Chapter 1

Introduction

1.1 Background

The poultry industry generates millions of tons of chicken feather waste annually, creating significant waste management and environmental challenges. Feathers, which consist of up to 90% keratin, are often discarded in landfills or incinerated, contributing to pollution and resource wastage (Chilakamarry et al., 2021). Despite its potential applications in tissue engineering, protein-based fertilizers, biodegradable plastics, and personal care products, keratin is difficult to extract due to its highly stable fibrous structure and strong disulfide bonds (Feroz et al., 2020). Traditional keratin extraction methods rely on harsh chemical treatments involving oxidative agents, acids, and alkalis. While effective in breaking down keratin, these methods degrade protein quality, generate hazardous waste, and consume large amounts of energy (Feroz et al., 2020). Given the increasing demand for sustainable and eco-friendly bioprocesses, there is a pressing need to develop a greener approach that maintains keratin integrity while minimizing environmental harm.

Microbial keratin degradation presents a promising alternative, leveraging keratinolytic bacteria that produce keratinase enzymes to hydrolyze keratin into usable proteins and peptides under controlled conditions (Qiu et al., 2020). Microorganisms' ability to biodegrade keratin is highly impacted by the fermentation medium's composition, specifically the type and concentration of carbon and nitrogen sources present. In addition to promoting microbial development, these nutrients control the production of keratinolytic enzymes (Sharma & Gupta, 2016; Syed et al., 2020). However, optimizing this process requires further exploration, particularly regarding the role of carbon and nitrogen sources in enhancing bacterial keratin degradation efficiency. *Bacillus* species are frequently utilized

among keratin-degrading microorganisms because of their strong keratinase activity and capacity for utilizing keratinous substrates in a variety of conditions. However, the nutritional environment has a significant impact on their efficiency, emphasizing the necessity of growth medium modification (Paul et al., 2022). This study aims to develop an optimized microbial keratin extraction method using fermentation, with a focus on sucrose and peptone as carbon and nitrogen sources. The findings of this research could contribute to environmentally sustainable waste management and promote the industrial, biomedical, and environmental applications of bio-based keratin.

1.2 Objective

This study aims to evaluate the keratin degradation capability of *Bacillus subtilis* on chicken feathers, with enhancement through the addition of varying sucrose concentrations, both alone and in combination with peptone, by assessing feather degradation and protein yield. To achieve this, bacterial growth, keratin degradation rate, and total protein concentration will be analyzed under varying fermentation conditions. Since conventional chemical methods often compromise the structural integrity of keratin, it is essential to assess the quality of microbially extracted keratin. Techniques such as SDS-PAGE and FTIR can help determine whether the native protein conformation is preserved (Abarca-Vargas et al., 2022). Converting feather waste into valuable keratin is in line with sustainable resource management and the circular bioeconomy. According to Agrahari et al. (2023), these methods not only lessen the load on landfills but also encourage the creation of renewable biomaterials. thereby contributing to sustainable waste management and resource utilization.

The study will involve bacterial fermentation using the *Bacillus subtilis* with highest keratinase degradation with different concentrations of carbon (sucrose) and with an addition of nitrogen (peptone), with a total of 6 variations including nutrient control which is only the nutrient broth. The extracted keratin will then undergo purification to ensure its quality and usability. The yield will be evaluated through total protein concentration assays and degradation rate analysis, while protein

characterization will be conducted using SDS-PAGE, FTIR, and other relevant techniques to determine the structural integrity of the extracted keratin.

1.3 Hypothesis

H0: Increasing carbon (sucrose) or nitrogen (peptone) will not enhance bacterial growth and keratin degradation in normal and nitrogen-rich (peptone) conditions.

H1: Increasing the concentration of carbon (sucrose) will enhance bacterial growth and keratin degradation, leading to higher keratin yield.

H2: Increasing the concentration of carbon (sucrose) in a nitrogen-rich environment (peptone) will enhance bacterial growth and keratin degradation, leading to higher keratin yield.