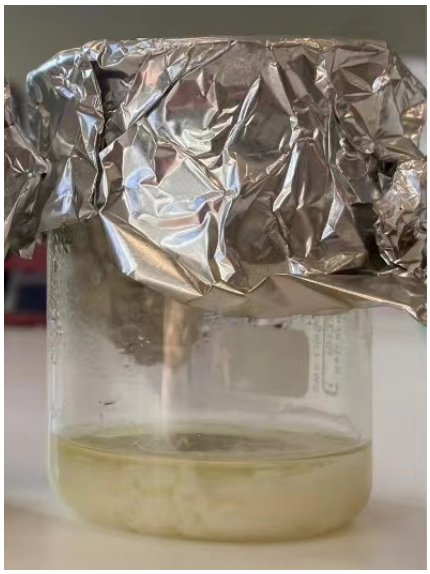
The value is present in mean±standard deviation. Standard deviation is displayed (n=3)

**Table 3:** Protein isolation yield

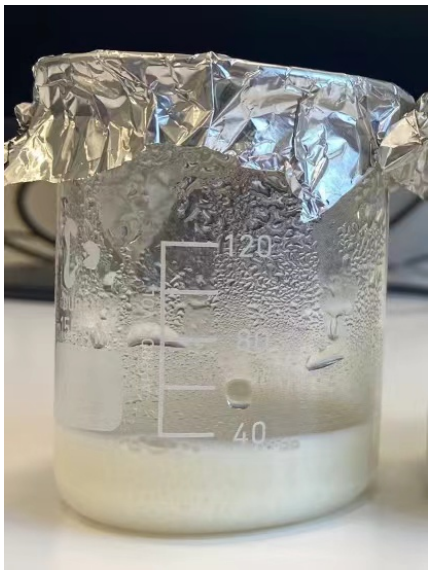
Compared with the protein isolation process investigated by Zeng (2021), the isolation yield from biomass was up to 71-77% which is considerably higher than the isolation yield obtained in this project (Zeng, 2021). Some possible reasons could be summarized for this lower yield. Firstly, compared with the protein isolation process conducted by Zeng (2021), there was no cell wall degradation process performed in this study due to equipment limitations. Hence, some intracellular protein in biomass could not be extracted which contributes to low protein isolation yield. Secondly, during alkaline extraction, the high sheer of blending could cause an increase in temperature which is not favorable for protein extraction. Protein coagulation, denaturation, and amino acid racemization could happen because of the increased temperature (Tangka, 2003) (Bals & Dale, 2011) (Zhu et al., 2010). These factors all contribute to the reduction of the protein extraction yield (Zhang et al., 2014). Although a study indicates that a high temperature accelerates protein recovery and productivity which means more protein can be recovered in a limited time but it conflicts with the general knowledge that high temperature can cause protein precipitation (Choi & Markakis, 1981). Thirdly, in the alkaline extraction step, the volume of solvent and the weight of material ratio, as well as alkaline concentration is regarded as the important parameter of extraction yield (Shen et al., 2008)(Lestari et al., 2010)(Harnedy & FitzGerald, 2013). In this study, these parameters did not investigate to optimize the isolation yield. Finally, the Proteolysis caused by the hypothesis of protease presence during the protein isolation process could be a key factor that leads to the reduction of yield. Based on a previous study investigated by Griffen et al (1997), the proteolytic activity was detected in the supernatant during exponential growth of fungi if the batch cultures were grown on a medium containing casein. During the stationary phase of fungi growth, the pH profile of proteases activity broadened and most of it was in the alkaline pH region(Griffen et al., 1997). In this project, MI stood at pH 10 for 1 hour for solubilizing protein into the water which may cause hydrolysis at the same time.

## 3.2 Protease activity

SSince the difference performance of gelation observed in pre-experiment (Figure 2). In short, the 15% w/w of MC can form a weak heat-induced gel (Figure 2b) if the protein-riched supernatant was transferred into the water bath immediately after it was collected, while 25% w/w of MC dispersion can not form a heat-induced gel (Figure 2a) if the protein-riched supernatant was collected and stored in the fridge (4°C) overnight.



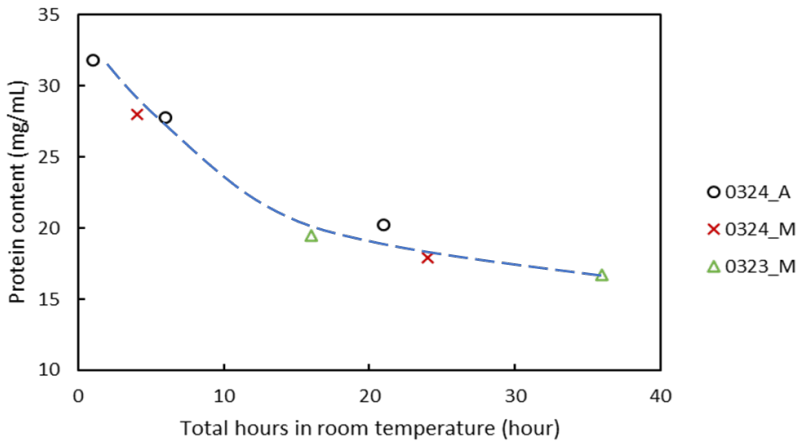
**(a)** 25% w/w supernatant of MC after 90°C water bath




**(b)** 15% w/w supernatant of MC after 90°C water bath

**Figure 2:** Gelation performance of 25% and 15% w/w MC supernatant

The hypothesis of protein content decreased over time was brought up, as well as considering the proteolytic activity described by Griffen (1997) (Griffen et al., 1997), a protein reduction curve was drawn by recording the time of sample that was placed at room temperature and the soluble protein determined using BCA analysis. Soluble protein content (mg/ml) was plotted against the time (h). The result is shown in Figure 3 which proved the hypothesis of soluble protein content decreased by the time put at the room temperature. As Figure 3 is shown, the protein content decreased by half in around 35 hours.




0324_A means the supernatant collected on 24 March.2022 afternoon, 0324_M means the supernatant collected on 24 March.2022 morning, and 0323_M means the supernatant collected on 23 March.2022 morning

**Figure 3:** Soluble protein content (mg/ml) verse time (hour)

The supernatant that was prepared on the afternoon of the 24 of March was divided

into three samples, one stored at room temperature (20°C), one stored in the fridge (4°C), and one stored in the freezer (-18°C). The storage time for these three samples in different temperatures was 17 hours. The soluble protein content was calculated by plotting the absorbance into BSA standard curve. The results show as Table 4. The results indicate that different storage temperatures could lead to different protein content even though all the samples were divided from the same supernatant. The sample which was measured protein content using BCA analysis immediately after being collected had the highest protein content, followed by the sample which was stored in the freezer (-18°C) for 17 hours, and followed by the sample stored in the fridge (4°C) for 17 hours and store in the room temperature (20°C) for 20 hours.

| **Sample** | **Protein content in supernatant (mg/ml)** |
|---|---|
| Supernatant measured immediately after collected | 31.8 |
| Supernatant placed at room temperature for 20 hours | 20.2 |
| Supernatant placed at room temperature for 3 hours and placed at fridge (4°C) for 17 hours | 25.9 |
| Supernatant placed at room temperature for 3 hours and placed at freezer (-18°C) for 17 hours | 27.8 |

**Table 4:** Supernatant collected on the afternoon of the 24 of March at different storage temperature

Some studies demonstrate that the protease activity inhibit by the lower temperature (Otroshi et al., 2014) (Tang et al., 2010). And in this project, the protein decrease could be slowed down by lower temperature which could speculate it was lower temperature inhibited the protease activity.

In addition, a study indicates that enzymatic modification can act as a functionalization strategy to improve the properties. For example, the addition of trypsin hydrolyzed soy protein can improve the gel strength by hydrophobic interactions and ionic bonds (Huang et al., 2010). However, another study showed that by hydrolyzing oat protein to a degree of hydrolysis of 5% by adding trypsin and pepsin no gelation was formed below 110°C at neutral or acidic pH (Nieto-Nieto et al., 2014). The conformational changes can be assumed to be responsible for no gel formed after enzymatic hydrolysis. More specifically, conformational changes affect protein solubility and thus affect the formation of soluble protein aggregates, which play an important role in the formation of gels after heat treatment proven by the study of Ma et al (2003) (Ma et al., 2003). Thus according to the previous studies and along with the proteolysis observed in this study, it can be concluded that a small amount of enzymatic hydrolysis of proteins can improve the functionality of protein, whereas the extensive hydrolysis could lead to opposite results. Furthermore, apart from the impact on functionality, it should also be noticed that extended enzymatic hydrolysis is producing some small hydrophobic peptides which result

in bitter taste and astringency (Ishibashi et al., 1988) (Saha & Hayashi, 2001). Therefore, in order to prevent protein from hydrolysis, for investigating the MC, protein solutions were used immediately after preparation.

## 3.3 Protein solubility

Solubility of MC (pH 5.7 and 7) and MI powder (pH7) together with pea protein isolate 1 were measured by BCA analysis. After 1% w/w protein solutions were prepared and centrifugated, the supernatant was collected and diluted 10 times for measurement. The results are shown in Table 5. The results indicate that the soluble protein content in MI (pH 7) is not significant differ ($p>0.05$) from that of MC (pH 7) which are both significantly higher than MC (pH 5) and pea protein isolate 1 (pH 6.7), containing 2.24 mg/ml and 2.21 mg/ml in the supernatant, respectively. And the soluble protein content of pea protein isolate 1 (pH 6.7) is significantly lower ($p<0.05$) than all the other protein solutions.

