CHAPTER I

INTRODUCTION

1.1 Background

The Indonesian archipelago is one of the largest fish producers in the world, which produces various fisheries from marine, freshwater, and brackish. In 2019, the Ministry of Marine and Fisheries of Indonesia reported a total national production of 15.5 million tons of fisheries, valued over 392 trillion Indonesian Rupiah, with tuna fisheries having one of the pivotal roles; contributing to approximately 16% of the world's tuna production (Khan *et al.,* 2024). However, a significant portion of the fish; such as the muscle, skin and fins, bones, heads, viscera, and scales, are oftentimes wasted. This waste accounts for 20 – 80% of the fish, posing both environmental and economic challenges (Coppola *et al.,* 2021).

The large volume of tuna production in Kota Bitung, North Sulawesi, Indonesia can lead to significant waste if not properly handled. Due to its high protein content, this waste holds potential for transformation into valuable products like fish meal or protein concentrates (De Asis *et al.,* 2023). However, if left unmanaged, tuna waste can rapidly deteriorate and produce a strong fishy smell caused by volatile nitrogen compounds. In order to prevent this, fermentation is a viable solution. Fermentation, typically involving proteolytic microorganisms, offers an eco-friendly and cost-effective way to make use of the waste (Nugroho *et al.,* 2020). Trimethylamine N-oxide (TMAO) is a compound in fish that can be further reduced into trimethylamine (TMA), which is responsible for the fishy odour in marine products. Despite that, *Saccharomyces cerevisiae* is one of many

microorganisms that can reoxidize TMA into TMAO through microbial activities, which in return can significantly reduce the odour (Park *et al.,* 2019). Previous studies by Tropea *et al.* (2021) found that fermenting fish waste with *S. cerevisiae, L. reuteri*, and lemon peel significantly increased the protein content, suggesting enhanced microbial activities and improved quality after fermentation. *Bacillus subtilis* is often used in fermentation due to its ability to produce good thermostable protease, which degrades protein and utilize the amino acids for its survival (Ramkumar *et al.,* 2016). The synergy between microbes during fermentation process was found to improve the quality of protein through the complex process of microbial activities and biochemical reactions (Zhu *et al.,* 2023).

Fermentation is usually carried out with substrate enhancers to provide nutrients for the microbes, with molasses as one of the most common substrates since it is easily found and rich in nutrients (Mangwanda *et al.,* 2023). Though Kota Bitung is not known for producing sugar, the surrounding areas are abundant in pineapple, producing 169,681 tons of pineapple in 2023 (BPS, 2023). Pineapple contains bromelain, a protease which can aid in breaking down the protein during the fermentation. Therefore, the utilization of pineapple waste as an alternative to molasses presents a sustainable solution.

The addition of pineapple and molasses during the fermentation not only acts as substrate enhancers, but might also as a prebiotic source that promotes beneficial microbes' growth while inhibiting undesirable microbes, resulting in improved fermentation characteristics and sensory qualities (Giyatmi and Irianto, 2017; Tropea *et al.,* 2021). Other research of fermentation using pineapple as substrate by Jiménez Alfaro *et al.* (2020) found that it significantly increased the

crude protein concentration by providing substantial amount of reducing sugars and essential nutrients, which facilitate microbial adaptation. The presence of bromelain, a proteolytic enzyme in pineapple, further assists in protein breakdown, hence improving the substrate availability and fermentation efficiency.

In this research, tuna waste is fermented with a combination of *S. cerevisiae* and *B. subtilis* along with bromelain and molasses as substrate enhancers to increase their protein extraction level and preserve the quality of extracted protein. The quality of fermented tuna waste will be analysed for its total volatile base nitrogen (TVBN) and trimethylamine (TMA) contents, which are chemicals responsible for indicating spoilage, along with protein content to assess the proteolytic activity of microbes and enzymes.

1.2 Research Problem

The tuna fishery industry in Kota Bitung, North Sulawesi, Indonesia produces a lot of waste during the processing since not all parts of the fish can be sold. Previous studies have found that the fermentation of tuna waste with *S. cerevisiae, L. reuteri*, and lemon peel could increase the protein content through microbial proteolytic activities. During fermentation, proteolytic activities from microbes and enzymes break down proteins into peptides and free amino acids, increasing measured protein in the sample. However, prolonged fermentation process of tuna waste might cause the deterioration of fish quality, indicated by the increased levels of total volatile base nitrogen (TVBN) and trimethylamine (TMA) in the sample. The yeast *S. cerevisiae* is able to reoxidize TMA into TMAO, with *B. subtilis* being a strong protease secretor, the synergize between the two may

produce better results rather than single-strain fermentation. Therefore the effect of different microbes were analyzed in this research.

In this research pineapple waste will be added instead of molasses. Unused pineapple waste contain reducing sugar such as fructose that might aid the fermentation process as readily available energy source. The quality of the extracted protein from fermented fish in this research would be done through analyzing the protein content alongside spoilage indicators (TVBN and TMA values) after being fermented with different microbes *(S. cerevisiae, B. subtilis,* or combination of both) and substrate enhancers (pineapple or molasses) for a set period of time (0, 1, 3, or 5 days).

1.3 Objectives

1.3.1 General Objective

The general objective of this research was to evaluate the potency of fermentation using *Saccharomyces cerevisiae* and *Bacillus subtilis*, and addition of molasses and pineapple as substrate enhancers in fermenting tuna waste over period of time to achieve the highest protein content outcome while assessing its deterioration quality to ensure it safety for further processing of protein extraction .

1.3.2 Specific Objectives

The specific objectives of this research are:

1. Determine the effect of microbes (*Saccharomyces cerevisiae* or *Bacillus subtilis* or combination of both), substrate enhancers (pineapple part or molasses), fermentation period (0, 1, 3, or 5 days) in the fermentation of tuna waste towards the total volatile basic nitrogen (TVBN) content.

2. Determine the effect of microbes (*Saccharomyces cerevisiae* or *Bacillus subtilis* or combination of both), substrate enhancers (pineapple part or molasses), fermentation period (0, 1, 3, or 5 days) in the fermentation of tuna waste towards the trimethylamine (TMA) content.
3. Determine the effect of microbes (*Saccharomyces cerevisiae* or *Bacillus subtilis* or combination of both), substrate enhancers (pineapple part or molasses), fermentation period (0, 1, 3, or 5 days) in the fermentation of tuna waste towards the protein content.
4. Determine the best treatment of the combination between microbes (*Saccharomyces cerevisiae* or *Bacillus subtilis* or combination of both), substrate enhancers (pineapple part or molasses), fermentation period (0, 1, 3, or 5 days) in the fermentation of tuna waste towards the protein, total volatile basic nitrogen (TVBN), and trimethylamine (TMA) content.