Chapter 1

Introduction

1.1 Background

Soy (*Glycine max* L. Merr.) has become one of the staple foods in Asia, especially in Indonesia. This legume is widely consumed due to its characteristic of being an inexpensive, high-protein source (Hendrawati et al., 2021). Annually, Indonesians consume around 3.2 million tons of soy, where 90% of it are converted into tempeh and tofu, and are expected to grow more in the upcoming years (Arwanto et al., 2022; Harsono et al., 2022; Sari et al., 2021). According to Mulyasari et al. (2022), there are around 15,000 factories that specifically produce tofu. With this high number of factories, a large quantity of soy pulp/okara and soy whey is generated. These wastes are still high in nutrients, making them possible for valorization. There are many valorization efforts for okara, shown in some studies (Aussanasuwannakul et al., 2023; Scarcella et al., 2024). However, the valorization of soy whey is not as widely done as okara due to its high-water content and lower nutrient content compared to okara, leading to the direct disposal of soy whey to the water stream. Even though the direct disposal seems simple, it harms the environment since the nutrient contents in the soy whey can cause oxygen depletion for aquatic life (Chua & Liu, 2019). Therefore, processing the soy whey after the tofu production is needed.

Liquid wastewater treatment is one of the solutions for processing the soy whey to make it less harmful for disposal. However, the cost for that is high, leading to a cost burden in the tofu production. Besides that, even though soy whey is considered a lack-of-nutrient compared to okara, it still contains soy isoflavones, a major bioactive compound of soy with various health benefits (Chua & Liu, 2019). Hence, valorization of soy whey is a better option for further processing after the tofu production. Fermentation of soy whey can be an alternative to the valorization since this process increases the beneficial properties of soy whey via secondary metabolites, with various benefits, such

as antimicrobial and antifungal properties. The fermentation of soy whey requires a strong cell factory and this can be found in *Aspergillus oryzae (A. oryzae)*. *A. oryzae* is a fungus that is widely used in the food industry due to its versatility, Generally Recognized as Safe (GRAS) characteristic, and is extensively used in the traditional soy fermentation process in East Asia (Sun et al., 2024). This fungus can secrete secondary metabolites that inhibit the growth of pathogens such as *Escherichia coli (E. coli)* and *Candida albicans (C. albicans)*, leading to the prolonged shelf life, which is beneficial in the food industry (Tu et al., 2025). Additionally, these secondary metabolites with antimicrobial and antifungal properties can modulate the gut microbiome and inhibit pathogenic microorganisms through selective inhibitory mechanisms (Dudek-Wicher et al., 2018).

Nevertheless, acknowledging the low concentration of nutrients inside the soy whey, the robust growth and productivity of *A. oryzae* might not be achievable. Hence, supplementation is needed to balance the nutrient profile of the soy whey, one of which is glucose supplementation. This is done to increase the carbon-to-nitrogen (C/N) ratio, which is important for biomass and product formation in fungi (Chen, Wan, et al., 2024). As mentioned previously, *A. oryzae* can produce secondary metabolites with antimicrobial and antifungal properties, as shown in the previous studies (Alshannaq et al., 2025; Rachma & Ulhaq, 2026; Son et al., 2018). These secondary metabolites have a complex mechanism in regulating their biosynthesis pathways. Jia et al. (2024) and Vorapreeda et al. (2021) mentioned that the carbon source can affect the *A. oryzae*'s metabolic pathways, where different carbon sources will upregulate different gene clusters, leading to different metabolites. Besides that, the concentration of the carbon source can also become another factor to manipulate the metabolic pathway of the cell factory (Wu et al., 2020). Hence, based on these premises, it is hypothesized that modifying the soy whey nutrient profile through glucose addition in different concentrations can influence the *A. oryzae* metabolic pathway to produce secondary metabolites with antimicrobial properties, due to the activation and upregulation of certain gene clusters

responsible for these secondary metabolites. Nevertheless, there are few to no published studies that investigate the hypothesis stated above.

1.2 Objective

This study aimed to explore the antimicrobial and antifungal properties of fermented soy whey at varying glucose concentrations, 0%, 1%, 3%, and 5% (w/v), and were evaluated in both crude and freeze-dried forms. The antimicrobial properties of the fermented soy whey were observed towards *Escherichia coli (E. coli), Staphylococcus aureus (S. aureus),* and *Salmonella typhimurium (S. typhimurium)*. The antifungal properties were observed towards *Candida albicans (C. albicans)*. Besides the antimicrobial and antifungal properties of the fermented soy whey, the pH level and biomass concentration after the fermentation were also analyzed.

1.3 Hypothesis

H_{0a} (Null Hypothesis): There will be no antimicrobial and antifungal properties of crude and freeze-dried fermented soy whey in different glucose concentrations.

H_{1a} (Alternative hypothesis): There will be antimicrobial and antifungal properties of crude and freeze-dried fermented soy whey in different glucose concentrations.

H_{0b} (Null Hypothesis): There will be no significant difference in the biomass concentration of fermented soy whey in different glucose concentrations.

H_{1b} (Alternative hypothesis): There will be a significant difference in the biomass concentration of fermented soy whey in different glucose concentrations.

H_{0c} (Null Hypothesis): There will be no significant difference in the pH level of fermented soy whey in different glucose concentrations.

H_{1c} (Alternative hypothesis): There will be a significant difference in the pH level of fermented soy whey in different glucose concentrations.