DEVELOPMENT OF SYNBIOTIC FEED PELLETS CONTAINING *Lactobacillus rhamnosus* GG WITH KANTAWAN (JERUSALEM ARTICHOKE) FOR NILE TILAPIA

UNCHALEEPORN SRIBOUNOY

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE PROGRAM IN APPLIED BIOLOGY

FACULTY OF SCIENCE AND TECHNOLOGY

RAJAMANGALA UNIVERSITY OF TECHNOLOGY THANYABURI

ACADEMIC YEAR 2020

DEVELOPMENT OF SYNBIOTIC FEED PELLETS CONTAINING *Lactobacillus rhamnosus* GG WITH KANTAWAN (JERUSALEM ARTICHOKE) FOR NILE TILAPIA

UNCHALEEPORN SRIBOUNOY

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE PROGRAM IN APPLIED BIOLOGY

FACULTY OF SCIENCE AND TECHNOLOGY

RAJAMANGALA UNIVERSITY OF TECHNOLOGY THANYABURI

ACADEMIC YEAR 2020

Thesis Title	Development of Synbiotic Feed Pellets Containing *Lactobacillus rhamnosus* GG with Kantawan (Jerusalem Articoke) for Nile Tilapia
Name – Surname	Miss Unchaleeporn Sribounoy
Program	Applied Biology
Thesis Advisor	Assistant Professor Arranee Chotiko, Ph.D.
Academic Year	2020

THESIS COMMITTEE

.....Chairman

(Associate Professor Nopadon Pirarat, D.V.M., Ph.D., D.T.B.V.P.)

.....Committee

(Assistant Professor Noparat Buddhakala, Ph.D.)

.....Committee

(Mr. Atsadawut Areesirisuk, Ph.D.)

.....Committee

(Assistant Professor Arranee Chotiko, Ph.D.)

Approved by Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi in Partial Fulfillment of the Requirement for the Master's Degree

.....Dean of Faculty of Science and Technology

(Assistant Professor Nipat Jongsawat, Ph.D.)

Date 1 Month December Years 2020

หัวข้อวิทยานิพนธ์ การพัฒนาอาหารเม็ดซินไบโอติกส์เสริม *Lactobacillus rhamnosus* GG และแก่นตะวันสำหรับปลานิล

ชื่อ – นามสกุล นางสาวอัญชลีพร สีบัวน้อย

สาขาวิชา ชีววิทยาประยุกต์

อาจารย์ที่ปรึกษา ผู้ช่วยศาสตราจารย์อารณี โชติโก, วท.ด.

ปีการศึกษา 2563

บทคัดย่อ

โพรไบโอติกส์สามารถช่วยเร่งการเจริญเติบโตและควบคุมการติดเชื้อของสัตว์น้ำ พรีไบโอติกส์แก่นตะวันสามารถป้องกันโพรไบโอติกส์จากสภาวะที่ไม่เหมาะสมได้งานวิจัยนี้มีวัตถุประสงค์เพื่อ 1) ศึกษาผลของแก่นตะวัน (Jerusalem Artichoke) ต่อการรอดชีวิต *Lactrobacillus rhamnosus* GG (LGG) ในแอลจิเนตแคปซูลหลังการทำแห้งแบบแช่เยือกแข็งในผงแห้งหลังการทำแห้งแบบพ่นฝอยและในสภาวะเลียนแบบทางเดินอาหารปลานิล 2) เพื่อพัฒนาอาหารเม็ดซินไบโอติกส์ที่เสริมด้วย LGG ที่ถูกห่อหุ้มและแก่นตะวันด้วยกระบวนการอัดเม็ดแบบไม่ใช้ความร้อน และ 3) เพื่อประเมินผลของอาหารเม็ดซินไบโอติกส์ที่เสริมด้วย LGG ที่ถูกห่อหุ้มและแก่นตะวัน ต่อสมรรถภาพการเจริญเติบโต ค่าเคมีในเลือด และลักษณะทางสัณฐานวิทยาของลำไส้ของปลานิล

LGG ผสมกับผงแก่นตะวันและถูกห่อหุ้มด้วยสารละลายแอลจิเนตผสมนมผงด้วยวิธีเจลเอ็กทรูชั่น (gel extrusion) ก่อนนำไปทำแห้งแบบแช่เยือกแข็งเพื่อให้ได้เม็ดแคปซูล LGG และนำไปทำเป็นผง LGG ด้วยวิธีทำแห้งแบบพ่นฝอย แคปซูลและผง LGG ถูกนำไปวิเคราะห์เพื่อหาจำนวนเซลล์ที่รอดชีวิตหลังจากการทำแห้งทั้งแบบแช่เยือกแข็งและแบบพ่นฝอยและเมื่อ LGG อยู่ภายใต้สภาวะเลียนแบบทางเดินอาหารของปลานิล หลังจากนั้น ตัวอย่างที่มี LGG รอดชีวิตสูงสุดถูกนำมาผสมกับส่วนผสมอาหารปลาและอัดเม็ดที่อุณหภูมิห้อง เพื่อผลิตอาหารเม็ดซินไบโอติกส์เสริม LGG และแก่นตะวัน อาหารเม็ดซินไบโอติกส์นี้ถูกนำไปเลี้ยงปลานิลเพื่อประเมินผลของ LGG และแก่นตะวันต่อสมรรถภาพการเจริญเติบโต ค่าเคมีในเลือด และลักษณะทางสัณฐานวิทยาของลำไส้ของปลานิล

ผลการทดลองชี้ให้เห็นว่า 1) แก่นตะวัน สามารถป้องกัน LGG จากการทำแห้งแบบแช่เยือกแข็งและการทำแห้งแบบพ่นฝอย และสามารถเพิ่มอัตราการรอดชีวิตของ LGG ภายใต้ระบบทางเดินอาหารของปลานิลได้ 2) LGG สามารถมีชีวิตอยู่รอดในเม็ดอาหารซินไบโอติกส์ได้ เมื่อนำผง LGG ผสมกับส่วนผสมอาหารปลา อัดเม็ดที่อุณหภูมิห้อง และทำแห้งเม็ดที่ 50 องศาเซลเซียสนาน 8 ชั่วโมง 3) เม็ดอาหารซินไบโอติกส์ทำให้ปลานิลมีน้ำหนักและอัตราการเติบโตจำเพาะที่เพิ่มขึ้น และมีวิลไลที่ยาวมากกว่ากลุ่มปลาที่กินอาหารควบคุม แก่นตะวันไม่มีผลต่อระดับกลูโคสในกระแสเลือดของปลานิล

ดังนั้น การทดลองนี้สรุปได้ว่าแก่นตะวันมีศักยภาพในการป้องกัน LGG จากภาวะที่ไม่เหมาะสม และ LGG ผสมแก่นตะวัน ช่วยส่งเสริมการเจริญเติบโตปลานิล อาหารเม็ดซินไบโอติกส์เสริมแก่นตะวันและ LGG มีศักยภาพในการใช้เป็นอาหารสำหรับปลานิล

คำสำคัญ: แก่นตะวัน เจรูซาเล็ม อาติโช๊ค ปลานิล *Lactrobacillus rhamnosus* GG

Thesis Title	Development of Synbiotic Feed Pellets Containing *Lactobacillus rhamnosus* GG with Kantawan (Jerusalem Artichoke) for Nile Tilapia
Name – Surname	Miss Unchaleeporn Sribounoy
Program	Applied Biology
Thesis Advisor	Assistant Professor Arranee Chotiko, Ph.D.
Academic Year	2020

ABSTRACT

Probiotics can help promoting growth and controlling infection in aquatic animals. Prebiotic from Kantawan (Jerusalem Artichoke, JA) can protect the probiotics from unsuitable conditions. The objectives of this study were to: 1) determine the effects of JA on survival of *Lactobacillus rhamnosus* GG (LGG) in alginate capsules after freeze drying process, in dried powders after spray drying process, and during exposure to stimulated in Nile tilapia gastrointestinal conditions; 2) develop synbiotic feed pellets containing encapsulated LGG with JA by using non-thermal pelleting process; and 3) evaluate the effects of synbiotic feed pellets containing encapsulated LGG with JA on growth performance, blood chemistry, and morphology of the Nile tilapia intestines.

LGG was mixed with JA powder and encapsulated with alginate solution and milk powder (MP) using gel extrusion method prior to the freeze drying process to obtain LGG capsules and the spray drying process to obtain LGG powders. LGG capsules and LGG powders were analyzed for the number of viable cells after freeze drying process, after spray drying process and when they were under stimulated in Nile tilapia gastrointestinal tract (GT). After that, the samples that had the highest number of viable LGG were mixed with fish feed ingredients, and then pelleted at room temperature to produce synbiotic pelleted feed supplemented with LGG and JA for Nile tilapia. The synbiotic pellets were fed to Nile tilapia to evaluate the effects of LGG and JA on the fish growth performance, the blood chemistry values, and the morphology of the Nile tilapia intestines.

The results indicated that: 1) JA could protect LGG from freeze-drying process and spray-drying process, and increase the survival of LGG under stimulated GT of Nile tilapia; 2) LGG could survive in the synbiotic pellets when the LGG powders were incorporated with fish feed ingredients, pelleted, and dried at 50 ºC for 8 hours; and 3) the synbiotic pellets could increase weight gain and specific growth rate of the fish, as well as the villi height which was higher than that was fed with a basal diet. In addition, JA had no significant effects on glucose levels of the fish. Therefore, it could be concluded that JA could potentially protect LGG from unsuitable conditions and LGG with JA could improve the tilapia growth. The synbiotic pelleted feed containing LGG and JA could be potentially used for Nile tilaplia.

Keywords: Kantawan, Jerusalem artichoke, Nile tilapia, *Lactobacillus rhamnosus* GG

Acknowledgements

This thesis is successful by the kind support of many people, without their help it would not be possible. I would like to greatly thank to my major advisor, Assistant Professor. Dr. Arranee chotiko for her kind supervision, excellent knowledge and valuable encouragement throughout the period of study.

