CHAPTER I

INTRODUCTION

1.1 Background

Shrimp processing industries are one of the biggest agro-industries in tropical and subtropical regions, thus causing a drastic increase in shrimp waste production. An example of an agricultural country is Indonesia, with *Penaeus monodon* shrimp as one of its major agroindustry commodity (Insan, *et al.*, 2015). Shrimp waste, more specifically peeled and disposed shrimp shells, contains many bioactive compounds such as chitin, pigments, amino acids, and fatty acids which have a wide range of application in medical and therapeutic fields, in cosmetics, in textile industries and many others (Kandra, *et al.*, 2011).

Chitin which can be commonly found in crustacean shells, is a non-toxic, biodegradable polysaccharide. Its derivatives, chitosan and glucosamine, present many benefits in various areas such as agriculture, water treatment, cosmetics, textile industries, paper technology, food and beverage, and pharmaceutics. Glucosamine is an amino sugar that can also be found naturally in the human body, and is a precursor for the biochemical synthesis of glycosaminoglycans found in cartilage, so it is extensively used in dietary supplements for the treatment of osteoarthritis, knee pain and back pain (Benavente, *et al.*, 2015). Chitin and its derivative, chitosan, can be applied in the food industry as edible and/or biodegradable films, and as bactericides and fungicides as they possess anti-microbial properties (Shahidi, *et al*., 1999).

Glucosamine is commonly present in 3 forms based on its preparation, which are glucosamine hydrochloride (G-HCl), glucosamine sulphate and N-acetylglucosamine (NAG). G-HCl and glucosamine sulphate have already been commercialized as food supplements. NAG has the potential to become an inexpensive food additive and functional material with a sweet taste, however it has not yet been approved as a natural material to be used as a food additive in some countries (Chen, *et al.*, 2010 and Kim, 2013[b]). NAG is more commonly used in the pharmaceutical field, especially for the treatment of osteoarthritis as it is a component of proteoglycans found in the cartilage.

The chemical production of G-HCL has many disadvantages as it is not environment friendly due to acidic wastes resulted, and the yield is low and hard to control (Sashiwa, *et al.*, 2002). Therefore, an alternative method is needed to overcome these disadvantages. The enzyme chitinase is found to be able to degrade chitin, however the enzymatic hydrolysis of chitin directly from crab or shrimp shell is not very effective, and the high cost and instability of enzymes also pose a challenge (Sashiwa, *et al.*, 2002). Thus, fermentation-based production of glucosamine by filamentous fungi and bacteria have gained increasing interest as it has been found to be environmentally friendly and requires relatively lower costs (Liu, *et al.*, 2013). Glucosamine produced by microbial fermentation of chitin is in the form of N-acetylglucosamine (NAG).

Trichoderma *virens* is one example of filamentous fungi which contains the chitinase enzyme (Vinale, *et al.*, 2008). Saskiawan and Handayani (2011) states that fungi can produce chitinase which possess endochitinase and exochitinase activities, while bacteria are able to produce endochitinase enzymes. Fungi is also claimed by Rachmawaty and Madihah (2013) to adapt better on solid substrates and able to penetrate the complex substrates to produce the extracellular enzymes. Several studies have researched the production of chitinase enzyme and its activity from *Trichoderma* species, however the study of optimum N-acetylglucosamine production by solid-state fermentation with *Trichoderma virens* has not yet been directly discussed.

1.2 Research Problem

Glucosamine, which is beneficial in the pharmaceutical field, can be produced through many methods such as chemical treatment, enzymatic hydrolysis or through fermentation-based production from shrimp wastes. Chemical hydrolysis results in acidic residues which poses an issue to the environment, low and obstreperous yields, while hydrolysis by enzymes require high costs and are relatively ineffective. Therefore, fermentation-based production by microbes, especially filamentous fungi, is a convenient alternative. *Trichoderma virens* is one species of fungi which can produce chitinase enzymes, and many researches conducted by Agrawal and Kotasthane (2012), Rachmawaty and Madidahh (2013), and Karthik, *et al.* (2014) have studied the yield and activity of the chitinase enzymes produced from *T. virens.* However, the study on fermentation-based production of glucosamine by *T. virens* and the optimization of influencing factors towards it have not yet been thoroughly studied.

1.3 Objectives

The objectives of this research can be divided into two, consisting of general objective and specific objectives.

1.3.1 General Objective

The general objective of this research was to produce N-acetylglucosamine by fermenting the chitin from shrimp shells using *Trichoderma virens*.

1.3.2 Specific Objectives

The specific objectives of this research were:

1. To determine the optimum fermentation temperature for N-acetylglucosamine production from fermentation of chitin powder.
2. To determine the optimum pH of media for N-acetylglucosamine production by fermentation of chitin powder.
3. To determine the optimum fermentation period for N-acetylglucosamine production from fermentation of chitin powder.