CHAPTER I

INTRODUCTION

1.1. Background of the Study

Over-fermented tempeh, or known as overripe tempeh, *tempe semangit*, or *tempe bosok,* is a term used for tempeh in which the natural tempeh mold growth began to decline and the solid-state fermentation is taken over by bacterial fermentation (Hassanein et al., 2015; Puteri et al., 2015). This tempeh has pungent odor, grey-brown color, and softer texture than the normal tempeh (Hassanein et al., 2015; Djunaidi et al., 2017). It is commonly used in traditional Javanese cuisines that use chili and/or coconut milk as basic flavor, such as *gudeg*, and may also be used as a food ingredient such as *oseng-oseng tempe bosok* (Puteri et al., 2015). Nowadays, researchers have been studying over-fermented tempeh as seasoning powder due to its high glutamic acid level which contributes to umami flavor (Puteri et al., 2015; Setiadharma et al., 2015). Thus, the application of over-fermented tempeh as cooking ingredients and seasoning has widened. In regards of the indigenous process, which involves less technological input, no control measure is applied during this over-fermentation process. This usually associates with inconsistency of the product quality (Sim et al., 2015). Therefore, a controlled over-fermentation process may be developed to obtain more consistent and desired product quality (Giraffa, 2004).

Essentially, tempeh fermentation occurred at two stages, during the soaking process and the mold fermentation process. During the soaking process, presence of lactic acid bacteria (LAB) and yeast were detected. LAB is present in high numbers during the soaking process to acidify the soybean under natural fermentation and plays a role in preventing pathogenic bacteria growth during tempeh production. Meanwhile, yeast grows together with LAB and their interaction can produce stimulatory or inhibitory effects on the pathogenic bacteria, depending on the combination of the species (Kustyawati, 2009; Nurdini et al., 2014). Without second boiling process before inoculation, the LAB developed during soaking may be carried over and continues to grow during mold fermentation and

contribute to the fermentation process (Nurdini et al., 2014). Mold fermentation process is essential for mycelium formation that binds the soybean cotyledons together, with *Rhizopus oligosporus* as the dominant species (Anggriawan, 2017; Pangastuti et al., 2019). Meanwhile, LAB is important to limit the natural pH increase during mold fermentation (Feng, 2006). Additionally, some LAB may grow together with *R. oligosporus* and may significantly inhibiting the growth of *R. oligosporus* after 24 hours over-fermentation process (Utami et al., 2016).

Generally, over-fermented tempeh is produced by letting fresh tempeh further fermented in room temperature for 2 – 5 days longer (Hassanein et al., 2015; Puteri et al., 2019). During 24 hours over-fermentation process, Nuraida et al. (2008) reported an increase in total viable bacteria (TVB) from 10^9 to 10^{10} CFU/g and an increase in total mold from 10^7 to 10^9 CFU/g. A follow up research by Nurdini et al. (2014) showed an increase in total mold 24 hours over-fermentation process, from 10^6 to 10^7 CFU/g. However, no changes in total lactic acid bacteria (TLAB) were observed in both studies after 24 hours over-fermentation process (Nuraida et al., 2008; Nurdini et al., 2014). At 72 hours over-fermentation process, TVB were observed at a range of 10^8 to 10^9 CFU/g, TLAB at 10^8 CFU/g, and total mold at a range of 10^7 to 10^9 CFU/g (Djunaidi et al., 2017). Study on microbial changes in over-fermented tempeh was limited to 24 and 72 hours over-fermentation process only. However, over-fermentation process may be held upto 2 (48 hours) to 5 (120 hours) days (Hassanein et al., 2015), thus the recent method does not describe the entire over-fermentation process. Therefore, the lack of information about microbial growth in the over-fermented tempeh would inhibit the development of optimal over-fermentation process (Giraffa, 2004).