Secondly, I would like to thank my gratitude is also extended to Associate Professor. Dr. Nopadon Pirarat, Assistant Professor Dr. Noparat Buddhakala and Dr. Atsadawut Areesirisuk for serving as a thesis committee and for their available and useful comments and suggestions.

Thirdly, I would like to thank the staff of division of Biology, Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi and Faculty of Veterinary Science Chulalongkorn University for the supports.

Finally, I mostly thank to my family members who always support and encourage me in every endeavor in my life. Without their loves, patience, and encouragement, I will not be whom I am today.

Unchaleeporn Sribounoy

Table of Contents

Table of Contents (Continued)

List of Tables

Page

List of Figures

Page

List of Figures (Continued)

Page

List of Figures (Continued)

Page

List of Abbreviations

FOS	Fructo - oligosaccharides
JA	Jerusalem artichoke
LGG	*Lactobacillus rhamnosus* GG
MP	Milk powder
SGF	Simulated Gastrointestinal tract
PFL	Pelleted Feed containing LGG

CHAPTER 1

INTRODUCTION

1.1 Important and background of thesis

Tilapias are one of the economical protein sources proving nutrition and food security to human. Thailand is one of the top six countries globally producing tilapias. Thai tilapias are commonly cultured at high density in ponds and floating cages placed on rivers or irrigation canals, which could promote susceptibility of tilapias to infectious bacteria and cause disease outbreaks. To control any possible outbreaks, antibiotics has been extensively used, leading to public concerns and criticisms about their health and environmental safety. This is because they can develop antibiotic-resistant bacteria spreading out to environments and possibly pass out to human through a food chain. Thus, development of antibiotic free fish farming has been recently emerged. The use of probiotics and prebiotics as alternatives of antibiotics has been introduced to increase the sustainability of the fish production. Probiotics are beneficial microorganisms such as *Lactobacillus rhamnosus* GG (LGG), which could effectively inhibit some tilapia pathogenic bacteria, promote the intestinal structure, enhance the mucosal immunity of tilapia, and improve tilapia stress coping capacity. On the other hand, prebiotics are non-digestible foods that can be fermented by certain selective colonic bacteria that could enhance growth performance, modulate intestinal microbiota, and improve immune systems. Jerusalem artichoke (*Helianthus tuberosus*), named is Thai as Kantawan, is a well-known source for prebiotics. They could increase viability of *Lactobacillus* spp. In lower pH conditions and the presence of bile salt in simulated digestive systems as well as extend the retention period of live probiotic existing in the fish gut. Generally, probiotics and/or prebiotics are administered to the fish through mixing with feeds. Development of instant feed pellets containing probiotic and prebiotics would be ideal, as it is ready to use. Combination of probiotics and prebiotics in the pellet feeds would provide dual effects to enhancing fish health and reduce the risk of bacterial infection diseases. However, pelleting process requires very high temperatures, which definitely harms the probiotics viability. Encapsulation of probiotic with prebiotics prior to pelleting

would be an approach to overcome the limitation. Encapsulation could serve physical barriers to the cells to protect them from unfavorable environments during not only pelleting but also passage through the gastrointestinal tract. Simultaneously prebiotics could help increase probiotic viability and their persistence in the gut system. However, incorporation of the encapsulated LGG with prebiotic JA into tilapia pellet feeds has not been conducted yet. Therefore, this research is aimed to encapsulate LGG combined with prebiotic JA in alginate-milk powder as a synbiotic supplement and subsequently incorporate it into feed pellets as well as examining the efficacy of the encapsulated synbiotic feed pellets on growth performance, blood chemistry and gut morphology in tilapias. This proposed research would yield a novel functional feed, sustainably contributing to the tilapia production industry and lay a foundation on aquaculture feeds supplemented with encapsulated synbiotics.

1.2 Objectives of thesis

Overall goal of this thesis is to develop Nile tilapia synbiotic feed pellets containing encapsulated *Lactobacillus rhamnosus* GG with Kantawan (Jerusalem artichoke, JA), which can provide beneficial effects to Nile tilapia. This research is consist of four internal studies. The objectives of each study are following:

1.2.1 To examine effects of JA on protection of LGG in alginate capsules during freeze drying and in simulated Nile tilapia gastrointestinal conditions.

1.2.2 To examine effects of JA on viability of LGG after spray drying and in simulated Nile tilapia gastrointestinal conditions.

1.2.3 To development of synbiotic feed pellets containing encapsulated LGG with JA by using non-thermal pelleting process.

1.2.4 To determine effects of synbiotic feed pellets containing encapsulated LGG with JA on growth performance, blood chemistry and morphology of Nile tilapia intestines.

1.3 Scope of thesis

1.3.1 Scope of research place

Faculty of Science and Technology, Rajamangala University of Technology Thanyaburi, Pathumthani, Thailand. And Faculty of Veterinary Science Chulalongkorn University, Thailand.

1.3.2 Scope of research

The Scope of research can be divided into four main parts. The first part was to study effects of JA on protection of *L. rhamnosus* GG in alginate capsules during freeze drying and in simulated Nile tilapia gastrointestinal conditions. The second part was to study effects of JA on viability of LGG after spray drying and in simulated Nile tilapia gastrointestinal conditions. The third part to produce and evaluation of synbiotic feed pellets containing encapsulated LGG with JA by using non-thermal pelleting process. The last part to development effects of synbiotic feed pellets containing encapsulated LGG with JA on growth performance, blood chemistry and morphology of Nile tilapia intestines.

1.4 Expectations of thesis

1.4.1 Jerusalem artichoke could increase the number of viable LGG after freeze drying and in simulated Nile tilapia gastrointestinal conditions.

1.4.2 Jerusalem artichoke could increase the number of viable LGG after spray drying and in simulated Nile tilapia gastrointestinal conditions.

1.4.3 Nile tilapia synbiotic feed pellets containing encapsulated *L. rhamnosus* GG with Jerusalem artichoke could be produced by using non-thermal pelleting process.

1.4.4 Nile tilapia synbiotic feed pellets containing encapsulated *L. rhamnosus* GG with Jerusalem artichoke could improve growth performance, blood chemistry, and morphology of Nile tilapia intestines.

CHAPTER 2

LITERATURE REVIEWS

2.1 Current situations of Nile Tilapia (*Oreochromis niloticus*) in Thailand

Nile tilapia (*Oreochromis niloticus*) is one of the most popular fishes used in aquacultures. They grow fast, reproduce easily, and resist to a variety of environment conditions [1]. Thailand is one of the top six Nile tilapia producers worldwide. Approximately 200,000 metric tons of Nile tilapia are produced annually in Thailand, accounting for approximately 10% of the world's annual tilapia market [2]. They are commonly cultured in the central, northeastern and northern parts of the country. As the demands of the fish has been increasing worldwide. Nile tilapia has been intensively cultured, causing a high risk of infectious disease devastation. The major diseases threatening tilapia farms have been identified from a vanity of bacteria such as *Streptococcus agalactiae* [3], *Flavobacterium columnare* [4], *Francisella* sp. [5], *Edwardsiella* spp. [6], and *Aeromonas* spp [7]. Significantly, *S. agalactiae* called as streptococcosis has brought severe problems to Nile tilapia cultures, especially for grow-out fish [8]. The outbreak is generally happened in the summer of Thailand (March to July) as the temperature is suitable for the bacteria to grow. After infection, the fish normally suffers a loss of appetite, and the mortality can increase by approximately 10-20% every day, depending upon the water quality [5]. Besides, *F. columnare* infection is one of a major outbreak found in Nile tilapia culture. The bacteria can kill various stages of fish, ranging from fries in hatcheries to adults in culture systems [9]. These two major pathogens have simultaneously hampered the Nile tilapia culture industry in Thailand and have caused severe economic losses to date. In order to manage these problems, the application of several chemicals and drugs by fish farmers is normally selected such as oxytetracycline, tetracycline, and chlortetracycline.

Obviously, antibiotics are generally used in the aquaculture industry not only for treatments but also for growth promotion and preventive purposes. This could bring about the emergence of drug resistant microorganisms and leave antibiotic residues in the fish and in the environment [10]. In addition, there is a risk associated with the transmission

of resistant bacteria from aquaculture environments to humans, and risk associated with the introduction in the human environment of nonpathogenic bacteria, containing antimicrobial resistance genes, and the subsequent transfer of such genes to human pathogens [11]. Antibiotics could also inhibit or kill beneficial microbiota in the gastrointestinal (GI) ecosystem [12]. Therefore, alternative methods of preventing bacterial infections have been seeking for, which could be immune stimulation through alteration of the composition of fish diet and feeding practices as well as immersion or via injection. Many substances from different sources (bacterial components, chemical agents, animal or plant extracts, etc.) have also been studied as prospective immune stimulants for fish [13]. Several immune stimulants such as levamisole [9], chitin [13], lactoferrin [14], Nisin [15], recombinant transferrin [16], modified carbohydrate [17], b-glucan [18], chitosan [19], and various kinds of probiotics [20] have been reported. They could be used by injection or dietary administration. The substances could enhance the resistance of cultured fish against diseases.

The probiotics administration is one of the strategies that has been launched. This approach provides several advantages over chemical and drug applications, especially for enhancing the growth and health of the host [21]. Probiotics could prevent several infected diseases and simultaneously enhance immune responses. They could improve fish growth performance by providing nutrients and enzymatic utilization and even help improve water quality [14]. However, the use of probiotics in aquaculture is limited as they have to be viable in sufficient numbers at in the gastrointestinal tract. Due to the diverse conditions in the fish gut, live probiotic bacteria often failed to reach the target site in the lower intestinal tract. Microencapsulation, a technology used to stabilize drugs for the controlled delivery and release of active ingredients, has been developed in aquaculture feed products, in order to improve the probiotics viability in the gastrointestinal tract environment.