The results shown in table 5 indicate at pH 7 the soluble protein content of MC was significantly higher ($p<0.05$) than at pH 5. However, MI isolated by pH shift method does not extract more soluble protein at pH 7 which illustrates by no significant difference ($p<0.05$) of soluble protein content measured at these two pH conditions.

| **Protein** | **pH** | **Protein content in supernatant (mg/ml)** | **Soluble protein in powder (%)** |
|---|---|---|---|
| Mycoprotein concentrate | 5.7 | $1.87\pm0.06^{b}$ | $17.94\pm0.58^{b}$ |
| Mycoprotein concentrate | 7 | $2.24\pm0^{a}$ | $21.46\pm0^{a}$ |
| Mycoprotein isolate | 7 | $2.21\pm0.05^{a}$ | $20.78\pm0.44^{a}$ |
| Pea protein isolate 1 | 6.7 | $1.43\pm0.16^{c}$ | $13.57\pm01.48^{c}$ |

$^{a-c}$ within a column with different lowercase superscript letters are significant different ($p<0.05$). The value is present in mean±standard deviation. Standard deviation is displayed (n=3).

**Table 5:** Soluble protein content in supernatant and powder

According to the similar soluble protein content of MC and MI, three reasonable explications could be speculated. Firstly, it could result from the changed solubility. After pH shift more NaCl was introduced to the protein powder which could change the solubility of the protein. Secondly, the assumption could be proteins extracted by this method were mostly insoluble at pH 7. Thirdly, since the protein was isolated at room temperature, protease-caused proteolysis could happen which resulted in decreased soluble protein content. Additionally, the presence of protease could also be the reason that changing the solubility of protein which was proved by a study indicating that the balance between hydrophobic and hydrophilic patches on the proteins' surface changed upon enzymatic action (Brückner-Gühmann et al., 2021).

## 3.4 Water holding capacity of MC

WHC of protein powder is the ability of the amount of water can be prevented from being released or expelled from their three-dimensional structure (Hermansson, 1986) (Zayas, 1997). As a result of a large amount of fiber present in MC, the WHC of MC was determined in duplicate and calculated. The results are present in Table 6.

| Protein | WHC (%) |
|---|---|
| MC | 170.10±10.22 |

**Table 6:** Water holding capacity of MC

## 3.5 Functionality study

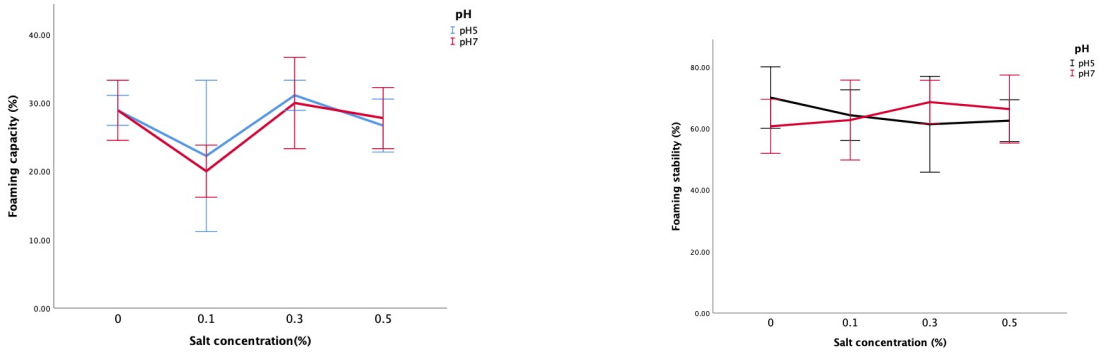
According to the pre-experiments investigated, the MI showed poor functionality. Thus, the following functionality study decided to use MC instead to determine how salt concentration, pH, and pre-thermal treatment affect the functionality of mycoprotein. More comparisons of the functionality of MC and MC are displayed in benchmarking parts.

### 3.5.1 Foaming properties

In this section, investigations of FC and FS affected by MC concentration, different salt concentrations and pH, and different pre-thermal treatments were measured. In addition, the benchmarking of FC and FS of 2% w/w dispersion of MC, MI, rice protein isolate, pumpkin protein isolate, fava bean protein concentrate, two pea protein isolates, and mung bean protein isolate at original pH were compared at the original pH.

#### 3.5.1.1 Foaming capacity and stability of MC with 0, 0.1, 0.3, and 0.5 % w/w salt at pH 5 and 7

The result on the effects of different salt concentration (0, 0.1, 0.3, and 0.5 % w/w) and two pH (5 and 7) treatment of 2% MC solution are presented in Figure 4. Both Figure 4a and Figure 4b indicate that there is no significant difference ($p>0.05$) among the difference salt range, as well as between 2 different pH condition.




**(a)** Foaming capacity **(b)** Foaming stability

**Figure 4:** Foaming ability with 0, 1, 3, 5 % w/w salt at pH 5 and 7

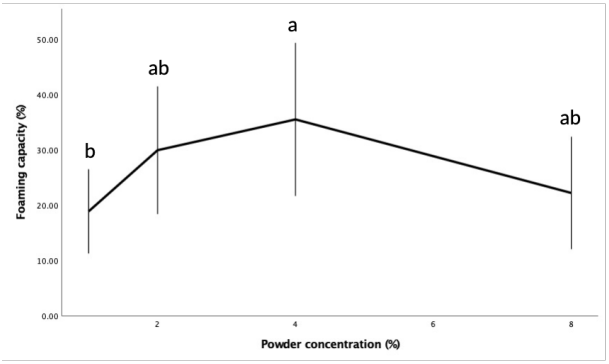
Studies showed that the initial addition of NaCl could enhance the FC because of the increment of protein solubility and dispersion which enhance the whipability and formation of stable cohesive films around air vacuoles. With continually increasing NaCl concentration, hydrophobic interaction between protein molecules increases which reduces the elasticity of protein molecules and consequently decrease the FC (Ndiritu et al., 2019)(Lawal, 2004). Also if continually adding NaCl, the FC could decrease resulting from the salting effect of NaCl (Khalid et al., 2003). These phenomenons are not in line with the results in this foaming properties test. The possible reason could be the salt content is not high enough to show the solubility and hydrophobicity changes. Due to the consideration of food application, a large amount of NaCl addition was not involved in the experiments.

The FS of protein depends on the cross-linking of protein molecules and the formation of film (Ohsodi, 1992). As a previous study showed, the FS of some proteins is very sensitive to ionic strength. With the presence of low NaCl concentration, FS can be enhanced concerning the salt-free sample. However, with the increase of NaCl concentration, the high ionic strength reduces the distance between protein stabilized bubble surfaces thereby facilitating bubble coalescence and reducing stable volume fraction. In addition, salt-induced protein aggregation interfaces with the formation of interfering with the formation of the interfacial network, which further destabilizes the foam.(Qiao et al., 2021). In this study, the FS was not changed significantly among different concentrations of salt added. We could speculate that MC foam may not be that sensitive to ionic strength changes or the amount of NaCl added in this study is not high enough to show the FS changes.

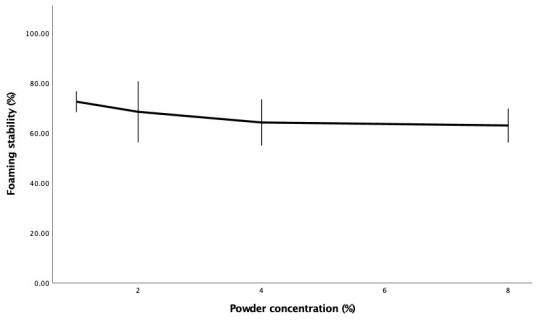
pH-dependent foaming properties for some proteins have been reported by Chel et al, and Lawal et al. At rather high or low pH, a high FC was observed attributed to the increase of net charge of protein molecules, which weaken hydrophobic interactions and increases protein flexibility. Therefore, the protein molecules can spread to the air-water interface more quickly, thereby encapsulating air particles and facilitating FS formation(Chel-Guerrero et al., 2002)(Lawal, 2004). The pH condition compared in this study was 5 and 7 which is around neutral so that could explain there was no significant difference ($p>0.05$) observed in FS. The 0.3% salt treatment at pH 7 was chosen following different MC concentrations and preheat treatment experiments.

#### 3.5.1.2 Foaming capacity and stability of different concentration of MC

Based on the common knowledge, protein is the main surface-active agent that works in the formation and stabilization of dispersed air while frothing (Paraskevopoulou et al., 2003). As Figure 5a shows, a significant difference ($p<0.05$) in samples observed within the group. There was an increase in FC of the samples with an increase in the concentration of MC from 1% to 4%, apart from the sample with the highest MC concentration. The FC of 8% w/w MC has no significant difference ($p>0.05$) from the other three concentrations of MC. Only FC of 4% w/w MC is significantly higher ($p<0.05$) than 1% w/w MC. In addition, as shown in Figure 5b, there is no significant difference ($>0.05$) in FS observed among all concentrations of MC.




**(a)** Foaming capacity




**(b)** Foaming stability

$^{a-b}$ illustrate the significant different ($p<0.05$). The error bars show the standard deviation (n=3).

**Figure 5:** Foaming ability of 0, 2, 4, 8% w/w MC

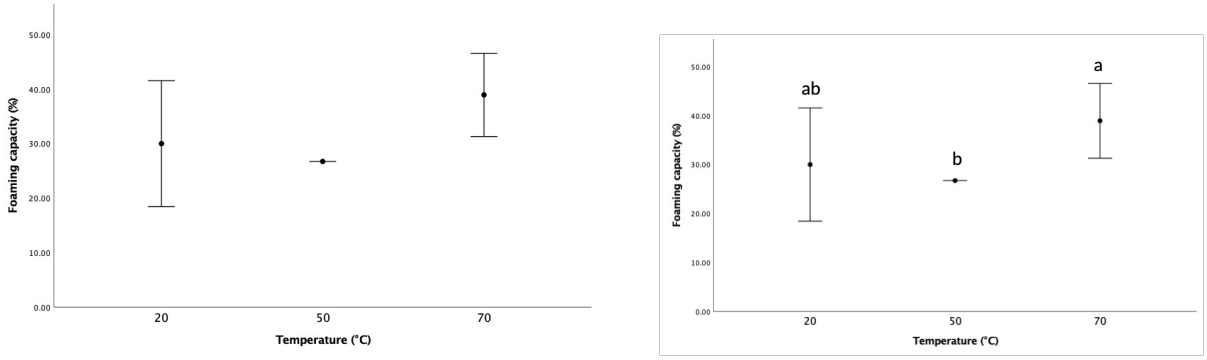
Based on previous foaming ability study described by Lonchamp et al (2019) indicate that the air bubble could be trapped by a dense network of mycelial and cell debris (Lonchamp et al., 2019). This phenomenon could explain that 4% w/w MC had significantly higher FC than 1% whereas 8% MC did not show a significant difference to 1% MC.

