CHAPTER 1: INTRODUCTION

1.1 Background

Soy sauce is a fermented liquid condiment that is popular because it has a unique taste and aroma. Soy sauce originated from China and is regularly consumed in East and Southeast Asian cuisines (Gao et al., 2010; Diez-Simon, Eichelsheim, Mumm & Hall, 2020). Soy sauce is made of fermented soybeans with mold, wheat flour, brine water, bacteria, and yeast. There are various types of soy sauce however, the general method of fermentation is the same where it needs to undergo a two-step fermentation called *koji* and *moromi*.

The process of soy sauce production begins with the *koji* fermentation and proceeds to the *moromi* fermentation and finally the refining and pasteurization of *moromi*. *Koji* fermentation is a solid-state fermentation of soybeans which relies on mold such as *Aspergillus oryzae* as the starter culture. The mold is responsible for producing hydrolytic enzymes to break down the protein and carbohydrate into amino acids and simple sugars, respectively (Liu et al., 2015). Generally, *koji* fermentation is finished after 72 hours of incubation which is characterized by the growth of the mycelium of mold that has fully covered the soybeans surface which indicates that the hydrolytic enzymes produced have been exhibited fully.

Meanwhile, *moromi* fermentation is a submerged fermentation of the mixture of mashed *koji* and brine solution which relies on a mixed culture of microorganisms such as bacteria and yeast. There are two stages within the *moromi* fermentation including the initial and later stages. During the initial stage, the bacteria start to grow and produce metabolites such as organic acids which will acidify the environment until the pH reaches below 5. Then at the later stage, the yeast starts to grow and produces most of the volatile compounds in the soy sauce. Typically, the duration of *moromi* fermentation varies between 3 months up to 4 years before being processed into soy sauce (Hoang et al., 2016). During these fermentation periods, the characteristics of soy sauce are being developed through the accumulation of the metabolites produced by the microorganisms in the *moromi*. The long fermentation period is caused by several factors such as high salt content in the brine ranging from 15-20%, fluctuating fermentation temperature, and antagonistic relationship between microorganisms (Kim & Lee, 2007; Hoang et al., 2016; Yang et al., 2017; Devanthi & Gkatzionis, 2019)

The long period of *moromi* fermentation can cause problems to both the consumers and the manufacturers due to the waiting time for the fermentation process. This problem has a severe impact on the supply chain of the soy sauce industry which causes loss to the industry due to the incapability to meet the market demand (Citraresmi & Rahmawati, 2020). Therefore, accelerating the *moromi* fermentation is needed in the soy sauce industry.

Several studies have been done to accelerate the soy sauce fermentation using different methods, such as salt content reduction, optimum temperature application, *koji* autolysis, and sonication (Muramatsu et al., 1993; Su et al., 2005; Hoang et al., 2016; Xu et al., 2016; Goh et al., 2017). All of these attempts were aimed to accelerate the growth of microorganisms and enhance the enzymatic reactions, which can further accelerate the fermentation process. By reducing the salt content and maintaining optimum temperature for fermentation, a study by Muramatsu et al. (1993), has shown to increase the amount of metabolites such as organic acids and total nitrogen within 8 days. By performing *koji* autolysis which is the cell wall destruction of mold could cause the content in the cytoplasm to burst out so the hydrolytic enzymes will be readily available to the environment. *Koji* autolysis has been reported by Xu et al. (2016), to cause an increase of sugar and free amino acids after 5 days of fermentation. Meanwhile, performing *moromi* sonication, which is the utilization of sound energy to agitate the particles in the *moromi* will reduce bacterial cell death due to cell clumping, has been reported by Goh et al., (2017) to cause a significant increase in the free amino acid content after 2 days of fermentation.

Another attempt is by deliberately adding starter culture into *moromi*, which is typically fermented spontaneously. An example is *Tetragenococcus halophilus* which is the most common LAB used as starter culture in the *moromi* fermentation (Kobayashi et al., 2010; Jayus et al., 2020). It can increase the production of lactic acid and acetic acid resulting in faster *moromi* acidification (Jayus et al., 2020). There is another potential bacteria that can be used for *moromi* starter culture which is *Bacillus* spp. because of the ability to produce relevant enzymes that break down the substrates in the soybean (Yang et al., 2017). *Bacillus subtilis* in particular has been found to produce a high amount of proteases and amylases which may improve the nutrient availability during the *moromi* fermentation which ultimately will increase the microorganisms activity (Jiang et al., 2019). *B. subtilis* was also reported to produce several important volatile compounds in the soy-based fermentation product (Visessanguan et al., 2005; Lee et al., 2017). However, the usage of *B. subtilis* as *moromi* starter culture was still not common yet. Therefore *B. subtilis* addition to the *moromi* fermentation might accelerate the physicochemical characteristics formation in the soy sauce.

1.2 Objective

The general objective of this study was to investigate the effects of *B. subtilis* inoculation onthe acceleration of soy sauce fermentation. In addition, specific objectives were set:

a) To evaluate the quality of soy sauce in the form of pH

b) To evaluate the quality of soy sauce in the form of total titratable acid (TTA)

c) To evaluate the quality of soy sauce in the form of total reducing sugar (TRS) d) To evaluate the quality of soy sauce in the form of free amino nitrogen (FAN)