2.2 Probiotics

A term of probiotic has been defined for decades. It was first used for the substances produced by microorganisms that stimulate the growth of man and animals. This name was taken from the latin words "pro" and "bios" [19]. The probiotic’s definition has been

revised for several times. Nowadays, it has been known as “live microorganisms that confer a health benefit to the host when administered in adequate amounts”, which was defined by the Food and Agriculture Organization of the United Nations and the World Health Organization [11]. Bacteria as probiotics are mostly a group of lactic acid bacteria, such as *Lactobacillus* sp., *Streptococcus* sp., *Enterococcus* sp. and *Lactococcus* sp. However, some *Bacillus* sp., fungi *Aspergillus* sp., and yeast, *Saccharomyces* sp., are also reported as probiotics. Probiotics provide many health promoting effects, such as adjusting the balance of microorganisms, stimulating and enhancing immunity [22], inhibiting the growth of bacteria that cause diseases [23], reducing serum cholesterol levels and blood pressure, providing anti-carcinogenic activity [24], and improving utilization of nutrients [25]. However, it was reported that probiotic could yield those health benefits when they were in sufficient amounts. International Dairy Federation (IDF) assigned to probiotic products that they should contain live bacteria at least 10^7 CFU/ml until they were consumed [26]. Probiotics contribute the health benefits to the host by the following modus of action.

2.2.1 Mode of action of probiotics

2.2.1.1 Competitive exclusion

Competitive exclusion means the scenario in which one species of bacteria more vigorously competes for receptor sites in the intestinal tract than another species. The mechanisms are that one spicy of bacteria exclude or reduce the growth of another one by creation of a hostile micro ecology, production and secretion of antimicrobial substances and selective metabolites, elimination of available bacterial receptor sites, and competitive depletion of essential nutrients. Lactobacilli and Bifidobacteria have been shown to inhibit a broad range of pathogens, including *Escherichia coli, Salmonella* spp., *Helicobacter pylori, Listeria monocytogenes* and Rotavirus [27]. They can modify the gut environment to make it less suitable for pathogenic bacteria by production of antimicrobial substances, such as lactic and acetic acid and hydrogen peroxide. Consequently, the pH of the gut is lowered below the point that pathogenic bacteria could survive [28]. Moreover, probiotics could produce the substance called bacteriocins, inhibiting growth of pathogenic microorganisms such as nisin, acidolina, acidofilina, lacatcyna, lacocydyna, reutryna, laktoline, and entrocine.

They showed high antibacterial activities against *E. coli, Salmonella* sp., *S. aureus, Clostridium perfringers,* and *Campylobacter* sp. [29]. Some lactobacilli and bifidobacteria could also share carbohydrate-binding specificities with some enter pathogens [30], competing with specific pathogens for the receptor sites on host cells [31]. In addition, they could eject the colonization of pathogenic bacteria by attaching themselves to the surface of the gut through the interaction between surface proteins and mucins, resulting in prevention of the pathogenic bacteria adhesion to gastrointestinal epithelium [32]. This effect has been found in *in vitro* human mucosal materials and *in vivo* pig mucosal material [33].

2.2.1.2 Production of Antimicrobial Substances

As mentioned above, probiotics can produce low molecular weight compounds, such as acetic acid, lactic acid, and bacteriocins. Acetic acid and lactic acid have been considered the main antimicrobial compounds responsible for the inhibitory activity of probiotics against pathogens especially gram-negative bacteria [33]. The undissociated form of the organic acids enters the bacterial cells and dissociates inside its cytoplasm, lowering the intracellular pH or the intracellular accumulation of the ionized form of the organic acid which leads to the death of the pathogen [34]. Bacteriocins including lactacin B from *L. acidophilus,* plantaricin from *L. plantarum* and nisin from *Lactococcus lactis* could destroy target cells by pore formation and/or inhibition of cell wall synthesis [35].

2.2.1.3 Enhancement of the epithelial barrier

Probiotics can prevent the potential pathogen bacteria by enhancement of intestinal barrier function through modulation of cytoskeletal and epithelial tight junction in the intestinal mucosa [36]. Under normal physiological conditions, intestinal barrier is maintained by several factors, such as mucus production, water and chloride secretion, and epithelial cells that form tight junction [37]. Disruption of epithelial barrier has been reported in several clinical conditions such as enteric infections, celiac diseases and infection bowl disease [37]. Enhancement of epithelial barrier integrity may be an important mechanism through which the probiotic bacteria benefit the host in these disease conditions. The probiotic bacteria could increase the secretion of mucus by triggering inflammation in enterocytes of the intestines [38].

2.2.1.4 Increased adhesion to intestinal mucosa

Adhesion to intestinal mucosa is regarded as a prerequisite for colonization and is important for the interaction between probiotics and the host [39]. Adhesion of probiotics to the intestinal mucosa is important for modulation of the immune system [40] and antagonism against pathogens [41]. Probiotics display various surface determinants that are involved in their interaction with intestinal epithelial cells (IECs) and mucus. IECs secrete mucin, which is a complex glycoprotein mixture that is the principal component of mucous, thereby preventing the adhesion of pathogenic bacteria [42]. This specific interaction has indicated a possible association between the surface proteins of probiotic bacteria and the competitive exclusion of pathogens from the mucus [34].

2.2.1.5 Stimulation of immune systems

It is well known that the immune system can be divided between the innate and adaptive systems. The adaptive immune response depends on B and T lymphocytes, which are specific for particular antigens. In contrast, the innate immune system responds to common structures called pathogen-associated molecular patterns (PAMPs) shared by the vast majority of pathogen [43]. Probiotic bacteria can exert an immunomodulatory effect. They have the ability to interact with epithelial and dendritic cells (DCs) and with monocytes/macrophages and lymphocytes, which have an important role in innate and adaptive immunity. Due to probiotic's ability of adhesion to the intestinal mucosa, it allows to create a natural barrier against potential pathogens, and thus enhances immunity by increasing production of immunoglobulins, activity of macrophages and lymphocytes, as well as stimulating the production of γ-interferon [44]. It was reported that administration of *L. rhamnosus* resulted in enhancement of non-specific humoral responses by increasing production of IgG, IgA and IgM from circulating lymphocyte [37].

2.2.2 Probiotic in aquacultures

The use of all sub-therapeutic antibiotics as growth-promoting agents in animal production has been banned since January 2006 by European Union. Alternative

growth promoters to be used in aquatic feeds have received much attention [45]. Development of new dietary supplementation strategies, including health and growth-promoting compounds as probiotics, prebiotics, synbiotics, phytobiotics and other functional dietary supplements have been heightened [46]. The research of probiotics and prebiotics in fish nutrition has been increasing with the demand for consumers and environment-friendly aquaculture. Many published reports demonstrated positive effects of probiotics and prebiotics in feeds for various fish species, including rainbow trout, Common carp [46] Indian major carp, Mozambique, Nile tilapia, Atlantic cod, and European Sea bass juveniles [47]. Probiotics helped increase feed conversion efficiency, live weight gain and confer protection against pathogens by competitive exclusion for adhesion sites [48], production of organic acids (formic acid, acetic acid, lactic acid), hydrogen peroxide and several other compounds such as antibiotics, bacteriocins, siderophores, lysozyme [49] and also modulation of physiological and immunological responses in fish [50]. This study investigated the effects of dietary inulin or Jerusalem artichoke (JA) on the growth performance, hematological, blood chemical and immune parameters of Nile tilapia fingerlings. Five treatment diets were designed to incorporate inulin at 0 (basal diet), 2.5 and 5.0 g/kg and JA at 5.0 and 10.0 g/kg. Two basal diets including fish meal and formulated experimental feed were used for fry and fingerling growing periods, respectively. Fingerlings were then nursed with the formulated experimental diets from weeks 5 to 12. Fingerlings fed on inulin at 5.0 g/kg or JA at either level had better growth performance and survival rate than that fed on the basal diets. There were no significant differences in body composition. Dietary prebiotic inulin and JA increased red blood cell number ($p < .05$). Among the five blood chemistry parameters examined, both inulin at 5.0 g kg and JA (5.0 and 10.0 g/kg) increased blood protein ($p < .05$). Taken together, dietary inulin at 5 g/kg and JA at 5.0 and 10.0 g/kg had beneficial effects on the growth performance, survival rate and immune of Nile tilapia fingerlings [45]. Apart from the nutritional and other health benefits [51], certain probiotics as water additives can also play a significant role in decomposition of organic matter, reduction of nitrogen and phosphorus level as well as control of ammonia, nitrite, and hydrogen sulfide [52]. Several probiotics either as monospecies or multispecies supplements are commercially available for aquaculture practices [53].

2.2.3 Factors affecting viability of probiotic

The ability of probiotics to survive and multiply in the host strongly influences their probiotic benefits. The bacteria should be metabolically stable and active in the products, survive passage through the upper digestive tract in large numbers and have beneficial effects when in the intestine of the host [22]. The standard for any foods and feeds sold with health claims from the addition of probiotics is that it must contain per gram at least 10^6-10^7 CFU of viable probiotic bacteria [11]. Therefore, probiotic survival is essential for organisms targeted to populate in the human gut. However, low viability of probiotic bacteria could happen due to several factors, namely pH, temperature, and oxygen [54].