Theoretically, with the increase in protein concentration, the foam stability could also increase because of the formation of stiffer foam which develops from higher viscosity. The FS is improved by greater protein concentration because it can increases viscosity and facilitate the formation of multiplayer cohesive protein films at the interface (Lawal, 2004). However, in this study, the result did not show this tendency.

#### 3.5.1.3 Foaming capacity and stability of MC with preheat-treated condition

Figure 6 shows at different pre-heat treatments FC and FS were significantly different ($p<0.05$) within the group. In Figure 6, the results of FC and FS two preheat treatment (50°C and 70°C) along with non-thermal treatment (20°C) is showed. Figure 6a illustrates that the sample applied 70°C pre-heated treatment has significantly higher ($p<0.05$) FC than the sample applied 50°C preheated treatment. And no there is no significant difference ($p>0.05$) was noticed among other pairwise comparisons.

The results in Figure 6b have shown that a sharp decrease of FS was observed as 70°C preheat treatment applied. The 70°C preheat treated sample was a highly significant difference ($p<0.001$) from the 50°C preheat treated sample and the non-heat treated sample, and no significant difference ($p>0.05$) observed between 50°C and 20°C samples.




**(a)** Foaming capacity **(b)** Foaming stability

$^{a-b}$ illustrate the significant different ($p<0.05$). The error bars show the standard deviation (n=3).

**Figure 6:** Foaming ability with non, 50°C, and 70°C preheat-treated

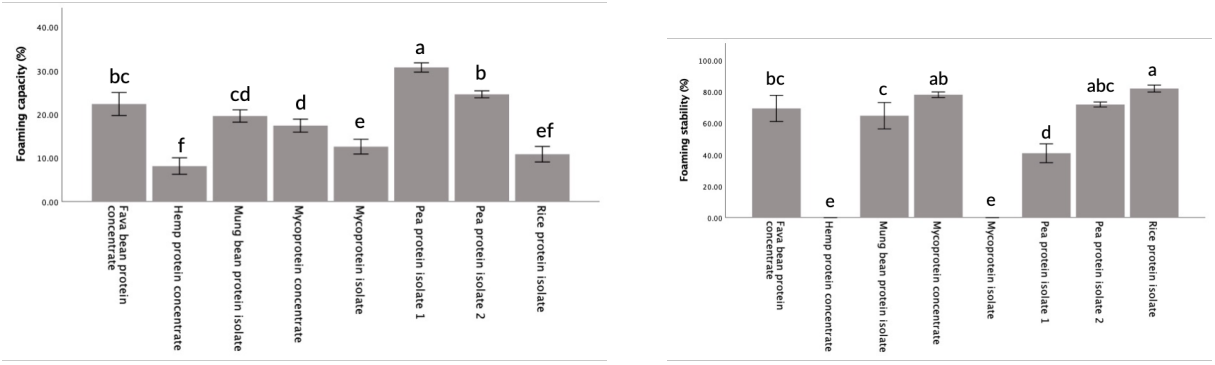
In terms of the difference in the temperature dependence on FC, the possible reason could be temperature dependence on protein molecular structure. At a higher temperature, the surface tension decreased with increased temperature which increases foamability. Also, the heat treatment could cause the unfolding of protein which increase its hydrophobicity and thus reduce the energy barrier for absorption at the air-water interface. Therefore the overall foaming capacity improved with the increased temperature of the thermal treatment. Although some detrimental effects result from the decreased viscosity and protein aggregation with temperature, the enhanced effect caused by increased temperature still predominates over the negative effects (Mitchell et al., 1986) (Indrawati et al., 2008). Moreover, many studies indicate that the occurrence of partial denaturation of protein by heating can cause the improvement of FC. However, the decrease in FC could also happen if the heating temperature is too high (DeVilbiss et al., 1974) (Haggett, 1976) (Graham & Phillips, 1979) (Kim et al., 2005). But this decrease in FC caused by high heating temperature was not observed in this study.

The possible reasons for FS decrease significantly with higher temperature could be due to the liquid viscosity being lower and the bubble size being larger at a higher temperature which accelerates the liquid drainage (Mita et al., 1977) (Hailing & Walstra, 1981) (Narsimhan & Wang, 2005). And this phenomenon of heating prior to foaming result in enhancing FC but decreasing FS was in agreement with previous mycoprotein functionality study conducted by Lonchamp et al (2019) (Lonchamp et al., 2019)

#### 3.5.1.4 Benchmarking of foaming ability

The FC and FS of MC and MI were compared with six other plant-based protein isolates and concentrate and the results were shown in Figure 7. As Figure 7a and Table A.2 show, the FC of MC has significantly lower ($p<0.05$) than two types of pea protein isolate and fava bean protein concentrate, significantly higher ($p<0.05$) than hemp protein concentrate, rice protein isolate, and MI. And the FC of MC had no significant difference from mung bean protein isolate ($p>0.05$). The FC of MI and rice protein were no significant ($p>0.05$) difference observed which both significantly lower ($p<0.05$) than all other proteins. FS shows in 7b, Hemp protein concentrate and MI collapsed in 30 min so both

FS were zero. MC, rice protein isolate, and pea protein isolate 2 show the highest FS which is 78.27%, 82.22%, and 72.5%, respectively. Overall, Both FC and FS of MC were significantly higher than MI ($p<0.05$). Pea protein isolate shows a good FC but the foam was not as stable as other proteins except for hemp protein concentrate and MI.




**(a)** Foaming capacity

**(b)** Foaming stability

$^{a-f}$ illustrate the significant different ($p<0.05$). The error bars show the standard deviation (n=3).

**Figure 7:** Foaming ability benchmarking

A study demonstrates that the presence of a dense mycelial network was responsible for FS, and particularly the high FS could be due to the concentration of cerato-platanin in protein fraction (Lonchamp et al., 2019). That could be the reason that MC had outperformed FS.

Apart from the foaming ability affected by protein, some other cosolutes could also affect it. A study reported that the presence of sucrose delays the formation and decreases the foaming powder (Lomakina & Mikova, 2006). It is because sugar affects the adsorption and aggregation behavior (Dickinson & Merino, 2002). The presence of sugar attributes to increased continuous phase viscosity, which is detrimental to air incorporation and the rapid diffusion and unfolding of proteins at the water-air interface. However, the good performance of FS of MC could partly result in presence of sugar. As sucrose enhances foam stability by increasing the viscosity of lamellar water and thus delaying liquid expulsion (Lau & Dickinson, 2005). As a result of the fermentation liquid residue present in the frozen biomass, MC could contain more sugar than other plant-based proteins which could result in negative affected FC but positively affected FS.

### 3.5.2 Emulsifying properties

The EC of 10% MC at pH 5 and 7 was compared with the that at original pH, and the EC and ES of 10% w/w dispersion of MC, MI, rice protein isolate, hemp protein concentrate, fava bean protein concentrate, two pea protein isolates, and mung bean protein isolate at original pH were compared at original pH.

#### 3.5.2.1 Emulsifying capacity at pH 5.7 (original), 5 and 7

The different concentrations of oil were added into MC at different pH (5.7, 5, and 7) of protein solution to prepare oil-in-water emulsions. The phase invasion point was recorded

which showed as conductivity reached zero. The emulsion break point of different pH conditions of the sample is shown in Figure 8. As can be seen, the EC of the original pH of MC is around 42.67 g oil /g powder and does not show any significant difference ($p>0.05$) from that of MC at pH 5 and 7.

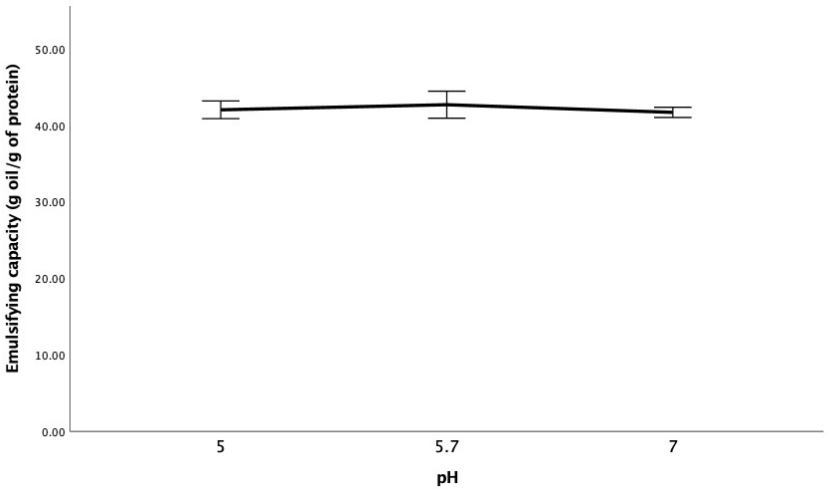


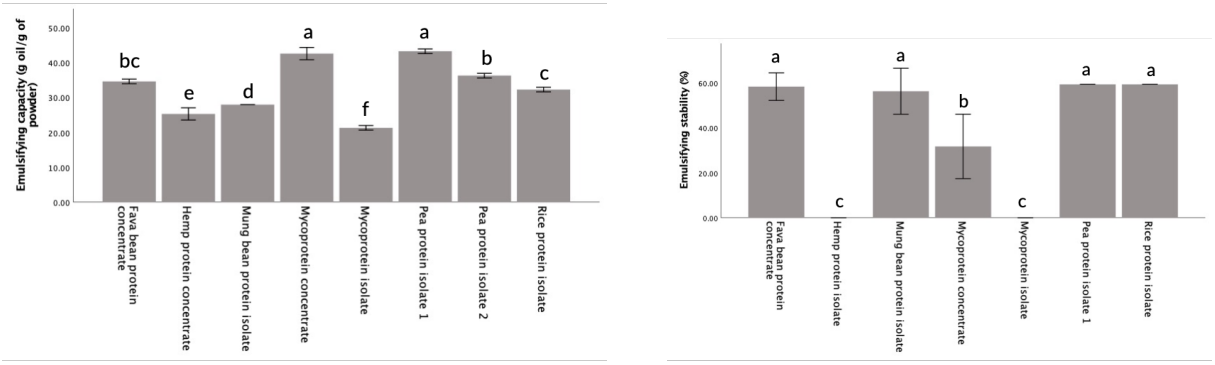

**Figure 8:** Emulsifying capacity at different pH

Generally, protein molecules are constituted of three types of amino acids non-polar amino acids, charged amino acids, and non-charged polar amino acids. All these amino acids induce hydrophobic and hydrophilic properties so that proteins can interact with oil and water acting as emulsifiers. The different performances of emulsifying properties could attribute to the protein solubility differing under different pH (LIADAKIS et al., 1998) (Ulloa et al., 2017). Considering the food application only pH 5 and 7 were chosen to compare with the original pH of MC. The results of EC do not show differences among these samples could be because of the similarity of solubility at pH 5.7, 5, and 7.

