Chapter 1

Introduction

1.1 Background

Environmental and health concerns associated with the consumption of meat, such as chronic health diseases and high volume pollution generation, necessitate a global shift from protein sourced from livestock produce towards a wider range of alternatives (Godfray et al., 2018). Several novel protein source alternatives are available to fill the gap of a growing demand. One of the developed meat analogues is mycoprotein, a protein-rich food source derived from the fermentation of filamentous fungi which is considered to be sustainable as its production may involve flexible substrate use such as waste or side products (e.g., soy whey, okara, sugarcane straw, and cassava peels) (Adetuwo & Naomi, 2025; Ahmad et al., 2022; Partha et al., 2024).

Soy whey, a recalcitrant wastewater generated from tofu production has seen potential for being utilized as a substrate for mycoprotein production using a non-pathogenic filamentous fungus *Aspergillus oryzae*. Results of a previous research details 53% (w/w) protein content per dry mycoprotein was capable of being reached using soy whey as a sole substrate and further supplementation of the soy whey media further enables the fungus to produce mycoprotein with 55.93% (w/w) protein content and almost triple the yield of unsupplemented soy whey from 234 to 637.68 mg/g DW substrate, highlighting the potential of soy whey supplementation to enable its development as an industrially viable fermentation medium (Partha et al., 2024; Pratama et al., 2025). However, the supplementation involves the addition of 0.8% (w/v) of ammonium sulfate, which in comparison to the average 0.6% (w/v) nitrogen content of soy whey, may prove to be a significant surplus to the nitrogen content of the supplemented soy whey media, thus possibly producing mycoprotein with a yield lower than its potential optimum yield (Chua & Liu, 2019; Li et al., 2021). Also, the lack of nitrogen concentration variety applied within the research may suggest

further identification of the optimum soy whey nitrogen supplementation to be necessary (Pratama et al., 2025). Additionally, no research has explored the use of carbon supplementation to increase the low 1% carbohydrate content of soy whey. The supplementation of both carbon and nitrogen proportions (C/N ratio) may also affect the nutrient composition of the resulting mycoprotein, potentially increasing the protein content per biomass with the optimal composition (Dutta & Das, 2017; Peteghem et al., 2022). As such, the potential of a further increase in production capacity in its yield and productivity while maintaining the protein-rich characteristic of the mycoprotein produced by *Aspergillus oryzae* within soy whey by optimizing the carbon and nitrogen content may still be undiscovered.

Various nutrient sources for media supplementation may also affect the outcome of fermentation capacity. Research findings suggest different forms of nutrient were uptaken differently for different microorganisms. Several studies involving different bacteria, yeast, and fungal strains determined various nutrient sources, particularly nitrogen, resulted in different rate of growths, macromolecule profile, and metabolite production (Arasaratnam et al., 1996; Eras-Muñoz et al., 2024; He et al., 2017). Similarly, the fermentation capability of *Aspergillus oryzae* may also depend on the compatibility of its metabolism with the substrate which highlights the importance of investigating different forms of nitrogen sources to supplement soy whey for promoting optimal growth of *Aspergillus oryzae* within the medium to produce mycoprotein with high quality nutrients and production capacities (Singh et al., 2017).

1.2 Objective

This research aims to determine the optimum carbon and nitrogen supplementation in different concentrations and varying nitrogen source types within a soy whey-based media to cultivate *Aspergillus oryzae* for mycoprotein production in aspects of production capacity, specifically yield and titer, and biomass protein content

1.3 Hypothesis

This research hypothesized several outcomes from this experiment which are as follows:

H0: Supplementation of soy whey with additional carbon and nitrogen sources does not significantly affect the productivity or proximate content of the mycoprotein produced by *Aspergillus oryzae*.

H1: Supplementation of soy whey with additional carbon and nitrogen sources significantly affects the productivity or proximate content of mycoprotein produced by *Aspergillus oryzae* both from concentration variation and types of nitrogen used.

H2: Supplementation of soy whey with varying carbon and nitrogen concentrations significantly affects the productivity and proximate content of the mycoprotein produced by *Aspergillus oryzae*, while the variation of nitrogen source does not.