I. INTRODUCTION

1.1. Background

Probiotics are non-pathogenic living microorganisms that have various health benefits if consumed in adequate amounts such as maintaining gastrointestinal microflora and helping in the digestion process. Besides this, probiotics also exhibit potential health benefits to the host such as enhancing the immune system, decreasing lactose intolerance, reducing serum cholesterol, and even reducing bladder and colon cancer (Williams, 2010; Shi et al., 2016). The most commonly studied microorganisms are *Saccharomyces boulardii* yeast and lactic acid bacteria (LAB), including *Lactobacillus* and *Bifidobacterium* species. However, recent studies have been interested in *Pediococcus* species, particularly *Pediococcus acidilactici*, due to its potential probiotic properties, such as antimicrobial activity towards pathogens, ability to adhere to intestinal cells, high tolerance in gastrointestinal tract conditions, and other great probiotic characteristics (Abbasiliasi et al., 2017; Ribeiro et al., 2014).

One of the most important prerequisites for probiotics, before they can confer health benefits, is their ability to adhere to the intestinal host cell. By adhering, it can reduce pathogenic bacteria adherence to the host cell, enhance gut microbiota, and also decrease the chance of the host getting diarrhea caused by pathogenic bacteria especially foodborne pathogens (Monteagudo-Mera et al., 2019). The factors that affect the adhesion mechanism of probiotics are non-specific binding that initiated the bacteria adhesion such as hydrophobicity, van der Waals interaction, the balance of electrostatic, and also aggregation ability, and followed strong adhesion by specific physical binding of cell wall components (Guan et al., 2020; Haddaji et al., 2015). All of the properties that *P. acidilactici* has especially that can protect the host gut health by reducing the adherence of pathogenic bacteria led to growing demand to include this bacteria in food and supplements.

Producing an easy-to-use product, probiotics are commonly dried to ensure their stability extend their shelf life, reduce transportation costs, and facilitate trade (Ratti, 2013). In this case, spray drying is the most common and potential drying technique as it is inexpensive, simple, fast, and produces a high yield. The principle of spray drying is by spraying a liquid feed containing probiotic and encapsulation material into hot and dry air ranging from 150^{o}C to 250^{o}C, to make the solution into small particles, then transformed into powder. The resulting powder will consist of encapsulated probiotic (Piñón-Balderrama, 2020). The moisture is removed quickly, leaving solid particles between 10 to 15 µm. However, spray drying is associated with several stresses such as heat, osmotic, oxidative, and desiccation that will affect the cell's viability and affect probiotic properties (Huang et al., 2017).

The spray-drying process used high temperatures that can significantly affect cell viability, therefore the other studies also evaluated the effect of inlet temperature of spray-drying towards probiotic viability (Kiekens et al., 2019; Tirta et al., 2023). This study included the inlet temperature of spray drying which is the temperature of heated drying air that enters the chamber (Chew et al., 2019). In probiotic encapsulation, the inlet temperature is an important factor that can decrease the moisture content in the yield (Chew et al., 2019; Ortega, 2017). Increasing the inlet temperature also can increase the productivity and drying rate when increasing the inlet temperature (Ozdikicierler, Dirim, & Pazir, 2014). However, increasing the inlet temperature contributes to exposing more heat stress to the probiotic, which can affect the probiotic's viability and functionality. Therefore this study evaluates the effect of inlet temperature spray drying on *P. acidilactici*.

The majority of the other studies related to probiotic spray drying mostly focused on assessing the effect of spray drying on the viability of the probiotics, while the effect of spray-drying on the functionality of probiotics was less studied (Tirta et al., 2023; Pradipta, 2018; Barbosa, Brandão, & Texeira, 2017). However, the effects of spray-drying encapsulation on

probiotic properties can be varied based on each strain, then the investigation to evaluate the ability of *P. acidilactici* after spray drying are important. The study conducted by Kiekens et al. (2020), evaluates the impact of spray drying on the pili of *Lactobacillus rhamnosus* GG. Based on this study, the result showed that spray-dried *L. rhamnosus* GG has a disruption in exopolysaccharides or pili expression. This impact is not affecting the survival of the probiotic but it significantly affects the adherence capacity of the *L. rhamnosus* GG (Kiekens et al, 2020).