#### 3.5.2.2 Benchmarking of emulsifying ability

To investigate the emulsifying properties of MC and MI, the EC and ES of six types of plant-based protein were measured to be compared. The results are shown in Figure 9 and Table A.2. As shown in Figure 9a, the EC of MC was 42.67±1.53 g oil/ g powder which shows as good performance as pea protein isolate 1 (43.33±0.58), and it is significantly higher ($p<0.05$) than fava bean protein concentrate, hemp protein concentrate, mung bean protein isolate, pea protein isolate 2, rice protein isolate and MI. ES measurements were conducted after 80° heat treatment followed by centrifugation and the results show in 7b . The EC of MI is significantly lower than all the proteins. There was no emulsion layer observed on Hemp protein concentrate and MI which means the emulsion of these two proteins is not stable at all. Besides, no significant difference ($p>0.05$) of ES was noticed among all other plant-based proteins which all significantly higher ($p<0.05$) than MC.

Generally, the EC of MC was much better than MI and most of the other plant-based proteins. However, its stability could be a problem when considering the food application.




**(a)** Emulsifying capacity **(b)** Emulsifying stability

$^{a-f}$ illustrate the significant different ($p<0.05$). The error bars show the standard deviation (for EC, n=3; for ES, n=2 ).

**Figure 9:** Emulsifying ability benchmarking

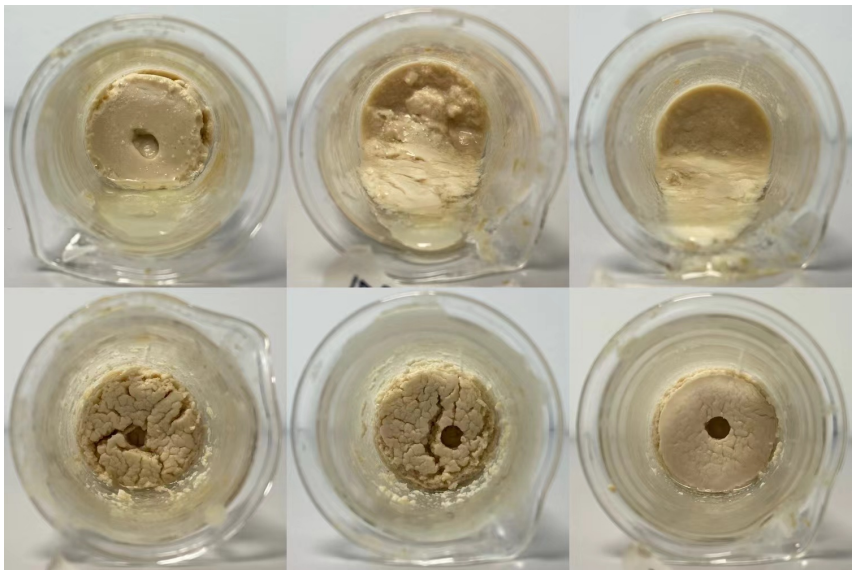
Similar to foaming properties, the presence of sugar also affects the emulsifying properties due to it can increase the diameter of droplets, and decrease the repulsive force between protein particles and between droplets. Also, the presence of sugar was proved to reduce the heat coagulation time, as well as the reducing sugars show faster coagulation which leads to instability of emulsion (Liang et al., 2014). Thus, we can draw an assumption that the residual sugar in MC could contribute to undesirable thermal stability.

### 3.5.3 Gelling ability

The gels made by 15% MC dispersion under different salt concentrations at pH 5 and 7 were presented in this section. The gelling ability of 25% w/w dispersion of MC, MI, rice protein isolate, pumpkin protein isolate, fava bean protein concentrate, two pea protein isolates, and mung bean protein isolate were compared at the original pH.

#### 3.5.3.1 Gelling ability of MC with 0, 0.5 and 1% w/w salt at pH 5 and 7

To determine whether salt concentration and pH influence the gel formation, penetration tests were performed to evaluate the gelling ability of 15% w/w MC dispersion. However, the tests were not run successfully due to the texture of the samples were not reach the lowest detection point. So no data was collected from the texture analyzer, and the visual observation was performed instead. The results shown as the Figure 10. The observation indicates that adding NaCl improves the texture of gel which showed a more smooth texture. When no NaCl and 0.5% w/w NaCl added cracks were observed, and as the NaCl concentration increased, the cracks on the surface of the sample decreased. Besides, as seen in Figure 10, the particulate structure was noticed at pH 7 while all the samples still had a paste-like texture after thermal treatment and cooling down which could be assumed that no gel formed at pH 5.



0, 0.5, and 1% w/w salt are displayed from left to right. pH 5 and 7 was displayed from the top down.

**Figure 10:** Rheology performance of 15% w/w MC with different salt concentration at pH 5 and 7

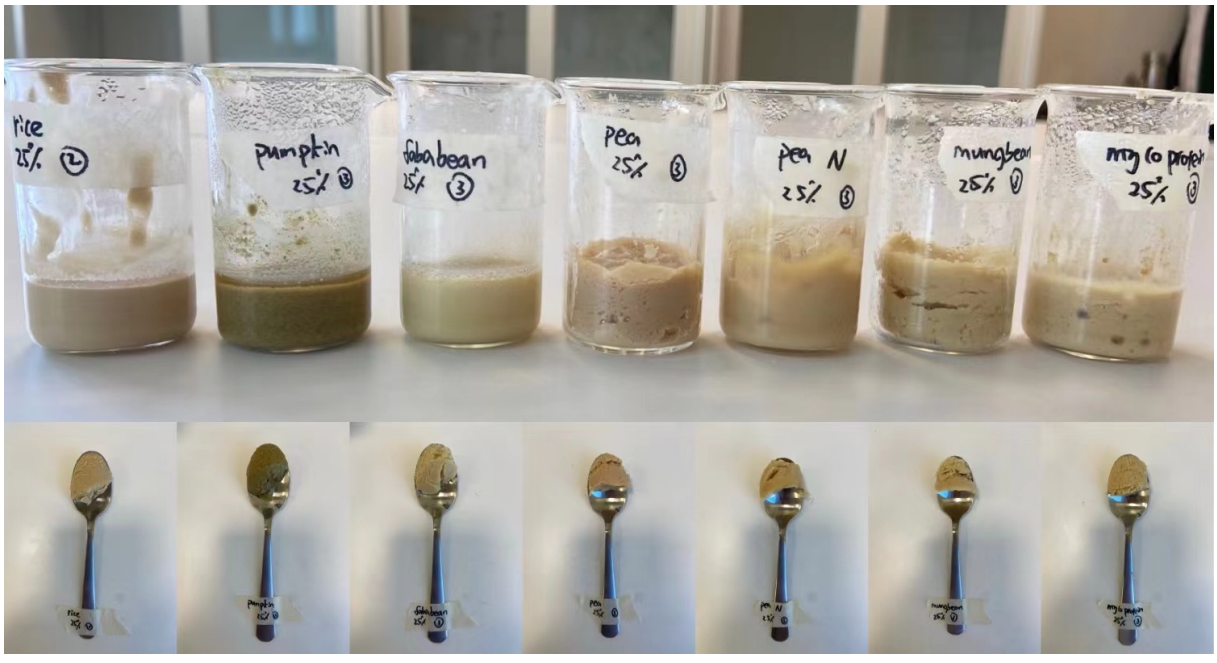
Based on the knowledge that gel can be made from many native proteins by heating them in a concentrated aqueous solution at suitable ionic strength and pH, thus adding NaCl and adjusting pH can influence gel formation (Van der Linden & Foegeding, 2009). The addition of salt leads to increased ionic strength which reduces the inter-molecular repulsion, thus the aggregation rate increases and it may happen more randomly (Schokker et al., 2000). However, it should be remarkable that the increased ionic strength by adding NaCl could result in increased denaturation temperature because of reduced intramolecular repulsion, thereby increasing conformational stability. So the protein molecules are more difficult to unfold, which is the initial reaction causing aggregation. In particular, this phenomenon is especially noticeable at around denaturation temperature (Clark et al., 2001). In this project, a more solid gel formed at a higher NaCl concentration ( higher ionic strength) which means the heating temperature (90°C) was far away from the denaturation temperature, and therefore, the first effect outweighed the second effect of MC in this study.

In addition, a study indicated that the moisture loss increased with increased pH, and this increase in moisture loss with increasing pH may be due to changes in network structure, from a finer stranded to a more coarse stranded. In a more coarse stranded network, water can move more freely so this type of network has less ability to trap water, thus a strong gel strength and more stable protein network could be explained by increased pH (Johansson, 2019). This phenomenon could also explain that some cracks occur at pH 7. Therefore, the addition of NaCl and increased pH could result in stronger three-dimensional network formation, thereby forming a stronger gel.

