INTRODUCTION

1.1 Background, Aim, and Scope of Study

Probiotics are living microorganisms that provide health benefits to their host (or consumer). It maintains its host's gut microbiome balance by producing beneficial metabolites and fighting foodborne pathogens in its gastrointestinal tract (GIT). Its benefits may include intestinal health improvement, immune response enhancement, and better serum cholesterol control (Kechagia et al., 2013). Probiotics are commonly incorporated in dietary supplements (e.g., probiotic capsule and tablet (Huq et al., 2016)) and functional foods (e.g., yogurt (Lourens-Hattingh & Viljoen, 2001), cheese (Rolim et al., 2020), cereal (Ogunremi, Agrawal & Sanni, 2015), chocolate (Mirković et al., 2018), and fruit juice (Dias et al., 2018)) for practical consumption.

A sufficient probiotic cell concentration must be administered in the food products to obtain optimum efficacy of its health benefits. The International Dairy Federation (IDF) suggests a minimum of 10^7 probiotic cells per gram or milliliter of product, maintained viable at consumption time (Corona-Hernandez et al., 2013). However, viable probiotics are significantly reduced in the production (food processing and storage) and consumption (in GIT) (Sangami & Sri, 2017; Ranadheera et al., 2019; Fiocco et al., 2020). This reduction is caused by the low probiotic survival against environmental stress, such as heat stress, desiccation stress, and low pH (Barbosa et al., 2015; Fernandez et al., 2014). This low survival issue maintains a challenge in the productivity and efficacy of the probiotic supplement. Hence, preservation methods were explored to improve probiotics' survival against mentioned stresses.

In the food industry, spray drying is widely used as an encapsulation method to preserve probiotics from environmental stresses (Fu et al., 2018; Lipan et al., 2020; Santos Monteiro et al., 2020). It is used for its simplicity, fast, cost-effective, and highly productive manner. During spray drying, probiotic suspension (probiotic added with encapsulation material) will be exposed to hot air (150°C to 250°C) and transformed into granulated powders. In short, the probiotic will be encapsulated with the material's physical matrix. The matrix protects the cells from upcoming environmental stress exposure, and the final powder form maintains probiotics at a low moisture content to improve storage stability (Makinen et al., 2012). Aside from its benefits, the drawback of spray drying includes exposure to heat and desiccation stress during its process. Such stresses lower encapsulation efficiency (due to viability reduction during spray drying) (Huang et al., 2017). Therefore, selecting encapsulation material to protect probiotics during spray drying is important.

Aside from the inherent properties of the probiotic strains (high tolerance against heat stress and pH), the selection of biopolymers as encapsulation material in spray drying is crucial to obtaining a high encapsulation efficiency (Flores-Belmont et al., 2015). No single biopolymer can provide all the ideal criteria for encapsulation materials (e.g., edible, low-cost, idle in nature, and good physicochemical properties) (Chandralekha et al. 2017). Hence, two or three materials are often used to obtain synergic properties to improve encapsulation efficiency (Leylak et al., 2021).

As probiotic encapsulation materials in spray drying, whey protein (WP) and gum arabic (GA) have gained research interest. It is suggested that WP and GA could lead to a potential encapsulation efficiency of 93.95% (for *Lactobacillus acidophilus*, predicted using response surface methodology) (Leylak et al., 2021). In combination with GA, the high protein content in WP can construct physically strong and stable matrices (Krunić, Obradović, & Rakin, 2019). Their interactions demonstrate excellent interfacial activity and emulsifying properties that promote high encapsulation efficiency in probiotic spray drying (Klein et al., 2010). Another study suggested that GA exhibits the best physicochemical properties (when used with WP) compared to other additional biopolymers (such as locust bean gum and maltodextrin) to provide better protection as cell encapsulation material (Leylak et al., 2021). Furthermore, GA is relatively cheap compared with other additional biopolymers such as alginate and locust bean gum.

