Chapter 1

Introduction

1.1 Background

Poultry is one of the most consumed types of meat around the world. According to a 2024 article published by the United State's Department of Agriculture, poultry is ranked as the second most consumed type of meat, accounting up to 33% of all the meat consumed in the world. It is just slightly behind pork, which sits at 36%. When processing chicken meat, chicken feathers are considered to be industrial waste and a source of disease. It accounts for several million tons of highly accumulating waste worldwide, creating a severe solid-waste problem (Mazotto et al., 2022). The most common methods of disposal are burning, dumping into a landfill, or burying. However, some of these methods are not always environmentally friendly and hygienic. Burning requires additional energy to dispose of the feathers and emits large quantities of carbon dioxide, while dumping and burying may result in the spread of pathogens due to the rich microflora present in the feathers (Sinkiewicz et al., 2017).

Though it is considered to be waste, chicken feathers actually contain 90% protein in the form of keratin (Almahasheer et al., 2022). Keratin is a type of protein found on epithelial cells, which line the inside and outside surfaces of the body. Keratins also constitute the tissues of the hair, nails, claws, turtle scutes, horns, whale baleen, beaks, and feathers of various animals (Wang et al., 2016). It is extensively used for various products such as animal feed, biofilm, fertilizer, and wood adhesive (Suharti 2023). It is also heavily used in healthcare products such as lotions, shampoos, hair conditioners, and biomedical items (Maurya & Singh, 2024).

Due to the current disposal methods and the potential of the keratin contained in chicken feathers, there is ongoing research on different methods that can be used to extract keratin. Several of these

are chemical hydrolysis, dissolution in ionic liquids, microwave technique, steam explosion technique, and thermal hydrolysis (Chilakamarry et al., 2021). However, these methods have several drawbacks. For example, chemical hydrolysis can destroy the native structure of keratin like in the case of acidic hydrolysis. Some chemicals like 2-mercaptoethanol are also toxic (Sinkiewicz et al., 2017). Ionic liquid hydrolysis is also a viable option due to its effectiveness, but expensive (Belhajja et al., 2024). Meanwhile, according to Zoccola et al. (2012), microwave heating techniques can result in a significant cysteine loss which increases up to 99% when using high temperatures up to 180 °C. The steam explosion technique is also reported by Shavandi et al. (2017) to destroy cysteine and reduce the quality of the final product.

Due to several of the aforementioned challenges when dealing with extraction, a more environmentally friendly and greener solution is needed. A possible solution to this is using microbes to extract keratin from the chicken feathers. Several species of bacteria, actinomyces, and filamentous fungi are able to break down keratin. However, the most commonly found keratinolytic microorganisms are several species of Bacillus bacteria (Tamreihao et al., 2019). Different bacterial strains of the genus Bacillus are known to produce keratinase, which are a class of proteolytic enzymes that can digest insoluble keratin in several forms such as feathers, nail, hair, and wool. They are also characterized by its high substrate specificity to keratin (Jaouadi et al., 2015). Well known keratinolytic Bacillus species include *Bacillus licheniformis, B. megaterium, B. subtilis, B. cereus*, and *B. pumilus* (Almahasheer et al., 2022). A common method of using the bacteria to digest the keratin from chicken feathers is by submerged fermentation (Sanghvi et al., 2019).

1.2 Objective

This research has two objectives. The first one is to screen for the best strain of Bacillus bacteria in terms of growth curve and keratinolytic activity. After choosing one strain, the second objective is to

optimize the fermentation process using the chosen strain by adding different increments of salt and sulfur sources in hopes of increasing protein yield and quality.

1.3 Hypothesis

1.3.1 Objective one: Screening for the best strain of Bacillus

H0: There is no significant difference in keratinolytic activity among the five Bacillus strains

H1: At least one Bacillus strain exhibits significantly higher keratinolytic activity compared to the others

1.3.2 Objective two: Optimization of the fermentation process using the chosen strain

H0: Increasing the concentration of salt and sulfur does not affect the keratinolytic activity of the chosen strain.

H1: Increasing the concentration of salt and sulfur affects the keratinolytic activity of the chosen strain.