Tempeh fermentation was found to be affected by several internal microbial growth factors, including pH, temperature, relative humidity, and water activity. Tempeh starter culture was found to grow optimally at pH 4 - 6.5 (Winanti et al., 2014). In normal condition, tempeh has pH varies from 4 - 6 (Tahir et al., 2018). However, most bacteria prefer the neutral pH of 7.0 (Isnawati & Trimulyono, 2017). Therefore, the acidic pH range will suppress bacterial contamination growth but support mold growth (Winanti et al., 2014). Tahir et al. (2018) mentioned the optimum incubation temperature for

tempeh fermentation is at a range of 37 – 38 ^{o}C, which can produce fresh tempeh after 22 hours fermentation. At lower temperature (25 ^{o}C), an acceptable tempeh may be produced with longer fermentation time (5 days) (Tahir et al., 2008). On the other hand, higher temperature (> 40 ^{o}C) may result in premature mold death (Nout & kiers, 2005). Optimally, tempeh fermentation is held at 75 – 78 % relative humidity (RH). At higher RH (> 75 %), tempeh will become too moist. Contradictary in lower RH (< 75 %), a black sporulation on the tempeh surface will be observed (Supriyono, 2003). Relative humidity need to be controlled to ensure optimum moisture content in the tempeh during fermentation process (McKinney et al., 2015). Meanwhile, water activity (a_w) is important during tempeh starter culture growth, in which the starter culture needs a_w as low as 0.8. Lower a_w in soaked soybean will result in hard-to-be-penetrated cotyledone by the mycelium. Meanwhile, higher a_w will inhibit oxygen dispersion, thus inhibiting mycelium growth (Winanti et al., 2014). Although internal microbial growth factors on tempeh fermentation had been studied, limited information regarding the internal microbial growth factors affecting tempeh over-fermentation is available. Changes in internal microbial growth factors could affect the growth, survival, and biochemical activity of microorganisms in foods (Giraffa, 2004). Therefore, studying the internal microbial growth factors changes could give more insight for the optimization of tempeh over-fermentation process.

1.2. Statement of the Problem

Based on the background of the study, several problems were formulated:

1. How are the changes of microbial and internal microbial growth factors during tempeh over-fermentation process (0, 24, 48, 72, 96, and 120 hours)?
2. How are the correlation between internal microbial growth factors changes of the fermentation towards microbial changes during tempeh over-fermentation process?

1.3. Objectives of the Study

Based on the research problems, the objectives of the study would be:

1. To evaluate the changes of microbial (Total Viable Bacteria, Total Lactic Acid Bacteria, and Total Yeast and Mold) and the internal microbial growth factors changes (internal temperature, pH, water activity, and moisture content) during tempeh over-fermentation process (0, 24, 48, 72, 96, and 120 hours).
2. To assess the correlation between internal microbial growth factors changes of the fermentation towards microbial changes during tempeh over-fermentation process.

1.4. Hypotheses

There are two hypotheses on this research, which were:

1. Null hypotheses (H_0)
    a. There is no significant difference in both microbial changes and internal microbial growth factors changes within tempeh over-fermentation process.
    b. There are no correlations between internal microbial growth factors changes of the fermentation towards microbial changes during tempeh over-fermentation process.
2. Alternative hypotheses (H_a)
    a. There is a significant difference in microbial changes and internal microbial growth factors changes within tempeh over-fermentation process.
    b. There are significant correlations between internal microbial growth factors changes of the fermentation towards microbial changes during tempeh over-fermentation process.

1.5. Significance of the Study

The findings of the study would provide great benefits to:

1. **The body of knowledge** through information regarding the changes of microbial and internal microbial growth factors throughout the tempeh over-fermentation process and the

information could be used as supplementary literature for further research on optimization of the over-fermentation process and microorganisms identification for starter culture development.

2. **The society** through information regarding the changes of microbial and internal microbial growth factors throughout the tempeh over-fermentation process that could be used to improve the quality of over-fermented tempeh consistently through starter culture development for controlled over-fermentation process.

1.6. Scope and Limitation of the Study

Tempeh samples used in this study were collected from two different tempeh industries, modern production condition and traditional production condition. Tempeh were over-fermented for 0 – 120 hours, with three biological replicates. The evaluation of microbial changes were limited to the estimation of total viable bacteria, total lactic acid bacteria, total yeast and mold. The writer did not do microbial identification due to budget and experiment time limitation. Additional measurement on internal temperature, pH, water activity, and moisture content were done as supporting data and to understand the correlations towards microbial dynamics.