CHAPTER 1

INTRODUCTION

1.1 Project Background

Soy sauce is a traditional fermented condiment that originated from China in the 3rd century and is widely consumed in Asian countries (Gao et al., 2010). Despite the variety of soy sauce and fermentation process from each country, this light brown to dark colour condiment consists of three main ingredients: soybeans, wheat, and brine solution, and also known to have an intense umami taste. Based on the proportion of materials used, soy sauce is mainly divided into two types, namely Chinese and Japanese soy sauce. Chinese soy sauce typically uses a high amount of soybeans and less wheat, while an equal amount of soybeans and wheat is used in the production of Japanese soy sauce. Opposed to Chinese soy sauce, which generally consisted of one type, Japanese soy sauce is classified into five different types, i.e. *koikuchi shoyu, usukuchi shoyu, saishikomi shoyu, tamari shoyu,* and *shiro shoyu*.

To produce Japanese soy sauce with its well-known distinct flavour and aroma, there are two main steps of the fermentation process. The first step involves koji fermentation by adding koji mould *Aspergillus oryzae* into a mixture of cooked soybeans and wheat flour. Following the koji fermentation is the second step, which is moromi fermentation. This step involves a process of mixing the koji with a brine solution containing 18-22% of NaCl (Devanthi, 2018). Salt plays an essential role in the production of soy sauce, as it inhibits the growth of undesirable bacteria and promotes the growth of desirable halotolerant microorganisms (Song, Jeong, & Baik, 2015).

However, this high salt concentration (18-22%) in soy sauce exceeds the level of recommendation from WHO (World Health Organization. According to WHO (2012), the recommended sodium intake is 2 grams, which is equivalent to 5 grams of salt, and yet the daily consumption of soy sauce in Indonesia is

10-15 ml per capita (Sasaki & Nunomura, 2003), which contributed to around 38% of sodium, according to the Recommended Daily Intake (RDI). With that amount of salt included in the daily consumption of this condiment, it could lead to hypertension and cardiovascular disease caused by excessive intake of NaCl (Singracha et al., 2017). Thus, the option for low-salt soy sauce needs to be developed.

Reducing salt in the production of low-salt soy sauce might give a plus side on the health benefits. Several studies regarding the reduction of salt in soy sauce were done by various approaches such as the production of low-salt soy sauce using amino acid-based saltiness enhancer (Segawa et al., 2014), and indigenous yeasts found in Korean soy sauce (Song, Jeong, & Baik, 2015), and so on. Both of these findings are focusing on reducing salt content and its impact on quality, however, the study on the effect of reducing salt towards the microbial safety needed to be done due to the potential survival of foodborne pathogens in the production of low-salt soy sauce. One study conducted by Song et al. (2015) showed the presence of putrefactive *Staphylococcus* and Enterococcus spp. during low-salt conditions. Another study exhibited the survival of *Staphylococcus aureus* and *Escherichia coli* O157:H7 even in high salt concentration (15.6%) and low pH (pH 4.6), in raw ready-to-eat crab marinated in soy sauce (Cho et al., 2016). These alarming findings confirmed the high potential of both bacteria contaminating in low-salt soy sauce production.

Staphylococcus aureus, a gram-positive bacteria, is one of the most dangerous foodborne pathogens. *S. aureus* has also been known to be able to tolerate high-salt concentration and has the ability to resist heat (Harris et al., 2002). Tamarapu et al. (2001) noted that 25% of all food-related disease in the US was caused by *S. aureus*. Another dangerous foodborne pathogen is Enterohemorrhagic *Escherichia coli* (EHEC) or known as *E. coli* O157: H7, which is a gram-negative enteropathogenic strain of *E. coli* that has been known as a foodborne human pathogen since 1982. This facultative anaerobe bacteria has the ability to produce Shiga-like toxins and cause haemorrhagic colitis (Wallace et al., 2016). This indicates that a study regarding the development of low-salt soy sauce with several treatments to prevent the growth of pathogens such as *S. aureus* and *EHEC* is needed.

1.2 Objective

The general objective of this research was:

To prevent the growth of two potential food pathogenic bacteria, namely *Staphylococcus aureus* and *Escherichia coli* O157:H7 during moromi fermentation in the production of low-salt soy moromi by applying various physical treatments

The objective of this study was achieved through:

a. Evaluating the effect of various salt concentration (6%, 12%, and 18%) on the growth of *S. aureus* and EHEC
b. Investigating the effect of temperature (ambient temperature and 30°C), pH (4.5, 5.5, and 6.5), and inoculum size (10^2, 10^5, 10^7 cells/mL) towards the survival of *S. aureus* and EHEC in low-salt conditions
c. Validating the most effective physical factors in real low-salt moromi fermentation