Survival of probiotics is considerably affected by pH and titratable acidity of the products during storage as well as in gastrointestinal tracts [55]. A very low pH value increases the concentration of undissociated organic acids, enhancing the bactericidal effects of these acids. It was found that *L. acidophilus* were unable to recover cells of after exposure to a pH of 2.0 for 45 min, while no significant reduction in the number of cells was observed even after 2 h exposure at a pH of 4.0 [56]. Similar trends were found in survival of *L. rhamnosus* GG in stimulated gastric juices at pH values between 1.0 and 7.0. The optimum range of pH for growth of *Lactobacillus* spp. and Bifidobacteria is in the range of 5.5–6.0 and 6.0– 7.0, respectively [57]. Lactobacilli are capable of growing and surviving in fermented products with pH values between 3.7 and 4.3 [58]. Bifidobacteria species are reported to be less acid tolerant, and a pH level below 4.6 is detrimental to their survival [23].

Temperature is one of the crucial factors affecting probiotic viability during formulating, processing and storage [59]. Probiotic products should preferably be stored at low temperatures of 4–5 °C and depend on types of the strains [60]. Highest viability of *L. acidophilus* LA-5 was observed for up to 20 days when stored at 2 °C, whereas for *Bifidobacterium lactis* BB-12, the optimum storage temperature was 8 °C [60]. However, for long-term storage freeze drying of probiotics was recommended. A much lower storage temperature of −18 °C could maximize viability of certain probiotic strains such as Bifidobacteria. Storage temperatures of 15-25 °C could result in significant reductions

in viable cell counts in the dried products. In addition, the low decrease in probiotic viability in sugar-containing products during storage was found when they were kept at high temperatures and/or relative humidity. This could be related to their glass transition temperatures [60]. Sugars could form high viscous glasses at room temperature when they are dehydrated, and the presence of a glassy state improves storage life of anhydrobiotes.

Besides, oxygen contents and redox potential are also the important factors affecting the viability of probiotics especially during the storage period [23]. Molecular oxygen is harmful to probiotic survival and growth, as most of the species are strictly anaerobic and saccharoclastic [58]. Oxygen affects probiotics in three ways as this following: it is directly toxic to some cells, certain cultures produce toxic peroxides in the presence of oxygen, and free radicals produced from the oxidation of components (e.g., fats) are toxic to probiotic cells [56]. Therefore, it has been recommended that the levels of oxygen within the package during storage of probiotic products should be as low as possible in order to avoid toxicity and death of the microorganism and the consequent loss of functionality of the product. The degree of oxygen sensitivity varies considerably among different species and strains of probiotics [61]. In general, Lactobacilli are more tolerant to oxygen than Bifidobacteria as Bifidobacteria are anaerobic naturally. To minimize effects of oxygen, vacuum packaging, using packaging materials with low oxygen permeability, adding antioxidants and oxygen scavengers to the product, and controlling the production process in such a way that minimum dissolved oxygen entered into product are suggested [62].

2.3 Prebiotics

Prebiotic was originally defined as “A non-digestible foods ingredients that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon, and thus improves host health” [48]. In the past decade a large number of studies have demonstrated that prebiotics have great potential as agents to improve or maintain a balanced intestinal microbiota to enhance health and wellbeing. The European market for health promoting prebiotics is growing rapidly [63]. Many food oligosaccharides and polysaccharides (including dietary fiber) have been claimed to have prebiotic activity, but not all dietary carbohydrates are prebiotics [62]. In

recent years, the use of prebiotics in fish aquacultures has been great interest [64]. Various mechanisms have been proposed to explain their specific action, such as selective stimulation of beneficial microbiota, and improvement of immune functions, fish health, and productivity

2.3.1 Selective stimulation of beneficial microbiota

Among the group of beneficial bacteria present in the gastrointestinal (GI) tract are those that most utilize prebiotic oligosaccharides being considered as the only microorganisms able to beneficially affect the host's health. Numerous human and animal feeding studies have shown that they selectively stimulate one or a limited number of beneficial bacteria thus causing a selective modification of the host's intestinal microbiota. [65]. Many prebiotics such as fructo oligosaccharides (FOS) and mannan-oligosaccarides (MOS) have been investigated for nutritional manipulation of the GI ecosystem of humans and animals, because they facilitate and support the symbiotic relationship between host and its microbiota [66]. FOS and MOS are two classes of prebiotic oligosaccharides that are beneficial to enteric health, but they do so by different means. For example, dietary supplementation of prebiotic oligosaccharides has been shown to provide a nutrient source for beneficial bacteria and may promote the maintenance of bifidobacteria and certain lactic acid bacteria in the gut of humans and animals [46]. FOS influence enteric microflora by 'feeding the good bacteria', which competitively excludes the colonization of pathogens and thus improving animal health and growth performance [67].

2.3.2 Improvement of immune functions

Prebiotics have been demonstrated to possess the capability of modulating the fish immune system either by direct or indirect mechanisms. The direct immunostimulatory properties of prebiotics may be explained when the prebiotic ligands interact with their associated receptors such as b-glucan and dectin-1 receptors present in macrophages [68]. A down-stream process could occur when the ligand-receptor interaction activates signal transduction molecules such as NF-kB, which stimulate a number of immune cells [69]. In another perspective, immune responses could be triggered when the saccharides interact with receptors in the form of microbe associated

molecular patterns (MAMPs) such as teichoic acid, peptidoglycan glycosylated protein, or the capsular polysaccharide of bacteria [70]. Prebiotics have been demonstrated to predominantly influence lysozyme, phagocytic, and respiratory burst activities in fish.

2.3.3 Effects on fish health and productivity

Evaluation of prebiotics as a feed additive and their effects on animal production has been developed. Inulin has been shown to have beneficial effects on growth and health status in mammals [71]. However, little is known about its effects on fish [72]. It was reported dietary supplementation with inulin had a positive effect on growth responses in various fish species, including Nile tilapia, Siberian sturgeon (*Acipenser baerii*), and rainbow trout (*Oncorhynchus mykiss*) [73]. However, dietary supplementation with inulin did not affect the grow the response in weaning turbot (*Psetta maxima*), Atlantic salmon (*Salmo salar*), hybrid striped bass (*Morone chrysops* x *Moronesaxatilis*) [74]. Moreover, dietary FOS supplementation led to increase survival rate of common carp fry although it did not significantly improve growth performance [66]. Comparative study on prebiotic effects *in vitro* between inulin and FOS revealed that they influenced different microbial community and proteolytic [75]. Dietary supplementation with either inulin or FOS had similar effects on growth performance in rainbow trout [76], whereas supplementation with FOS had a more positive effect than inulin on the growth rate of turbot larvae [77].

2.4 Kantawan (Jerusalem artichoke)

Kantawan (Jerusalem artichoke; JA) or *Helianthus tuberosus* is a plant native to North America. JA is a perennial plant which consists of a stem about 1–3m tall, small yellow flowers, hairy oval shaped leaves and an underground rhizome system which bears small tubers (Figure 2.1). It is an angiosperm plant species of the composite family, which is commonly referred to as the sunflower or daisy family [78]. The stems are stout and ridged which can become woody overtime. Its leaves alternate near the top of the stem, the lower leaves are larger and broader, and can grow up to 30 cm long while the higher ones are smaller and narrower. In terms of flower heads, each is 5–7.5 cm wide and formed by small, yellow, tubular disk flowers in the center and surrounded by florets, which occur separately or in groups at the end of alar branches and main stems. As for

tubers, they are uneven and elongate varying from knobby to round clusters. The colors of tubers range from pale brown to white, red and purple [76]. JA has a number of advantageous characteristics over traditionally agricultural crops, including high growth rate, good tolerance to frost, drought and poor soil, strong resistance to pests and plant diseases, with minimal to zero fertilizer requirements [78]. Conventionally, JA has been used for food or animal feeds [79], and for the past two decades, alternative uses have been explored especially for the production of functional food ingredients including inulin and fructose [80]. JA contained high proportion of shorter fructan comparing to inulin from chicory. While high proportion (43–52%) of furctan in JA were short chain fructan (<9 degree of polymerization (dp)), 64–71% of fructan in inulin were medium chain fructan (10–40 dp) [76].

Figure 2.1 Plant and tubers of Jerusalem artichoke [76].

Inulin is a polysaccharide, similar to starch, and exists as a white powder with neutral taste. Chemically, it is a linear biopolymer of D-fructose units connected by glycosidic linkages, and terminated with one D-glucose molecule linked to the fructose chain [81]. The degree of polymerization of inulin generally ranges from 2 to 60. To date, inulin has been increasingly used as functional ingredients in processed foods and feeds due to its unique characteristics [82]. Inulin cannot be digested by human intestinal

enzymes. When orally ingested, inulin passes through the mouth, stomach and small intestine without being metabolized, until it enters into the large intestine where it becomes fermented by the colonic microflora. Inulin as a prebiotic simulates the growth of existing strains of beneficial bacteria in the colon which enhances the absorption of important mineral components like calcium and magnesium as well as the synthesis of B vitamins [68]. In addition, it has been shown to exert a protective effect toward *L. acidophilus*, *L. casei*, *L. paracasei*, *L. rhamnosus*, *L. plantarum* and *Bifidobacterium* spp. [83] by improving their survival and activity during storage. It was also found that the incorporation of inulin in a diet reduced the lipid content of blood and liver in saturated fat-fed rats. Recent studies observed that inulin played an important role in the prevention and inhibition of colorectal, colon and breast cancers [84]. In addition, it was reported that JA had positive effects on growth performance in Nile tilapia that were comparable to those of inulin. The growth performances (including final weight, specific growth rate, and Feed conversion ratio) of Nile tilapia fed the JA diets were superior to those of fish fed the inulin diets. Moreover, JA also contains various minerals and vitamins including iron, calcium, potassium, vitamin B complex, vitamin C, and vitamin A [85]. These micronutrients in JA also may have had addition al positive effects on growth response and feed utilization in the Nile tilapia.