#### 3.5.3.2 Benchmarking of gelling ability

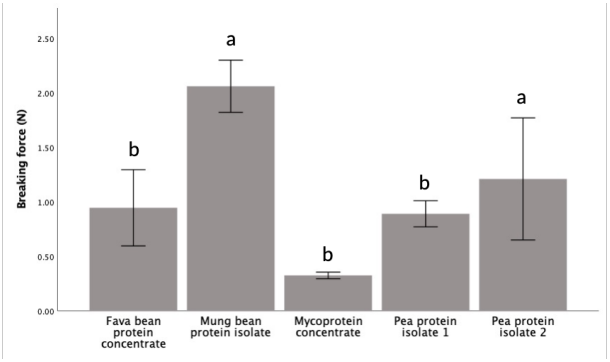
To obtain comparable parameters including breaking force (N), deformation (mm), and firmness (N/m), 25% w/w of protein dispersions were prepared followed by thermal treatment and penetration test. According to these three parameters, the strength of protein and protein interaction was expressed by the breaking force value, the elasticity of gels was shown by the deformation of gel at breaking point value, and the potential to against deform and tendency to flow rather than break (Galante et al., 2017) (Arfat & Benjakul, 2012). As shown in Figure 11, the gelling properties of MC and MI were compared to the other six plant-based proteins. The force-displacement curves were drawn from proteins than can form the gels which are shown in 11. Pumpkin protein isolate, rice protein isolate, and mycoprotein isolate can not form a gel at 25% w/w.

Figure 12a shows the breaking force of the gels which indicates the strength of protein and protein interactions. The results indicate that the mung bean protein isolate and pea protein isolate 2 had significantly higher ($p<0.05$) breaking force than the fava bean protein isolate, pea protein isolate 1, and mycoprotein concentrate which means stronger protein and protein bonds established (Arfat & Benjakul, 2012). And among fava bean protein isolate, pea protein isolate 1, and mycoprotein concentrate, there is no significant difference ($p>0.05$) observed. Same results were found in deformation (12b) in which noticed the significantly higher ($p<0.05$) value in mung bean and pea protein isolate. Figure 12c indicates mung bean had the highest firmness (0.63 N/mm) which was significantly higher than ($p<0.05$) mycoprotein concentrate and pea protein isolate 2. Generally, mung bean isolate showed significantly the highest ($p<0.05$) value among the measured three parameters, whereas the mycoprotein was significantly the lowest($p<0.05$).

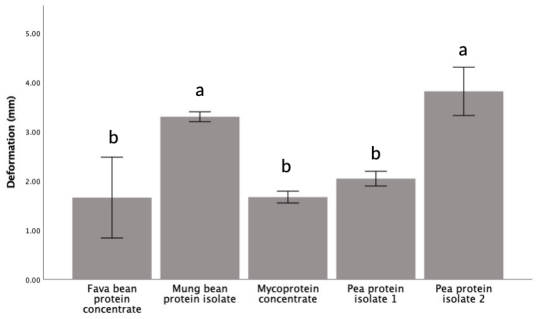



After heat treatment, rice protein isolate, pumpkin protein isolate, fava bean protein concentrate, pea protein isolate 1, pea protein isolate 2, mung bean protein isolate, and MC samples are displayed from left to right (the picture of MI was missing).

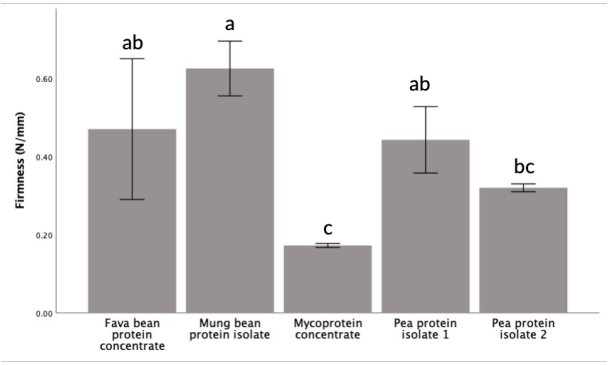
**Figure 11:** Gelling performance of 25% w/w proteins




**(a)** Breaking force




**(b)** Deformation




**(c)** Firmness

$^{a-c}$ illustrate the significant different ($p<0.05$). The error bars show the standard deviation ($n=2$).

**Figure 12:** Breaking force, deformation, and firmness of gels

Except for the protein participant three-dimensional network of gel formation, some studies show that the addition of fiber with strong hydration is an efficient way to improve the aggregation of protein network (Zhuang et al., 2018). Meanwhile, with the addition of fiber content water loss decreased significantly, and the hardness and gel strength were noticeably enhanced because of the formation of a more uniform and compact microstructure (K. Li et al., 2020).

In addition, the amount of starch present in different protein samples could also influence gel formation. After heating and cooling down, the gelatinized granules can create flaws in the microstructure which result in a weakening gel formation if there is a small amount of starch present. On the contrary, a higher amount of starch in thermal-induced gel leads to reinforcement of the gel which indicates an interpenetrating network between protein and the gelatinized starch is formed (Khan et al., 2020).

Therefore, considering the different content of protein and different fiber and starch in different gel systems, the breaking force, deformation and firmness were differing.

## 3.6 Hypothesis of the decreased functionality in MI

Firstly, after the pH-shift process, the structure of protein could be changed to some extent. A study proposed that both at alkaline and acidic pH, the head of the myosin

refold happened, and the refolded head is looser than the native structure (Raghavan & Kristinsson, 2007). Besides, another study demonstrates that traditional alkaline extraction and isoelectric precipitation result in more damaging the native conformation of protein compared with other protein isolation methods such as extraction by reverse micelles (Zhao et al., 2008). Consequently, during the pH-shift process in this study, the refold very likely happened, thus the native protein structure changed. This changed secondary structure could lead to the changes in hydrophilicity, hydrophobicity, and structural stability of protein which are all closely associated with the functional properties (Utsumi et al., 2002) (Zhao et al., 2008).

Secondly, some intracellular proteins could have better functionality but did not extract in this study. In this study, the protein isolation yield was only around 37% which is relatively lower than the isolation yield (71-77%) reached by a previous study conducted by Zeng (2021) using the pH-shift method together with cell wall degradation method (Zeng, 2021).

Thirdly, the soluble protein content was observed to decrease by half in 35 hours due to proteolysis. Some protein that has better functionality could be broken down into amino acid, thereby poor functionalities were shown.

Last but not least, during the pH shift process, sodium hydroxide (NaOH) and hydrochloric acid (HCl) were added which introduced more salt content to MI. Some researches indicate that the presence of salt affects the foaming, emulsifying and gelling performance (Ulloa et al., 2017) (Andualem & Gessesse, 2013). A small amount of salt could increase the solubility (salting in effect) resulting from the increased ionic strength of salt allowing dissociation and consequent interaction with proteins. However, at a higher concentration of salt, it produces a dehydration effect on protein leading to protein aggregation, thereby decreasing the solubility of the protein.

# 4 Conclusion

The objective of this study was to determine the functionality of MC and its foaming, emulsifying, and gelling ability to evaluate how different factors such as salt, pH and prethermal treatment, and benchmark MC and MI with other plant-based proteins influence its functionality.

The pH shift method was conducted in this project to extract MI which included alkaline solubilization, isoelectric precipitation, and neutralization steps. The isolation yield from two raw materials, MC powder and biomass, were compared. Isolated protein directly from biomass can reach a significantly higher ($p<0.05$) yield. The pH-driven isolation method achieved an overall yield of 36.70% of biomass with 20.78% soluble protein content (dry matter basis) which is no significant difference ($p>0.05$) compared with MC if adjusting the pH of MC to 7.

The soluble protein content of 25% w/w MC solution was decreased in half after 35 hours which was the result from the proteolysis. The protease was speculated present in MC and active at pH 5.7, and responsible for that protein decrease.

For foaming properties at different concentrations of MC, the results indicated that different concentrations (1%, 2%, 4%, and 8%) of MC affect FC. 4% w/w of MC solution showed the highest FC which was significantly higher ($p<0.05$) than that of 1% solution. The addition of 0, 0.1, 0.3, and 0.5% w/w salt at pH 5 and 7 did not show any significant difference ($p>0.05$). It was found that the preheat treatment at 70°C showed a significantly higher ($p<0.05$) FC than the preheat treatment at 50°C and the non preheat treatment. The preheat treatment at 70°C compromised the FS which was significantly lower ($p<0.05$) than the other two conditions. Regarding emulsifying properties, the EC of MC was measured at the original pH of 5.7, pH 5, and pH 7. No significant difference ($p>0.05$) was found among these pH conditions. For gelling ability, it was found that 15% MC dispersion added could not form a gel at pH 5 which displayed a paste-like texture. At pH 7, the 15% MC dispersion could form a solid-like particulate gel. With the addition of salt, a smoother texture and fewer cracks were observed which could have implied an increase in aggregation. This aggregation could have resulted from an increase in ionic strength that reduced the inter-molecular repulsion.

In terms of benchmarking, the MC only showed significantly higher ($p<0.05$) FC than hemp protein concentrate, MI, and rice protein isolate. Its FS had no significant difference ($p>0.05$) to the rice protein isolate which displayed the highest FS. Regarding EC, then the MC and the pea protein isolate 1 presented the significantly highest ($p<0.05$) values compared to fava bean protein concentrate, hemp protein concentrate, mung bean protein isolate, MI, pea protein isolate 2, and rice protein isolate. There was no significant difference observed between MC and pea protein isolate 2 which were up to 42.67 g oil/ g of powder and 43.33 g oil/ g of powder, respectively. However, the ES of MC was significantly lower ($p<0.05$) than fava bean protein isolate, mung bean protein isolate, pea protein isolate 1, and rice protein isolate. For gelling ability, MC did not show any significantly higher breaking force, deformation, and firmness than other plant-based proteins that can form gel at 25% w/w protein dispersion. In contrast, the MI showed significantly lower ($p<0.05$) FC, FS, EC, and ES than MC, and it could not form a detectable heat-

induced gel at 25% w/w dispersion. Additionally, its FC was only significantly higher ($p<0.05$) than hemp protein concentrate. Also, its FS, EC, and ES were not significantly higher ($p<0.05$) than any of the proteins.