Pediococcus acidilactici has robust characteristics against low pH in the GIT (Fernandez et al.,2014) and potent characteristics as probiotics, including diverse antimicrobial activity (Abbasiliasi et al., 2017), great adherence to intestinal cells (Abbasiliasi et al., 2017), and capability to produce useful metabolites such as bacteriocin and gamma-Aminobutyric acid (Porto et al., 2017; Anggraini et al., 2019). Furthermore, it has wide applications in the food industry, ranging from fish feed supplementation (Merrifield et al., 2011 & Standen et al., 2013), starter culture of traditional sausage (Ruiz-Moyano et al., 2011), orange juice supplementation (Barbosa, Borges, & Teixeira, 2015; de Oliveira Vieira et al., 2020), until bioprotective culture as a preservative agent in a food product (İncili, Karatepe, & İlhak, 2020).

Unfortunately, the current study about *P. acidilactici* spray drying encapsulation is limited. Only two studies were found to focus on its encapsulation efficiency. First, Reddy, Madhu, & Prapulla (2009) used maltodextrin and nonfat skimmed milk separately for *P. acidilactici* CFR 2193. Although sufficient encapsulation efficiency after spray drying was obtained (roughly 95%), the survival throughout the storage period is low (50% loss during 60 days at 4^{o}C), and no physicochemical evaluation was done. Next, Barbosa et al. (2015) used orange juice and maltodextrin as an encapsulation material for *P. acidilactici* HA-6111-2. Altho sufficient encapsulation efficiency (100%) was obtained, the nature of orange juice could affect the taste of its final powder and limit its application in the food industry. Its survival during gastrointestinal tract simulation or any acid and bile salt stress was not reported.

The use of WP and GA as spray drying encapsulation material for *P. acidilactici* has not been covered. Hence, to find encapsulation material alternatives for *P. acidilactici,* this study investigated WP and GA as encapsulation materials for *P. acidilactici* regarding its spray drying encapsulation efficiency, viability during storage, and survival during GIT simulation. WP to GA ratios was tested with three different formulations: 1 to 1, 3 to 1, and 1 to 3. The formulations were set to represent encapsulation material formulation with equal WP to GA ratio, higher WP ratio, and higher GA ratio. The effect of varying WP and GA ratios for *P. acidilactici* spray drying encapsulation material was investigated towards spray drying efficiency, survival during storage, and survival during GIT simulation. Additionally, physicochemical properties of all formulations were measured, including production yield, moisture content, water activity, Fourier-transform infrared spectroscopy (FT-IR) analysis, and scanning electron microscope (SEM).

1.2 Research Questions

The following research questions were set to fulfill the aim of this study:

a. Do WP and GA formulations provide sufficient spray drying encapsulation efficiency for *P. acidilactici*? (Efficiency >50% is considered efficient (Barbosa et al., 2015)). Does varying WP and GA ratio affects the spray drying encapsulation efficiency? If yes, which formulation yields the best outcome? (indicated by higher encapsulation efficiency).
b. Does spray drying encapsulation using WP and GA provide significant protection for *P. acidilactici* during GIT simulation? Does varying WP and GA ratio affects its survival during GIT simulation? If yes, which formulation yields the best outcome? (indicated by higher survival during GIT simulation).
c. How is the viability of spray-dried *P. acidilactici* during storage? Does varying WP and GA ratio affects its survival during GIT simulation? If yes, which formulation yields the best outcome? (indicated by higher viability during storage).
d. What are the physicochemical properties (product yield, moisture content, water activity, FT-IR analysis, and SEM analysis) of the three formulations of spray-dried *P. acidilactici*? Does varying WP and GA ratio affects its physicochemical properties? If yes, which formulation yields the best outcome? (indicated by higher product yield, lower moisture content, lower water activity, interacting WP and GA in FT-IR profile, and smooth microcapsule surface in SEM result).