2.5 Encapsulation techniques

Encapsulation is a process to entrap an active substance within wall materials. The encapsulated substance can be called the core, fill, active, internal or payload phase, while the wall material that is encapsulating is often called the coating, membrane, shell, capsule, carrier material, external phase, or matrix [86]. In recent years, several functional compounds such as antioxidants, vitamins, phytosterols, and probiotics are added into food and feed products. They are usually highly susceptible to environmental processing and/or gastrointestinal conditions. Therefore, encapsulation has imposed an approach for effective protection [87]. This technology provides barriers between sensitive bioactive agents and the environment. In addition, the techniques have been applied to modify physical characteristics of the original materials to allow easier handling, help separate the components of the mixture that would otherwise react with one another, and to provide

an adequate concentration and uniform dispersion of an active agent [86]. Many encapsulation technologies, such as emulsion technology, extrusion method (Figure 2.2) and spray drying technology (Figure 2.3), have been reported [88].

2.5.1 Emulsion techniques

The emulsion method is commonly used for encapsulation of microbial cells. This technique involves dispersion of the cells mixed in a polymer solution (dispersed phase) in an oil (continuous phase). The mixture forms a water-in-oil (W/O) emulsion with the aid of surfactant and stirring followed by the step in which the phases are separated and the dispersed phase encapsulates the probiotic microorganism as core material [65]. Ingredients such as milk proteins or caseinate as well as alginate have mostly been used in the emulsion method. The main advantages of this technique are large particle size ranging from 0.2 to 5000 mm and upscale easy [89], achieved by controlling the stirring speed and water/oil ratio [89]. Encapsulation using the emulsion technique enhances protection of *L. plantarum* DPC206, *L. reuteri* DPC16, *Pediococcus acidilactici* DPC209 and *Bifidobacterium lactis* HN019 from gastrointestinal tract conditions and during storage [48].

2.5.2 Extrusion techniques

The extrusion technique is used to convert hydrocolloids into capsules. The technique consists of a core material completely mixed with the wall material which is projected into a nozzle in a hardening solution [61]. The droplet formation occurs in a controlled manner, which is done by pulsation of the jet or vibration of the nozzle [90]. The main ingredients/materials used for this technique are alginate, carrageenan and pectin, which are able to form gels in presence of minerals such as, calcium and potassium and have been used successfully to entrap probiotic microorganisms inside the capsule. Gel is formed by the bonding of multiple free carboxylic radicals by gelling ions [62]. Also, the beads with probiotic microorganisms made by means of the extrusion technique can be mixed at the same time with different ingredients such as starch, milk protein and coated with chitosan to enhance the stability of the capsules throughout the process and better protect the probiotic in the gastrointestinal tract [62]. The extrusion technique is simple and gentle and does not harm probiotic cells. It does not require high temperatures and the use of organic solvents, which contribute to high probiotic viability. Moreover, it

is cheap. All conditions can be controlled, and it works well in either aerobic or anaerobic condition. Mass production of beads can either be achieved by multi-nozzle system or using a rotating disc. However, their use in large-scale production to date is limited due to slow formation of beads. These extrusion capsules have been added in different foods, mainly dairy products, especially cheese, yogurt drink and fermented milks [91]. This type of technique has also been tested in fermented meat products [91].

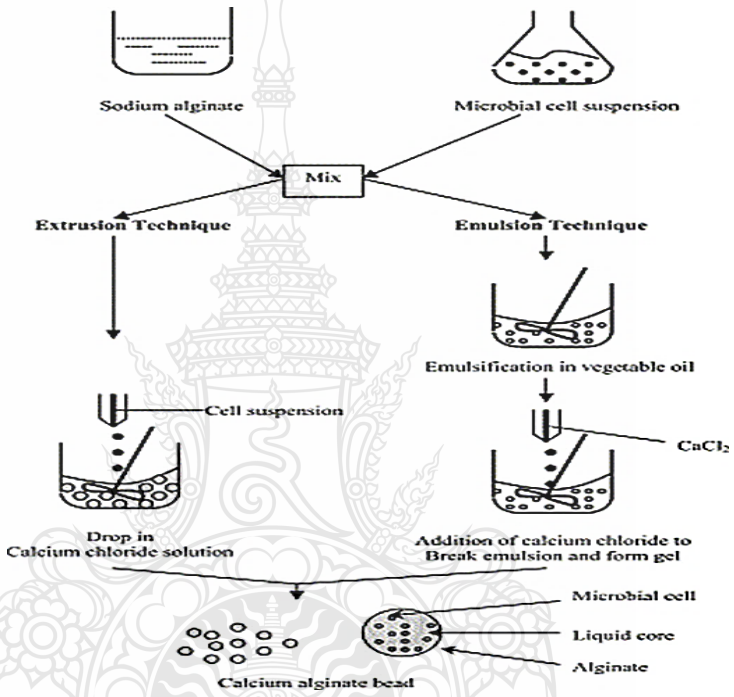


Figure 2.2 Flow diagram of encapsulation of bacteria by the extrusion and emulsion techniques [89].

Alginate is a naturally derived polysaccharide extracted from various species of algae and composed of D-mannuronic and L-guluronic acids. The composition of the polymer chain varies in amount and in sequential distribution according to the source of the alginate. This influences functional properties of alginate as a supporting material [92]. Alginate is an extensively used biopolymer for encapsulation, Alginate bead gelation occurs by cross-linking of Ca_2+ gelling ions with the alginate and calcium alginate is ideal at concentrations of 0.5–4% for encapsulating probiotics, the structure of

the gel looks like an egg box. Alginate is widely used for its simplicity, biocompatibility, low cost, non-toxicity, easier gel matrices formation around bacterial cells and mild processing condition [93]. However, some disadvantages are attributed to the use of alginate. For example, alginate beads are sensitive to the acidic environment [58], which is not compatible for the resistance of the micro particles in the stomach conditions. Others disadvantages concern the scaling-up of the process that is very difficult. In addition, the micro particles obtained are very porous which a drawback is when the aim is to protect the cells from its environment [94].

Nevertheless, the defects can be compensated by mixing alginates with other polymer compounds, coating the capsules by another compound or applying structural modification of the alginate by using different additives [95]. For example, mixing alginate with starch is commonly used and it has been shown that this method results in an improvement of probiotic encapsulation effectiveness [95]. Skim milk was regarded as a kind of material and proved to have remarkable effects on cell viability during dehydration [64]. Inulin, which has a 10-60 of polymerization (DP), serves as a prebiotic and indirectly offers many benefits to the host by selectively stimulating the growth and/or activity of intestinal bacteria [96], and many previous reports proved that inulin improved the viability of *Lactobacillus plantarum* [6]. Alginate gel matrices enclose the bacterial cells to produce beads in the range of 1–3 μm diameter and pore sizes of the surface up to 7 nm. Bacteria encapsulated using alginate improve their survival rates in skim milk by one log compared to free cells until storage of 24 h [67]. Encapsulation using blend of alginate-glycerol significantly increases the viability of LAB even after deep freezing process [68]. [97] prepared poly-L-lysine cross linked alginate microparticles for encapsulating bifidobacteria to protect the cells without losing their survivability in gastric fluid, due to stability of cross linked microparticles. Probiotic cultures such as *L. casei, L. acidophilus, B. bifidum* and *B. longum* encapsulated using a blend of prebiotics (fructooligosaccharides or isomaltooligosaccharides), growth promoter (peptide) and sodium alginate in the ratio of 3:1:1 reported the highest counts [97]. Edible coating using sodium alginate or sodium alginate/whey protein concentrate combined with LGG in probiotic pan bread significantly improved its viability until 7 days storage [70]. Microencapsulation of many laboratory was studied using alginate

in combination with other coating materials such as *L. lactis* using composite alginate/pectin [98], *L. plantarum* and Staphylococcus xylosus using alginate-starch mixture [99] and *Lactobacillus rhamnosus* GG in chitosan-coated alginate bead [100]. *L. plantarum* encapsulated using inulin-sodium alginate-skim milk (ISA) by freeze-drying method enhanced the viability as well as probiotic functionality compared to free cells under both gastric and bile conditions [101].

2.5.3 Spray drying

Spray drying is defined as the transformation of a fluid from a liquid state into a dried particulate form by spraying the fluid into a hot drying medium [102]. It is a suitable one-step process for the conversion of various liquid formulations into dry powders. The spray drying process consists of three fundamental steps as following; atomization of the liquid feed, drying of the sprayed droplets in the drying gas and formation of dry particles, and separation and collection of the dry product from the drying gas. Spray drying is a simple, fast, and scalable drying technology [103]. The equipment is commercially available and the production cost is lower compared to that of other drying technologies, such as freeze drying [104]. The powders produced are high in quality and have low moisture content, resulting in high shelf stability [102]. Spray drying is expected to be an alternative method for production of probiotic powders at an industrial scale. However, the harsh spray drying conditions, in particular high temperature exposure at the last stage of drying, limit the applicability of spray drying in probiotic production [64]. Therefore, using a protective matrix as a drying medium represents has been used as an strategy to protect bacteria from spray drying [105].

Skim milk is regarded as a kind of material and proved to have remarkable effects on probiotic viability during dehydration [101]. There has been an increasing number of reports on the application of skim milk in the protection of probiotic cells in recent years, relating to roles in protection both while drying is occurring as well as during exposure to gastrointestinal or bile fluids [106]. These have demonstrated good survival; however, these studies utilized either whey skim milk in combination with carbohydrate or liquid whey which also contains lactose [107]. For example, skim milk coated inulin sodium alginate (ISA) encapsulation beads improved the survival of *L. plantarum* during exposure to adverse environment. The viability of *L. plantarum* from ISA encapsulation

beads did not change after 2 h incubation in simulant gastric fluid (SGF). In 1% bile salt solution, the *L. plantarum* from ISA encapsulation beads only had 1.21 log CFU/mL reduction after 2 h treatment. After 7 weeks storage, the survival rate of *L. plantarum* was 20.89%, which showed reduction from 8.52 to 7.84 log CFU/mL [82].