Conclusively, the MI extracted by the pH shift method in this project performed lower functionality than MC which means the isolation process compromised the functionality of the native protein. A better isolation process is worth further research to extract functional protein fractions. Particularly, the proteolysis during the isolation process needs to be addressed.

# 5 Future outlook

This project aimed to measure the functional properties of mycoprotein, including foaming properties, emulsifying properties, and gelling ability. The salt, pH, and pre-heat treatment were applied to investigate their influence on the functionality of mycoprotein. The functionality of MC and MI were compared along with other plant-based proteins for benchmarking.

In this project, the functionality of MI was not shown as better functionalities than MC and other plant-based proteins. So it would be interesting to further explore a better protein isolation method without compromising its functionality which also should be suitable to produce for industrial production. Additionally, It is also worth analyzing the protein fragments by conducting the gel electrophoresis under different conditions such as different pH and extract methods. Moreover, the cell wall degradation is necessary to be applied to reach a higher yield such as enzymatic degradation, ultrasonication, and high pressure homogenization.

For future application, emulsifying capacity seems the most promising functionality of mycoprotein produced at Mycorena. But to the time limit in this project, the addition of salt and pre-heat treatment did not investigate. It could be of interest to know which external condition is the best for emulsifying applications. And some methods to further stabilize the mycoprotein emulsion would be of interest. Furthermore, as a lot of fiber is present in MC, the effect of fiber content on functionality is also worth investigating.

Further investigating about how to reduce or inhabit the proteolysis influence on functionality is another area of interest. Studies in this area should start with detect the protease activity by more convincing method using protease assay kit.

# A Appendix-tables

## A.1 Dilutions of BSA standards

| Tube | Volume of BSA | Volume of diluent | Final BSA concentration |
|---|---|---|---|
| 1 | 250 $\mu$l from 2 mg/ml solution | 250 $\mu$l | 1000$\mu$g/ml |
| 2 | 250 $\mu$l from tube 1 | 250 $\mu$l | 500 $\mu$g/ml |
| 3 | 250 $\mu$l | 250$\mu$l | 250 $\mu$g/ml |
| 4 | 300 $\mu$l | 300$\mu$l | 125$\mu$g/ml |
| 5 | 100 $\mu$l | 400 $\mu$l | 25 $\mu$g/ml |
| 6 | 0 | 400 $\mu$l | 0 $\mu$g/ml |

## A.2 Foaming and emulsifying properties of different proteins

Summary of the foaming, emulsifying ability of proteins. $^{a-f}$ within a column with different lowercase superscript letters are significant different ($p<0.05$). The value is present in mean±standard deviation. Standard deviation is displayed (for FC, FS, and EC, n=3; for ES, n=2)

| **Protein** | **FC (%)** | **FS (%)** | **EC (g oil/g of powder)** | **ES (%)** |
|---|---|---|---|---|
| Fava bean protein concentrate | 22.37±2.28$^{bc}$ | 69.55±7.22$^{bc}$ | 34.67±0.58$^{bc}$ | 58.5±4.35$^{a}$ |
| Hemp protein concentrate | 8.14±1.64$^{f}$ | 0$^{e}$ | 25.33±1.53$^{e}$ | 0$^{c}$ |
| Mung bean protein isolate | 19.63±1.23$^{cd}$ | 64.90±7.28$^{c}$ | 28.00±0.00$^{d}$ | 56.45±7.26$^{a}$ |
| Mycoprotein concentrate | 17.42±1.30$^{d}$ | 78.27±1.55$^{ab}$ | 42.67±1.53$^{a}$ | 31.82±10.16$^{b}$ |
| Mycoprotein isolate | 12.60±1.46$^{e}$ | 0$^{e}$ | 21.33±0.58$^{f}$ | 0$^{c}$ |
| Pea protein isolate 1 | 30.76±0.92$^{a}$ | 41.00±5.20$^{d}$ | 43.33±0.58$^{a}$ | 59.52±0.00$^{a}$ |
| Pea protein isolate 2 | 24.60±0.69$^{b}$ | 72.05±1.38$^{abc}$ | 33.00±5.20$^{b}$ | did not measure |
| Rice protein isolate | 10.87±1.54$^{ef}$ | 82.22±1.92$^{a}$ | 32.58±7.61$^{c}$ | 59.52±0.00$^{a}$ |

## A.3 Breaking force, deformation, and firmness of different proteins

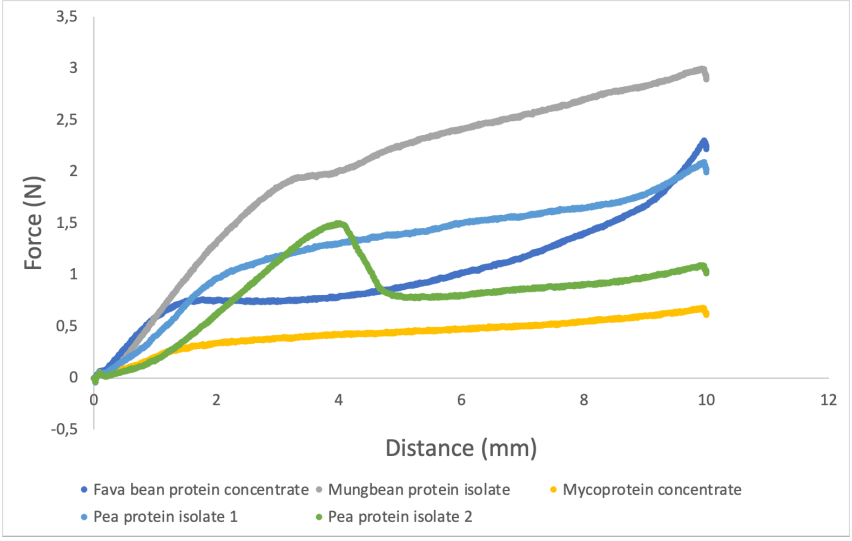
Summary of the breaking force, deformation, and firmness. $^{a-c}$ within a column with different lowercase superscript letters are significant different ($p<0.05$). The value is present in mean±standard deviation. Standard deviation is displayed (n=2)

| Protein | Breaking force (N) | Deformation (mm) | Firmness (N/mm) |
|---|---|---|---|
| Fava bean protein concentrate | $0.95\pm0.25^{b}$ | $1.66\pm0.58^{b}$ | $0.47\pm0.13^{ab}$ |
| Pumpkin protein isolate | / | / | / |
| Mung bean protein isolate | $2.06\pm0.17^{a}$ | $3.30\pm0.07^{a}$ | $0.63\pm0.05^{a}$ |
| Mycoprotein concentrate | $0.32\pm0.02^{c}$ | $1.67\pm0.85^{b}$ | $0.17\pm0.004^{c}$ |
| Mycoprotein isolate | / | / | / |
| Pea protein isolate 1 | $0.89\pm0.08^{bc}$ | $3.81\pm0.35^{b}$ | $0.44\pm0.06^{b}$ |
| Pea protein isolate 2 | $1.21\pm0.40^{b}$ | $2.05\pm0.11^{a}$ | $0.32\pm0.007^{bc}$ |
| Rice protein isolate | / | / | / |

# B Appendix-figure

## B.1 Penetration test result of 25% w/w protein dispersion

The force (N)-distance (mm) curve collected by texture analyzer.



# References


AACC. (2022). *56 physicochemical tests methods*. Retrieved May 5, 2022, from https://www.cerealsgrains.org/resources/Methods/Pages/56PhysicochemicalTests.aspx

Ahmad, M. I., Farooq, S., Alhamoud, Y., Li, C., & Zhang, H. (2022). A review on mycoprotein: History, nutritional composition, production methods, and health benefits. *Trends in Food Science & Technology*, 14–19.

Aiking, H. (2014). Protein production: Planet, profit, plus people? *The American Journal of Clinical Nutrition*, *100*(suppl_1), 483S–489S.

Alonso-Miravalles, L., & O'Mahony, J. A. (2018). Composition, protein profile and rheological properties of pseudocereal-based protein-rich ingredients. *Foods*, *7*(5), 73.

Andualem, B., & Gessesse, A. (2013). Effects of salt (nacl) concentrations on the functional properties of defatted brebra (millettia ferruginea) seed flour. *Middle-East Journal of Scientific Research*, *13*(7), 889–897.

Arfat, Y. A., & Benjakul, S. (2012). Gelling characteristics of surimi from yellow stripe trevally (selaroides leptolepis). *International Aquatic Research*, *4*(1), 1–13.

Avanza, M. V., Puppo, M. C., & Añon, M. C. (2005). Rheological characterization of amaranth protein gels. *Food hydrocolloids*, *19*(5), 889–898.

Bals, B., & Dale, B. E. (2011). Economic comparison of multiple techniques for recovering leaf protein in biomass processing. *Biotechnology and bioengineering*, *108*(3), 530–537.

Bertasini, D., Binati, R. L., Bolzonella, D., & Battista, F. (2022). Single cell proteins production from food processing effluents and digestate. *Chemosphere*, *296*, 134076.

Bottin, J. H., Swann, J. R., Cropp, E., Chambers, E. S., Ford, H. E., Ghatei, M. A., & Frost, G. S. (2016). Mycoprotein reduces energy intake and postprandial insulin release without altering glucagon-like peptide-1 and peptide tyrosine-tyrosine concentrations in healthy overweight and obese adults: A randomised-controlled trial. *British Journal of Nutrition*, *116*(2), 360–374.

Brückner-Gühmann, M., Kratzsch, A., Sozer, N., & Drusch, S. (2021). Oat protein as plant-derived gelling agent: Properties and potential of modification. *Future Foods*, *4*, 100053.

Campbell, G. M., & Mougeot, E. (1999). Creation and characterisation of aerated food products. *Trends in food science & technology*, *10*(9), 283–296.