Considering there is a limited study that evaluates the effect of spray-drying on probiotics functionalities, this study evaluates the functionalities of *P. acidilactici* after spray-drying. One of the important probiotic abilities is the ability to adhere to the intestinal epithelial cells and inhibit pathogen adhesion. The adherence rate of *P. acidilactici* before and after spray-drying is already been trialed and recorded in unpublished data. The unpublished data evaluate the adherence ability using adherence assay, aggregation, and co-aggregation ability. However, based on the unpublished data the ability of *P. acidilactici* before (0.175 ± 0.0010%) and after spray-drying with 150°C inlet temperature (0.085 ± 0.0008%) has a low adherence rate compared to the other studies (Novella, 2023). This experiment did the adherence assay by inoculating the probiotic into pre-incubated HT-29, incubating in a 37°C incubator for 2 hours, then harvesting the bacteria by centrifuging, and cell counting the adhered bacteria. This method has limitations because the equipment and the parameter that is used in this experiment are not suitable for adherence assay. The most suitable equipment and parameter are incubating in a 37°C incubator with 5% CO_2 and 95% air, and using 3,500 rpm for 10 minutes for harvesting the probiotics after incubating 2 hours with the cells. Other than unsuitable equipment, the aggregation and co-aggregation assay also can not picture the *P. acidilactici's* ability to adhere. Therefore in the present study, this study evaluates the ability of *P. acidilactici* before and after spray-drying used cell surface hydrophobicity assay, repeating adherence assay, and inhibit pathogen adhesion assay.

1.2. Research Questions

Based on the Introduction, several research questions were formulated.

1. What are the effects of spray drying encapsulation on the cell hydrophobicity of *P. acidilactici*?
2. What are the effects of spray drying encapsulation on the adherence ability of *Pediococcus acidilactici* to the intestinal epithelial cells (HT-29 cells)?
3. What are the effects of spray drying encapsulation on the ability of *Pediococcus acidilactici* to reduce food-borne pathogens such as *S. aureus* and *L. monocytogenes* adherence to the surface of mammalian intestinal epithelial cells?

1.3. Hypothesis

These are the hypothesis related to the research questions.

1. H_0: There will be no significant difference in the cell hydrophobicity ability of *Pedioccocus acidilactici* before and after spray drying

   H_1: There will be a significant difference in the cell hydrophobicity ability of *Pedioccocus acidilactici* before and after spray drying
2. H_0: There will be no significant difference in the adherence ability of *Pedioccocus acidilactici*

   H_1: There will be a significant difference in the adherence ability of *Pedioccocus acidilactici*
3. H_0: There will be no significant difference in *Pedioccocus acidilactici*'s ability to reduce food-borne pathogens' adherence on the surface of mammalian intestinal epithelial cells

   H_1: There will be a significant difference in *Pedioccocus acidilactici*'s ability to reduce food-borne pathogens' adherence on the surface of mammalian intestinal epithelial cells

1.4. Research Aim & Scope

This research aims to investigate the effects of spray drying encapsulation on *Pedicoccus acidilactici* probiotic properties, which include:

1. Cell hydrophobicity of *Pediococcus acidilactici*
2. Ability to adhere on the surface of intestinal epithelial cells (HT-29)
3. Ability to reduce food-borne pathogens' especially *S. aureus* and *L. monocytogenes* adherence on the surface of intestinal epithelial cells (HT-29)

The research scope for this study will be:

1. Spray drying of *Pediococcus acidilactici* using gum arabic and whey protein as their wall material, with different inlet temperatures of 120°C, 150°C, and 170°C
2. Comparing the probiotics properties of *Pediococcus acidilactici* before and after spray drying *in vitro*, including
    a. Cell hydrophobicity of *Pediococcus acidilactici*
    b. Ability to adhere on the surface of intestinal epithelial cells (HT-29)
    c. Ability to reduce food-borne pathogens' adherence on the surface of intestinal epithelial cells (HT-29)
3. Food-borne pathogens tested in this experiment included
    a. Staphylococcus aureus
    b. Listeria monocytogenes