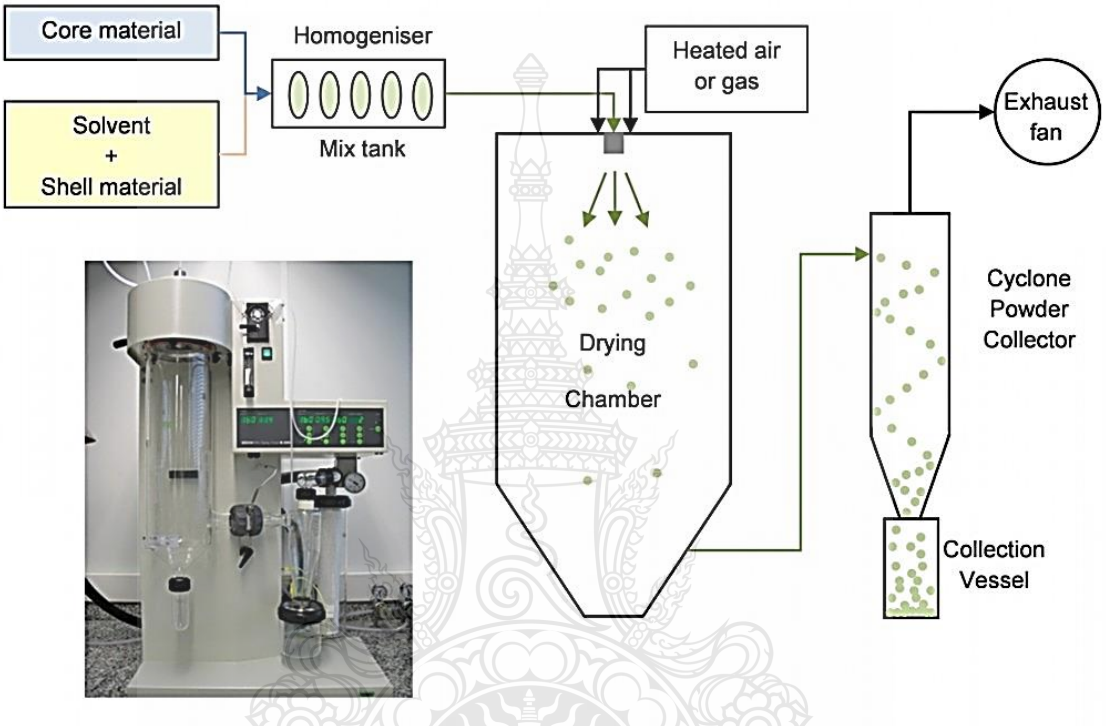


Figure 2.3 The spray drying process consists of three fundamental steps [219].

2.6 Related research

The effects of dietary inulin and Jerusalem artichoke (JA) on intestinal microbiota and morphometry of Nile tilapia fingerlings were investigated. Five treatment diets were designed to supplement inulin at 0 (basal diet), 2.5 and 5.0 g/kg, and JA at 5.0 and 10.0 g/kg. The result showed that the fish fed with inulin and JA had different microbial community profiles compared to the basal diet group. The number of bacteria and *Bifidobacterium* spp. were increased in the fish fed with dietary inulin at 5.0 g/kg and JA (at both levels) while the number of *Vibrio* spp. was reduced. The inulin (5.0 g/kg) and JA at both levels improved intestinal villi height of the fish as well as a goblet cell number. The results indicated that dietary inulin (5.0 g/kg) and JA (5 and 10.0 g/kg) since the first

feeding positively affected the intestinal microbiota and morphology of Nile tilapia fingerlings [108].

The application of microencapsulation technique by alginate extrusion on a human-derived probiotic, *Lactobacillus rhamnosus* GG (LGG) and determination of the cell viability and morphology of the microcapsules while in transit through the tilapia gastrointestinal tract were studied. The *in vitro* results showed that the microencapsulated probiotics in alginate matrix and skim milk–alginate matrix under simulated had higher viability under gastric conditions and in tilapia bile than that of the free probiotics. The cell viability of both the alginate matrix and skim milk–alginate matrix microencapsulated probiotics was maintained after storage at room temperature for 14 days, while viable free LGG could not be detected after only 7 days. The results from the *in vivo* study revealed that the overall intestinal structure, growth performance and significant protection against *Streptococcus agalactiae* challenge of the fish were improved when they were fed with microencapsulated probiotic. These results suggested the potential application of alginate-microencapsulated LGG in tilapias [109].

Modulation of intestinal morphology and immunity in Nile tilapia by *L. rhamnosus* GG (LGG) were evaluated. The results suggested that LGG could promote the intestinal structure and the mucosal immunity of tilapia. The probiotic could increase the fish villous height in all parts of the intestines and enhanced the population of intraepithelial lymphocytes. The population of acidophilic granulocyte in the fish fed with LGG was significantly greater at the proximal and distal parts when compared with the control group. It was found that LGG could serve as an important regulator of gut associated immune systems indicated by the increase of serum complement activity as well as the phagocytosis and killing ability of the head kidney leukocytes in the probiotic supplemented fish [110].

Synbiotics, a synergistic combination of probiotics and prebiotics, are currently regarded as one of the most practical nutritional supplements in tilapia farms. In this study, the effect of supplementing the diet of red tilapia (*Oreochromis spp*.) with Jerusalem artichoke (*Helianthus tuberosus*) and *Lactobacillus rhamnosus* GG (LGG) was evaluated. Growth performance, serum biochemical parameters, intestinal morphology, goblet cell counts, immune parameters and protection against *Aeromonas veronii*

challenge were determined. The results showed that fish fed with synbiotic supplemented diets had a significantly higher ($P < 0.05$) feed conversion ratio (FCR), specific growth rate (SGR), and average daily gain (ADG) than fish fed with a control diet. The symbiotic supplemented diet increased glucose, total protein and the total cholesterol levels. The absorptive area of the proximal and distal intestine of fish fed on the synbiotic diet was significantly higher ($P < 0.05$) than in those fed with probiotics (LGG), prebiotic supplemented diets (JA), and the control diet. Goblet cell counts revealed that the numbers of acid mucous cells, neutral mucous cells and double-staining mucous cells of fish fed the synbiotic supplemented diet (JA + LGG) were significantly higher ($P < 0.05$) in the proximal and distal intestine. Fish fed the synbiotic supplemented diets also exhibited significantly higher ($P < 0.05$) lysozyme activity. The cumulative mortalities of fish fed with a synbiotic supplemented diet were significantly lower than those of fish fed other diets. The results suggested the beneficial effect of JA and LGG synbiotic diet on growth performance and health status of red tilapia. Direct administration of JA and LGG in fish feed can be used as a practical nutritional supplement in tilapia [111].

CHAPTER 3

METHODOLOGY

3.1 Materials

3.1.1 Equipments

3.1.1.1 Autoclave	Model NB-1100, N-BIOTEK, Korea
3.1.1.2 Centrifugation	Sigma 2-16PK, Sartorius, Germany
3.1.1.3 Freeze dryer	FDU-8624, Operon, Korea
3.1.1.4 Hot-air oven	Contherm, Thermotec 2000,Germany
3.1.1.5 Incubated	BE 500, Schwa Bach, Germany
3.1.1.6 Spray dryer	Buchi B-290, Buchi, Germany
3.1.1.7 Stomacher	Stomacher®400 Circulator, Seward, UK
3.1.1.8 Water bath	JSGI-250J, Schwa Bach, Germany

3.1.2 Chemicals

3.1.2.1 Alginate	Kasetsart University, Thailand
3.1.2.2 Bile salt	Market, Thailand
3.1.2.3 Calcium chloride	Univar®, Ajax Finechem, Australia
3.1.2.4 Cassava chips	Market, Thailand
3.1.2.5 Corn meal	Market, Thailand
3.1.2.6 Coconut meal	Market, Thailand
3.1.2.7 De Man Rogosa Sharpe	BD Difco™, Maryland, USA
3.1.2.8 Fish meal	Market, Thailand

3.1.2.9 Hydrochloric solution Lab scan, Analytical, Thailand

3.1.2.10 Jerusalem artichoke (JA) Kasetsart University, Thailand.

3.1.2.11 Milk powder Market, Thailand

3.1.2.12 Premix Market, Thailand

3.1.2.13 Rice bran Market, Thailand

3.1.2.14 Soybean meal Market, Thailand

3.1.3 Microorganisms

3.1.3.1 *Lactobacillus rhamnosus* GG

3.1.3.2 Nile tilapias (*Oreochromis niloticus*)

3.2 Methods

3.2.1 Effects of Jerusalem artichoke on viability of *Lactobacillus rhamnosus* GG in alginate capsules after freezing, freeze drying, and in simulated Nile tilapia gastrointestinal conditions

3.2.1.1 Preparation of *L. rhamnosus* GG (LGG)

Frozen cultures of *L. rhamnosus* GG (LGG) in 20% glycerol was reactivated into de Man Rogosa Sharpe (MRS) broth (BD Difco™, Maryland, USA) and incubated (BE 500, Schwa Bach, Germany) at 37 °C for 24 h. The bacteria (5%) was then subsequently inoculated into MRS broth and incubated at 37 °C until it reaches to stationary phase. The cells were harvested by centrifugation at 8000 rpm, for 10 min at 4 °C (Sigma 2-16PK, Sartorius, Germany). Afterwards, the cell pellets were dispersed into saline solution and used for encapsulation.

3.2.1.2 Preparation of jerusalem artichoke powders

Jerusalem artichoke (JA) powders will be obtained from Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. Proximate analysis of JA and the content of fructan will be determined prior to use.