Capacidade, D. F. E. E. D., Emulsão, E., Espuma, D., & Proteínas, D. S. S. (2015). Foaming and emulsifying capacity, foam and emulsion stability of proteins of porcine blood: Determination at different values of ph and concentrations. *Revista Brasileira de Tecnologia*, *9*(1), 1797–1809.

Chel-Guerrero, L., Pérez-Flores, V., Betancur-Ancona, D., & Davila-Ortiz, G. (2002). Functional properties of flours and protein isolates from phaseolus lunatus and canavalia ensiformis seeds. *Journal of Agricultural and Food chemistry*, *50*(3), 584–591.

Choi, Y. R., & Markakis, P. (1981). Blue-green algae as a source of protein. *Food Chemistry*, *7*(4), 239–247.

Clark, A., Kavanagh, G., & Ross-Murphy, S. (2001). Globular protein gelation—theory and experiment. *Food Hydrocolloids*, *15*(4-6), 383–400.

Coelho, M. O., Monteyne, A. J., Dunlop, M. V., Harris, H. C., Morrison, D. J., Stephens, F. B., & Wall, B. T. (2020). Mycoprotein as a possible alternative source of dietary

protein to support muscle and metabolic health. *Nutrition Reviews*, *78*(6), 486–497.

Damodaran, S. (1997). Protein-stabilized foams and emulsions. *Food science and technology-new York-marcel dekker-*, 57–110.

Derbyshire, E., & Ayoob, K.-T. (2019). Mycoprotein: Nutritional and health properties. *Nutrition today*, *54*(1), 7–15.

Derbyshire, E. J., & Delange, J. (2021). Fungal protein–what is it and what is the health evidence? a systematic review focusing on mycoprotein. *Frontiers in Sustainable Food Systems*, 18.

DeVilbiss, E., Holsinger, V., Posati, L., & Pallansch, M. (1974). Properties of whey protein concentrate foams. *Food Technology*, 40.

Dickinson, E. (1992). *Introduction to food colloids*. Oxford university press.

Dickinson, E. (1999). Adsorbed protein layers at fluid interfaces: Interactions, structure and surface rheology. *Colloids and surfaces B: Biointerfaces*, *15*(2), 161–176.

Dickinson, E., & Merino, L. M. (2002). Effect of sugars on the rheological properties of acid caseinate-stabilized emulsion gels. *Food Hydrocolloids*, *16*(4), 321–331.

Finnigan, T. J., Wall, B. T., Wilde, P. J., Stephens, F. B., Taylor, S. L., & Freedman, M. R. (2019). Mycoprotein: The future of nutritious nonmeat protein, a symposium review. *Current developments in nutrition*, *3*(6), nzz021.

Foegeding, E. A., Bowland, E. L., & Hardin, C. C. (1995). Factors that determine the fracture properties and microstructure of globular protein gels. *Food Hydrocolloids*, *9*(4), 237–249.

Foegeding, E. A., & Davis, J. P. (2011). Food protein functionality: A comprehensive approach. *Food hydrocolloids*, *25*(8), 1853–1864.

Galante, M., Boeris, V., Álvarez, E., & Risso, P. (2017). Microstructural and textural properties of rennet-induced milk protein gel: Effect of guar gum. *International journal of food properties*, *20*(sup3), S2569–S2578.

Graham, D., & Phillips, M. (1979). Proteins at liquid interfaces: I. kinetics of adsorption and surface denaturation. *Journal of colloid and interface science*, *70*(3), 403–414.

Griffen, A. M., Wiebe, M. G., Robson, G. D., & Trinci, A. P. (1997). Extracellular proteases produced by the quorn® myco-protein fungus fusarium graminearum in batch and chemostat culture. *Microbiology*, *143*(9), 3007–3013.

Guo, A., & Xiong, Y. L. (2021). Electrical conductivity: A simple and sensitive method to determine emulsifying capacity of proteins. *Journal of Food Science*, *86*(11), 4914–4921.

Haggett, T. (1976). The whipping, foaming and gelling properties of whey protein concentrates. *New Zealand Journal of Dairy Science and Technology (New Zealand)*, 244–250.

Hailing, P. J., & Walstra, P. (1981). Protein-stabilized foams and emulsions. *Critical Reviews in Food Science & Nutrition*, *15*(2), 155–203.

Harnedy, P. A., & FitzGerald, R. J. (2013). Extraction of protein from the macroalga palmaria palmata. *LWT-Food Science and Technology*, *51*(1), 375–382.

Hasenhuettl, G. L., Hartel, R. W. et al. (2008). *Food emulsifiers and their applications* (Vol. 19). Springer.

He, J.-S., Mu, T.-H., Guo, X., Zhu, S., Azuma, N., & Kanno, C. (2013). Comparison of the gel-forming ability and gel properties of $\alpha$-lactalbumin, lysozyme and myoglobin in the presence of $\beta$-lactoglobulin under high pressure. *Food Hydrocolloids*, *33*(2), 415–424.

Hermansson, A. (1986). Functional properties of food macromolecules. *by JR Mitchell and DA Ledward, Elsevier Applied Science Publishers, London*, 273.

Huang, X., Li, C., Yang, F., Xie, L., Xu, X., Zhou, Y., & Pan, S. (2010). Interactions and gel strength of mixed myofibrillar with soy protein, 7s globulin and enzyme-hydrolyzed soy proteins. *European Food Research and Technology*, *231*(5), 751–762.

Hultin, H., & Kelleher, S. (1999). *Process for isolating a protein composition from a muscle source and protein composition* (US Patent 6,005,073). Espacenet.

Hultin, H., & Kelleher, S. (2000). *High efficiency alkaline protein extraction* (US Patent 6,136,959). Espacenet.

Hultin, H., Kristinsson, H., Lanier, T., Park, J., et al. (2005). Process for recovery of functional proteins by ph shifts. *Surimi and surimi seafood*, 107–139.

Indrawati, L., Wang, Z., Narsimhan, G., & Gonzalez, J. (2008). Effect of processing parameters on foam formation using a continuous system with a mechanical whipper. *Journal of food engineering*, *88*(1), 65–74.

Ishibashi, N., Ono, I., Kato, K., Shigenaga, T., Shinoda, I., OKAi, H., & Fukui, S. (1988). Role of the hydrophobic amino acid residue in the bitterness of peptides. *Agricultural and Biological Chemistry*, *52*(1), 91–94.

Johansson, M. (2019). *Pea protein-systems for plant based protein products, the effect of insoluble and soluble lentil fractions on rheology, microstructure and gelation properties of heat induced pea protein gels* (Master's thesis). Chalmers University of Technology. the Sweden.

Khalid, E., Babiker, E., & Tinay, A. E. (2003). Solubility and functional properties of sesame seed proteins as influenced by ph and/or salt concentration. *Food chemistry*, *82*(3), 361–366.

Khan, F. A., Arshad, M. U., Rizvi, S. S. H., Ameer, K., Anjum, F. M., Imran, M., & Imran, A. (2020). Optimization of potato starch gel formulation as green alternative of animal-sourced gelatin. *Food Science and Technology*, *41*, 626–634.

Kim, D. A., Cornec, M., & Narsimhan, G. (2005). Effect of thermal treatment on interfacial properties of $\beta$-lactoglobulin. *Journal of Colloid and Interface Science*, *285*(1), 100–109.

Kristinsson, H. G., & Hultin, H. O. (2003). Changes in conformation and subunit assembly of cod myosin at low and high ph and after subsequent refolding. *Journal of Agricultural and Food Chemistry*, *51*(24), 7187–7196.

Lam, R. S., & Nickerson, M. T. (2013). Food proteins: A review on their emulsifying properties using a structure–function approach. *Food chemistry*, *141*(2), 975–984.

Lau, C. K., & Dickinson, E. (2005). Instability and structural change in an aerated system containing egg albumen and invert sugar. *Food Hydrocolloids*, *19*(1), 111–121.

Lawal, O. S. (2004). Functionality of african locust bean (parkia biglobossa) protein isolate: Effects of ph, ionic strength and various protein concentrations. *Food Chemistry*, *86*(3), 345–355.

Lestari, D., Mulder, W., & Sanders, J. (2010). Improving jatropha curcas seed protein recovery by using counter current multistage extraction. *Biochemical Engineering Journal*, *50*(1-2), 16–23.

Li, K., Liu, J.-Y., Fu, L., Zhao, Y.-Y., Zhu, H., Zhang, Y.-Y., Zhang, H., & Bai, Y.-H. (2020). Effect of bamboo shoot dietary fiber on gel properties, microstructure and water distribution of pork meat batters. *Asian-Australasian journal of animal sciences*, *33*(7), 1180.

Li, X., Li, Y., Hua, Y., Qiu, A., Yang, C., & Cui, S. (2007). Effect of concentration, ionic strength and freeze-drying on the heat-induced aggregation of soy proteins. *Food Chemistry*, *104*(4), 1410–1417.

LIADAKIS, G. N., TZIA, C., OREOPOULOU, V., & THOMOPOULOS, C. D. (1998). Isolation of tomato seed meal proteins with salt solutions. *Journal of Food Science*, *63*(3), 450–453.

Liang, Y., Matia-Merino, L., Patel, H., Ye, A., Gillies, G., & Golding, M. (2014). Effect of sugar type and concentration on the heat coagulation of oil-in-water emulsions stabilized by milk-protein-concentrate. *Food Hydrocolloids*, *41*, 332–342.

Lomakina, K., & Mikova, K. (2006). A study of the factors affecting the foaming properties of egg white–a review. *Czech Journal of Food Sciences*, *24*(3), 110–118.

Lonchamp, J., Akintoye, M., Clegg, P. S., & Euston, S. R. (2020). Sonicated extracts from the quorn fermentation co-product as oil-lowering emulsifiers and foaming agents. *European Food Research and Technology*, *246*(4), 767–780.

