CHAPTER I: INTRODUCTION

1.1 Background

In recent years, consumption on fermented dairy products, including fermented milk and cheese, projected an inclining trend due to the health benefits they offered (Garcia-Burgos et al., 2020). This trend then ignites growing development of alternative starter culture from lactic acid bacteria (LAB) to initiate the fermentation and give additional characteristics of fermented dairy products (Laranjo, Potes, & Elias, 2019). The fermented dairy products from the starter culture should fulfill certain basic requirements for commercial probiotic products: acidification, viable cell count, physico-chemical attributes, volatile compounds, and sensory analysis (Muelas et al., 2022). Starter culture with good probiotic properties is highly demanded to achieve fermented products with beneficial health effects to consumers. One of LAB, *Pediococcus acidilactici*, recently gained popularity for its probiotic and fermentation properties (Holland et al., 2022).

P. acidilactici was discovered as one prominent player in some food fermentation, including meat (Hye et al., 2018); *gajami-sikhae* (Jang et al., 2021); *wara* (Olajugbagbe et al., 2020); and also gouda cheese (García-Cano et al., 2020). Specifically in milk fermentation, *P. acidilactici* BK01 was able to reduce the pH until 4.48 in goat milk, where it is within the range of optimum pH for yoghurt due to ideal amount of lactic acid (Sharma et al., 2021). These properties are also exerted by *P. acidilactici* Kp10 on skim milk as well *P. acidilactici* BD16 on buttermilk and soymilk (Abbasiliasi et al., 2017; Melia et al., 2020). This is also exhibited by *P. acidilactici* BE with adequate lactic acid production, pH, viscosity, also antioxidant activity in milk (Widodo et al., 2023). *P. acidilactici* is also receiving attention as starter culture for its ability to produce bacteriocins, namely Pediocin PA-1 and Pediocin AcH, which are profound to give wider antimicrobial activity in comparison to many existing bacteriocins (Crow & Curry, 2011). These pediocins give inhibition to *Bacillus, Clostridium, Lactococcus, Listeria* spp. and some strains of *Staphylococcus aureus*, also highly effective against

Listeria monocytogenes (Bédard et al., 2018; Mandal et al., 2010; Anastasiandou et al., 2008). These antimicrobial properties are important to inhibit pathogen growth in the food. Furthermore, it plays a major role to eliminate pathogen inhabitants in hosts and promote intestinal microflora regulation (Qiao et al., 2022). In addition, *P. acidilactici* offers cholesterol lowering properties through bile salt deconjugation by bile salt hydrolase (BSH) with higher deconjugation efficiency compared to several strains of other LAB, such as *Lactobacillus acidophilus* and *Lactobacillus casei* (Gil-Rodriguez & Beresford, 2021; Mandal et al., 2009).

For commercial production, liquid and frozen starter culture have several disadvantages, where both require storage within low temperature at all times to prevent faster viability reduction by halting bacteria growth (Durso & Hutkins, 2003; Peighambardoust et al., 2011). Specific storage conditions thus decrease the movability of the starter culture and push the transportation cost (Peighambardoust et al., 2011). This led to increased popularity of powdered starter culture in commercial production for the enhanced shelf-life with lower contamination rate, and one popular method is through spray drying (Bhagwat et al., 2020). Spray drying is considered to have lower specific cost compared to other methods (e.g. freeze-drying) to produce powdered starter culture, which is preferred for commercial production (Huang et al., 2017; Peighambardoust et al., 2011).

In order to create desirable final fermented milk, many factors should be adjusted accordingly (Mengesha et al., 2022). Major aspects including inoculation size are known to highly influence the fermentation and the final product, as excessive or insufficient concentration could lead to undesirable fermentation quality (Wardani et al., 2017; Mengesha et al., 2022). Previous study reported on distinct acidification rate and the curd formation produced by implementing different inoculum size of *Lactobacillus plantarum* (Wardani et al., 2017). Most of the inoculation sizes of starter culture are in the range 1-15% v/v (Kaur et al., 2020). Determination on optimum inoculation size enables achievement of greater fermentation efficiency and productivity (Kaur et al., 2020). Few studies have observed the effect of inoculation size of liquid free culture onto the milk fermentation, yet the effect of inoculation size of spray dried *P. acidilactici* is especially limited.

Past work by Tirta et al. (2022) have developed spray dried *P. acidilactici* for fermentation production, where testing for fermentation has not yet been conducted. Therefore, this study focused on measuring the effect of inoculation size in order to give insights upon utilization of spray dried *P. acidilactici* in dairy food production, specifically fermented milk. To assess the fermented milk quality, main characteristics were investigated such as pH, titratable acidity, microbial growth, and the syneresis level. The milk used was the commercial UHT cow's milk that is widely available in the store, which was fermented with different inoculation sizes ranging from 1%, 2%, and 4% (v/v). Fermented cow's milk was obtained for the fermentation characteristics every 3-4 hours for 30 hours.

1.2 Objectives

This study is aiming to give insights upon different inoculation size (1, 2, 4%, w/v) of spray dried *P. acidilactici* to the fermented milk characteristics:

1. The growth kinetics of *P. acidilactici*
2. Acidification properties of *P. acidilactici*
3. Texture of fermented milk derived from *P. acidilactici*

1.3. Research Questions

In regards to the objectives of the study, the following research questions are to be investigated:

1. What are the effects of inoculation sizes of spray dried *P. acidilactici* on the culture growth, acidification rate, and texture of the fermented milk?

1.4. Hypothesis

These were following hypotheses in relation to the research questions:

1. H_0: The difference in acidification between different inoculation sizes of spray dried *P. acidilactici* is not significant.

$H_{1:}$ The difference in acidification between different inoculation sizes of spray dried *P. acidilactici* is significant.

2. H_0: The difference in microbial growth between different inoculation sizes of spray dried *P. acidilactici* is not significant.

   $H_{1:}$ The difference in microbial growth between different inoculation sizes of spray dried *P. acidilactici* is significant.

3. H_0: The difference in texture between different inoculation sizes of spray dried *P. acidilactici* is not significant.

   $H_{1:}$ The difference in texture between different inoculation sizes of spray dried *P. acidilactici* is significant.

1.5. Research Scope

This study includes several research scopes as listed below:

1. Preparation of *P. acidilactici* culture derived from i3L culture collection and spray dried *P. acidilactici* encapsulated with 20% (w/v) gum arabic and whey protein in 150^{o}C air inlet temperature.
2. Milk fermentation using three different inoculation sizes (1, 2, and 4%, w/v) of spray dried *P. acidilactici*
3. Comparative study between fermented milk quality of different inoculation sizes of spray dried *P. acidilactici* through aforementioned methods:
   a. Microbiological analysis
      i. Cell enumeration using Miles-Misrah method
      ii. Generation number calculation
   b. Acidification evaluation
      i. pH measurement with pH meter
      ii. Titratable acidity measurement using titration method

c. Syneresis evaluation