3.2.1.3 Encapsulated alginate capsule preparation using gelation-extrusion method

The extrusion technique of encapsulation was modified from [93]. The cell pellets were resuspended in 10%w/v of milk powder (MP) mixed with 1.8%w/v food grade alginate and JA powders at different concentrations (10 g/100 mL, 15 g/100 mL, and 20 g/100 mL). JA powders were obtained from Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. JA was composed of 28 % of sugar, 39.09 % of inulin, 15.8 % of FOS [112]. The sample was mixed until it was homogenous. After that, the mixture was dropped into 0.1 M Calcium chloride ($CaCl_2$) (Univar®, Ajax Finechem, Australia) solution and kept hardening in the solution for additional 30 min. The $CaCl_2$ was decanted to collect the alginate capsules. After encapsulation, the capsules were determined for their encapsulation efficiency (%) = $(N/N_0) \times 100$, where N and N_0 were the number of viable cells encapsulated in alginate capsules after alginate encapsulation and the number of viable cells before encapsulation, respectively. The capsules were then placed in glass chamber and frozen in alcohol bath at -86 °C. All samples were subsequently dried in a freeze dryer (FDU-8624, Operon, Korea) for 48 h and stored in sealed aluminum bags at 4 °C. After freezing and freeze drying, the cell viability was measured. Unencapsulated cells (UC) and only alginate encapsulated LGG (AL) were used as controls.

3.2.1.4 Determination of LGG viability in wet and dried AG-capsules under simulated gastrointestinal (GI) tract of Nile tilapia

1) Viability of freeze dried alginate capsules in simulated gastric conditions

Simulated gastric fluids (SGF) of Nile tilapia were prepared using normal saline solution, adjusted pH to 1.5, 2.0, and 3.0 (adjust pH by using 5 M hydrochloric solution (Lab scan, Analytical, Thailand)). Exactly 0.5 mL of free cells and 0.5 g of freeze dried alginate capsules were placed into separated test tubes containing 4.5 mL of the simulated gastric solutions. Triplicate samples were taken after incubation (JSGI-250J, Schwa Bach, Germany) at 25 °C in a water bath for 0, 1, and 2 h. The capsules were then disintegrated by soaking in 1 M phosphate buffer pH 7.0.and subsequently placing in a stomacher at 200 rpm for 3 min.

2) Viability of freeze dried alginate capsules in Nile tilapia bile salt solutions

Bile tolerance, 0.5 mL of un-encapsulated cells and 0.5 g of freeze dried capsules were added into 4.5 mL of SGF at pH 2.0 and incubated at 25 °C (BE 500, Schwa Bach, Germany) for 60 min. After the incubation, the SGF was removed and replaced with 4.5 mL of 3%w/v, 5%w/v, and 10%w/v tilapia bile salt. Triplicate samples were taken after further incubations of 1, 2, and 3 h at 25 °C. The capsules were then disintegrated as mentioned above. The cell counts of LGG were enumerated on MRS agars by using a pour-plate method. Un-encapsulated cells were used as a control.

3.2.2 Effects of Jerusalem artichoke on viability of *Lactobacillus rhamnosus* GG after spray drying , and in simulated Nile tilapia gastrointestinal conditions

3.2.2.1 Spray drying of *L.rhamnosus* GG

JA powders was kindly provided by Phetchabun Research Station, Agro-Ecological System Research and Development Institute, Kasetsart University, Thailand. It contained 28 g/100 g of sugar, 39 g/100 g of inulin, and 16 g/ 100 g of fructo-oligosaccharide [76]. Five feed solutions containing LGG were prepared including alginate solution (18 g/L), alginate solution (18 g/L) with milk powder (200 g/L), and alginate solutions (18 g/L) with milk powder (200 g/L) and JA at different concentrations (100 g/L, 150 g/L, and 200 g/L). Firstly, JA solution were prepared at the concentration of 100 g/L, 150 g/L, and 200 g/L by dissolving the JA powder in distilled water until the total soluble solid of the solution reached 10, 15, or 20 ^{o}Brix, respectively. The JA solutions were then centrifuged at 5,000xg for 5 min to remove the remained particles. After that, 200 g/L of milk powder (containing 10.8 g/100 g of protein, 25.3 g/100 g of fat, and 57g/100 g of lactose) was then added and magnetically stirred at room temperature until homogenously. Food-grade alginate (18 g/L) was then added into the solutions prior to resuspend the LGG cell pellets (~10^9 CFU/mL). Only alginate solution and alginate with milk powder solution were used as controls. The alginate solution was prepared by dissolving the alginate in distilled water and subsequently used to resuspend the LGG cells, while alginate with milk powder solution was prepared by adding alginate into milk powder solution before resuspending LGG. Each feed solution was fed into in a laboratory scale spray dryer (Buchi B-290, Buchi, Germany) at inlet temperature of

125 °C and outlet temperature of 65 °C with the flow rate of 300 mL/h) to obtain encapsulated LGG powders, namely encapsulated LGG in alginate (AL), encapsulated LGG in alginate and milk powder (AM), encapsulated LGG in alginate, milk powder, and JA at 100, 150, and 200 g/L (AMJ10, AMJ15, and AMJ20, respectively). After spray drying, the number of viable LGG in the powders were enumerated as well as under the simulated gastrointestinal tract and the cell survival (%) was calculated by the following equation, Cell survival (%) = $(N/N_0) \times 100$, where N and N_0 were the number of viable LGG in the spray dried powders (log CFU/g) and the number of viable cells before spray drying (log CFU/g), respectively.

3.2.2.2 Determination of LGG viability after drying processes and under simulated Nile tilapia gastrointestinal tract.

1) After spray drying

One gram of each LGG powders, namely AL, AM, AMJ10, AMJ15, and AMJ20, were resuspended in 9 mL of normal saline solution (0.85 g/100 mL). The serial dilution was then conducted. A pour-plate method was used to enumerate the cell number of LGG powder on MRS agars. The samples were then incubated at 37 °C for 48 h.

2) *In vitro* simulated gastric conditions

Simulated gastric fluids (SGF) of Nile tilapia were prepared using normal saline solution, adjusted pH to 1.5, 2.0, and 3.0 by using 5 M hydrochloric solution (Lab scan, Analytical, Thailand). Exactly 0.5 g of spray dried LGG powders (AL, AM, AMJ10, AMJ15, and AMJ20) were placed into separated test tubes containing 4.5 mL of SGF. One mL of each sample was taken after incubation at 25 °C in a water bath for 0, 1, and 2 h (JSGI-250J, Schwa Bach, Germany) and enumerated for the cell counts on MRS agars using a pour-plate method.

3) *In vitro* bile salt solutions

For bile tolerance determination, 0.5 mL of LGG fresh cells as control and 0.5 g of LGG powders (AL, AM, AMJ10, AMJ15, and AMJ20) were added into 4.5 mL of SGF at pH 2.0 and incubated at 25 °C for 1 h. After the incubation, the samples were then centrifuged. SGF was removed and separately replaced with 4.5 mL

of 3 mL/100 mL, 5 mL/100 mL, and 10 mL/100 mL of tilapia bile salt. One mL of each sample was taken after further incubations of 1, 2, and 3 h at 25 °C and enumerated on MRS agars by using a pour-plate method to determine for the number of viable cells.

3.2.3 Development of Nile Tilapia Pelleted Feed Containing LGG and JA

3.2.3.1 Production of Nile tilapias pelleted feed containing LGG

To prepare the pelleted feed, dry feed ingredients, including fish meal (200 g/kg of total weight), soybean meal (130 g/kg of total weight), rice bran (150 g/kg of total weight), corn meal (120 g/kg of total weight), cassava chips (120 g/kg of total weight), and premix (50 g/kg of total weight) were ground, sterilized at 121°C for 15 min, and dried in a hot-air oven (Contherm, Thermotec 2000, Germany) at 55°C until their moisture contents reached 12-13%. Dry feed ingredients were then mixed with starch binding agent (30 g/kg of total feed ingredients), dried coconut meal as a floating aid (200 g/kg of total feed ingredients) and the selected LGG powder, AMJ20 (100 g/kg of feed ingredients). Tap water (300 g/kg of total feed ingredients) was then added to obtain a feed mash. The mash was conveyed to a single screw extruder and proceeded at room temperature to obtain pelleted feed containing LGG (PFL). The pellets were then dried at 50 °C for 8 h. PFL was evaluated for proximate analysis and cell viability after drying and under simulated gastrointestinal tract.

3.2.3.2 Cell viability in Nile tilapia pelleted feeds containing LGG

Exactly one-gram PFL were disintegrated by soaking in 1 M phosphate buffer pH 7.0 in a stomacher bag and subsequently placing it in a stomacher (stomacher®400 Circulator, Seward, UK) for 3 min prior to performing serial dilutions in normal saline solution (0.85 g/100 mL). LGG fresh cells and the selected LGG power were used as controls. The cell counts of LGG in all samples were enumerated on MRS agars by using a pour-plate method.

3.2.3.3 Proximate analysis of Nile tilapia pelleted feed containing LGG

PFL samples were analyzed for their nutritional compositions. Moisture and protein contents were analyzed by using standard methods of ISO 6496:1999 and ISO 5983-2:2005, respectively. Ash and total fat were determined by following the methods of AOAC (2016) 942.05 and AOAC (2016) 954.02, respectively.

The total carbohydrate was calculated by the following equation; Total carbohydrate content (%) = 100-(moisture content + protein content + total fat content + ash content).

3.2.4 Effects of Nile Tilapia pelleted feed containing LGG and JA on growth performance, blood chemistry and intestines of Nile tilapia

3.2.4.1 Fish, dietary supplementation and *in vivo* experimental designs

Three hundred tilapias (*Oreochromis niloticus*) with 20-30 g body weight were acclimatized for 7 days and randomly placed in 90-L tanks (30 fish per tank) for the control groups and the dietary supplementation groups. The tanks were filled with recycled water which was controlled for temperature (25-28 ºC), dissolved oxygen (5.8–6.8 ppm), and pH (6.5–7.0) throughout the experiment. Five experimental groups were established as below and done in duplicate. The fish was fed approximately 5% of body weight twice a day [110].