Lonchamp, J., Clegg, P., & Euston, S. R. (2019). Foaming, emulsifying and rheological properties of extracts from a co-product of the quorn fermentation process. *European Food Research and Technology*, *245*(9), 1825–1839.

Ma, C.-Y., Rout, M., & Phillips, D. (2003). Study of thermal aggregation and gelation of oat globulin by raman spectroscopy. *Spectroscopy*, *17*(2-3), 417–428.

Mäkinen, O. E., Wanhalinna, V., Zannini, E., & Arendt, E. K. (2016). Foods for special dietary needs: Non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical reviews in food science and nutrition*, *56*(3), 339–349.

Marmon, S. (2012). *Protein isolation from herring (clupea harengus) using the ph-shift process–protein yield, protein isolate quality and removal of food contaminants* (Master's thesis). Chalmers University of Technology. the Sweden.

Maurya, N. K., & Kushwaha, R. (2019). Chapter-7 novel protein foods: Alternative sources of protein for human consumption.

Méx, B. S. Q. (2008). Emulsifying properties of proteins. *Boletıḱn de la Sociedad*, *2*(2), 80.

Mita, T., Nikai, K., Hiraoka, T., Matsuo, S., & Matsumoto, H. (1977). Physicochemical studies on wheat protein foams. *Journal of Colloid and Interface Science*, *59*(1), 172–178.

Mitchell, J. et al. (1986). Foaming and emulsifying properties of proteins. *Developments in food proteins*, *4*, 291–338.

Murray, B. S. (2020). Recent developments in food foams. *Current Opinion in Colloid & Interface Science*, *50*, 101394.

Narsimhan, G., & Wang, Z. (2005). Stability of thin stagnant film on a solid surface with a viscoelastic air–liquid interface. *Journal of colloid and interface science*, *291*(1), 296–302.

Nations, U. (2019). *World population trends*. Retrieved May 25, 2022, from https://www.un.org/en/observances/world-population-day

Ndiritu, A. K., Kinyuru, J. N., Gichuhi, P. N., & Kenji, G. M. (2019). Effects of nacl and ph on the functional properties of edible crickets (acheta domesticus) protein concentrate. *Journal of Food Measurement and Characterization*, *13*(3), 1788–1796.

Nicolai, T., & Durand, D. (2013). Controlled food protein aggregation for new functionality. *Current Opinion in Colloid & Interface Science*, *18*(4), 249–256.

Nieto-Nieto, T. V., Wang, Y. X., Ozimek, L., & Chen, L. (2014). Effects of partial hydrolysis on structure and gelling properties of oat globular proteins. *Food research international*, *55*, 418–425.

Nolsøe, H., & Undeland, I. (2009). The acid and alkaline solubilization process for the isolation of muscle proteins: State of the art. *Food and Bioprocess Technology*, *2*(1), 1–27.

Ohsodi, A. (1992). Proximate composition, nutritionally valuable minerals ad functional properties of adenopus brevriflorus bent seed flour. *Food Chem*, *45*, 79–83.

Otroshi, B., Anvari, M., Shariarinour, M., et al. (2014). Study on activity and stability of proteases from bacillus sp. produced by submerged fermentation. *Int. J. Adv. Biol. Biom. Res*, *2*(7), 2283–2287.

Paraskevopoulou, A., Athanasiadis, I., Kanellaki, M., Bekatorou, A., Blekas, G., & Kiosseoglou, V. (2003). Functional properties of single cell protein produced by kefir microflora. *Food research international*, *36*(5), 431–438.

Prins, A. (1988). Principles of foam stability. *Advances in food emulsions and foams* (pp. 91–122). Elsevier Applied Sience.

Qiao, X., Miller, R., Schneck, E., & Sun, K. (2021). Influence of salt addition on the surface and foaming properties of silk fibroin. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *609*, 125621.

Raghavan, S., & Kristinsson, H. G. (2007). Conformational and rheological changes in catfish myosin as affected by different acids during acid-induced unfolding and refolding. *Journal of agricultural and food chemistry*, *55*(10), 4144–4153.

Rybak, K., Parniakov, O., Samborska, K., Wiktor, A., Witrowa-Rajchert, D., & Nowacka, M. (2021). Energy and quality aspects of freeze-drying preceded by traditional and novel pre-treatment methods as exemplified by red bell pepper. *Sustainability*, *13*(4), 2035.

Saha, B. C., & Hayashi, K. (2001). Debittering of protein hydrolyzates. *Biotechnology advances*, *19*(5), 355–370.

Schokker, E., Singh, H., Pinder, D., & Creamer, L. (2000). Heat-induced aggregation of $\beta$-lactoglobulin ab at ph 2.5 as influenced by ionic strength and protein concentration. *International Dairy Journal*, *10*(4), 233–240.

Sharif, M., Zafar, M. H., Aqib, A. I., Saeed, M., Farag, M. R., & Alagawany, M. (2021). Single cell protein: Sources, mechanism of production, nutritional value and its uses in aquaculture nutrition. *Aquaculture*, *531*, 735885.

Shen, L., Wang, X., Wang, Z., Wu, Y., & Chen, J. (2008). Studies on tea protein extraction using alkaline and enzyme methods. *Food Chemistry*, *107*(2), 929–938.

Souza Filho, P. F., Andersson, D., Ferreira, J. A., & Taherzadeh, M. J. (2019). Mycoprotein: Environmental impact and health aspects. *World Journal of Microbiology and Biotechnology*, *35*(10), 1–8.

Sun, X. D., & Arntfield, S. D. (2011). Gelation properties of salt-extracted pea protein isolate induced by heat treatment: Effect of heating and cooling rate. *Food Chemistry*, *124*(3), 1011–1016.

Tadpitchayangkoon, P., Park, J. W., Mayer, S. G., & Yongsawatdigul, J. (2010). Structural changes and dynamic rheological properties of sarcoplasmic proteins subjected to ph-shift method. *Journal of agricultural and food chemistry*, *58*(7), 4241–4249.

Tang, X.-Y., Wu, B., Ying, H.-J., & He, B.-F. (2010). Biochemical properties and potential applications of a solvent-stable protease from the high-yield protease producer

pseudomonas aeruginosa pt121. *Applied Biochemistry and Biotechnology*, *160*(4), 1017–1031.

Tangka, J. K. (2003). Analysis of the thermal energy requirements for the extraction of leaf protein concentrate from some green plants. *Biosystems engineering*, *86*(4), 473–479.

Tessari, P., Lante, A., & Mosca, G. (2016). Essential amino acids: Master regulators of nutrition and environmental footprint? *Scientific reports*, *6*(1), 1–13.

Troiano, D., Orsat, V., & Dumont, M. (2020). Status of filamentous fungi in integrated biorefineries. *Renewable and Sustainable Energy Reviews*, *117*, 109472.

Tsutsui, T. (1988). Functional properties of heat-treated egg yolk low density lipoprotein. *Journal of Food Science*, *53*(4), 1103–1106.

Ulloa, J. A., Villalobos Barbosa, M. C., Resendiz Vazquez, J. A., Rosas Ulloa, P., Ramıḱrez Ramıḱrez, J. C., Silva Carrillo, Y., & González Torres, L. (2017). Production, physico-chemical and functional characterization of a protein isolate from jackfruit (artocarpus heterophyllus) seeds. *CyTA-Journal of Food*, *15*(4), 497–507.

Undeland, I., Kelleher, S. D., & Hultin, H. O. (2002). Recovery of functional proteins from herring (clupea harengus) light muscle by an acid or alkaline solubilization process. *Journal of agricultural and food chemistry*, *50*(25), 7371–7379.

Utsumi, S., Maruyama, N., Satoh, R., & Adachi, M. (2002). Structure-function relationships of soybean proteins revealed by using recombinant systems. *Enzyme and Microbial Technology*, *30*(3), 284–288.

Van der Linden, E., & Foegeding, E. A. (2009). Gelation: Principles, models and applications to proteins. *Modern biopolymer science* (pp. 29–91). Elsevier.

Vishwanatha, K., Rao, A. A., & Singh, S. A. (2010). Acid protease production by solid-state fermentation using aspergillus oryzae mtcc 5341: Optimization of process parameters. *Journal of Industrial Microbiology and Biotechnology*, *37*(2), 129–138.

Wilde, P., Mackie, A., Husband, F., Gunning, P., & Morris, V. (2004). Proteins and emulsifiers at liquid interfaces. *Advances in colloid and interface Science*, *108*, 63–71.

Wu, G. (2009). Amino acids: Metabolism, functions, and nutrition. *Amino acids*, *37*(1), 1–17.

Yamagishi, T., Miyakawa, A., Noda, N., & Yamauchi, F. (1983). Isolation and electrophoretic analysis of heat-induced products of mixed soybean 7s and 11s globulins. *Agricultural and Biological Chemistry*, *47*(6), 1229–1237.

Zayas, J. F. (1997). Gelling properties of proteins. *Functionality of proteins in food* (pp. 310–366). Springer.

Zeng, B. (2021). Single cell protein extraction for protein functional properties study and food applications.

Zhang, C., Sanders, J. P., & Bruins, M. E. (2014). Critical parameters in cost-effective alkaline extraction for high protein yield from leaves. *Biomass and Bioenergy*, *67*, 466–472.

Zhao, X., Chen, F., Xue, W., & Lee, L. (2008). Ftir spectra studies on the secondary structures of 7s and 11s globulins from soybean proteins using aot reverse micellar extraction. *Food Hydrocolloids*, *22*(4), 568–575.

Zhu, K.-X., Sun, X.-H., Chen, Z.-C., Peng, W., Qian, H.-F., & Zhou, H.-M. (2010). Comparison of functional properties and secondary structures of defatted wheat germ proteins separated by reverse micelles and alkaline extraction and isoelectric precipitation. *Food Chemistry*, *123*(4), 1163–1169.

Zhuang, X., Han, M., Bai, Y., Liu, Y., Xing, L., Xu, X.-l., & Zhou, G.-h. (2018). Insight into the mechanism of myofibrillar protein gel improved by insoluble dietary fiber. *Food Hydrocolloids*, *74*, 219–226.