(1) Fish fed with tilapia feed pellets (control group)	30 fish
(2) Fish fed with LGG free encapsulated capsules (AL+MP)	30 fish
(3) Fish fed with tilapia feed pellets incorporated with LGG (LGG)	30 fish
(4) Fish fed with tilapia feed pellets with JA (JA)	30 fish
(5) Fish fed with encapsulated synbiotic tilapia feed pellets (PFL)	30 fish

3.2.4.2 Growth performance determination

The fish was weighed at day 0 and 30 of feeding and calculated for weight gain, specific growth rate and feed conversion ratio. The equations are described below. By according to the following equations [113].

$$\text{weight gain (\%)} = \frac{(\text{final mean body weight - initial mean body weight})}{\text{initial mean body weight}} \text{ x } 100$$

$$\text{specific growth rate} = \frac{\ln(\text{final body weight}) - \ln(\text{initial body weight})}{\text{day}} \text{ x } 100$$

$$\text{feed conversion ratio} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}$$

3.2.4.3 Blood chemistry analysis

At the end of the experimental period, fish was not fed for 18 h prior to blood collection. Seven fish from each group was removed from the tank and anesthetized with 0.2% of 2-phenoxyethanol. Blood samples were collected from the caudal vein using a hypodermic syringe and analyzed for glucose, triglyceride, cholesterol, total protein, glucose, triglyceride, cholesterol, total protein, albumin, blood urea nitrogen (BUN), total bilirubin, direct bilirubin, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, calcium, chloride, magnesium, and iron.

3.2.4.4 Measurement of villous height

To carry out intestinal histopathology, intestinal samples were fixed in 10% buffered formalin. The fixed tissues were embedded into paraffin blocks according to standard histological techniques and the 4 μm tissue sections were stained with hematoxylin and eosin. The villous height was measured from the villous tip to the bottom of the 10 tallest villi per section. An average of these10 villi per section was expressed as the mean villous height for each section [15].

3.3.1 Statistical analysis

All values were means and standard deviations of determinations. Means values from statistical analysis was conducted with SPSS Statistics software (version 24). One-Way ANOVA and Duncan test were carried out to determine differences all treatments at the significance level of $P \leq 0.05$.

CHAPTER 4

RESULTS AND DESCUSSION

4.1 Effects of Jerusalem artichoke on viability of LGG in alginate capsules after freezing, freeze drying, and in simulated Nile tilapia gastrointestinal conditions

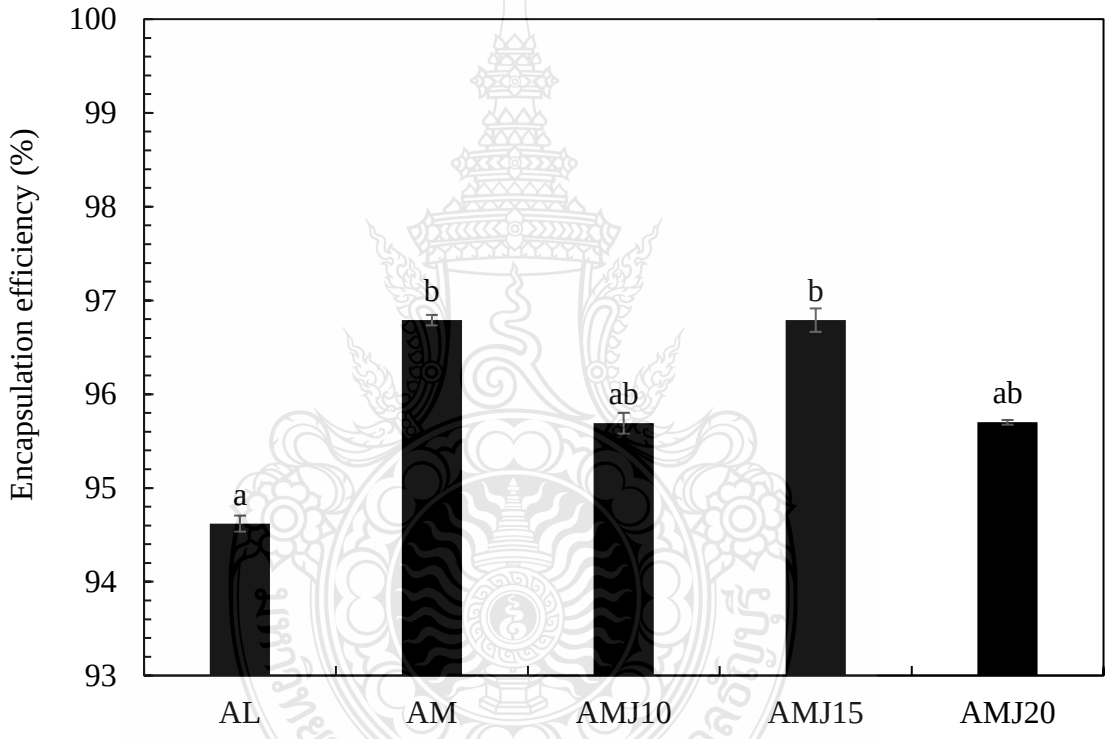
4.1.1 Encapsulation efficiency of alginate encapsulated capsules containing LGG

The result indicated that all treatments had less than one log reduction in cell viability after encapsulation, which were 0.53, 0.31 0.42, 0.45, and 0.42 log reduction, respectively. This suggested that the extrusion technique was suitable for encapsulation of LGG, this technique is simple and gentle. It did not harm probiotic cells and did not require high temperatures and the use of organic solvents [95]. Moreover, it was reported that alginate was categorized as a nanoporous polymer having pore size between 5 and 200 nm [114]. As well known, bacteria size is about 0.2 μm in diameter and 2-8 μm in length were entrapped inside of the capsules. The %EE of all treatments was between 94.62±0.09% and 96.79±0.03% (Figure 4.1). The highest %EE was found in AM, while JA caused slight reduction of %EE. However, it was not significantly different. This was probably due to densely packed structure of added JA, causing partly block the cell entrapment [115].

4.1.2 Effects of freezing and freeze drying on viability of LGG

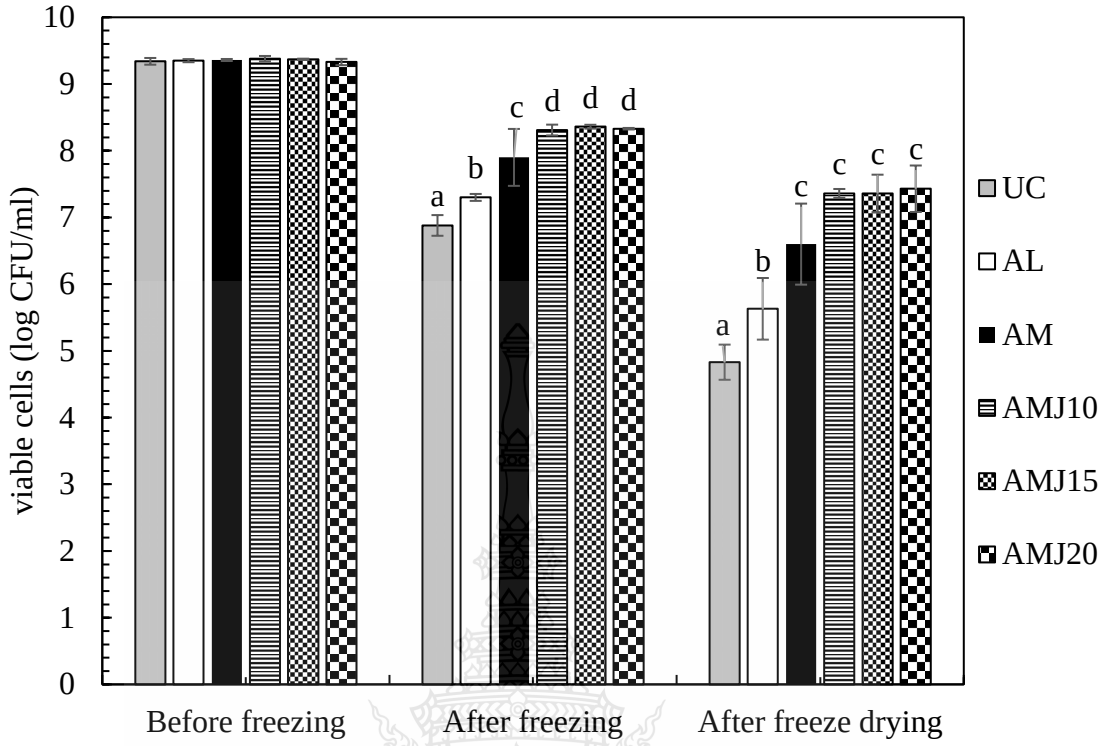
For long-term storage, probiotics are usually preserved by freeze drying. In this study, the alginate capsules were freeze-dried. As shown in Figure 4.2, after freezing, the viability of LGG in UC, AL and AM was reduced by 2.46, 2.05, and 1.46 log CFU/mL, respectively. Addition of JA could significantly increase the number of viable cells after freezing. However, the concentrations of JA did not significantly affect the cell viability. The number of viable cells in AMJ10, AMJ15, and AMJ20 was reduced by 1.07, 1.01, and 1.00 log CFU/mL, respectively. According to, [116] the loss of cell viability after freezing was mainly caused by extracellular ice formation. Alginate encapsulation functioned as a physical barrier protecting the cells from the freezing process. Milk powder offered a surface for probiotic adherence, helping the cells avoid effects of ice crystallization and extracellular osmolality [54].

In addition, it was reported that JA contained large amount of inulin, which were 16-20% inulin with both low and high degree of polymerization (DP) [117]. Mentioned that Low DP inulin in JA could penetrate into the cell membrane and acted as a buffer layer against ice formation when the beads encapsulated cells were frozen [118]. Concurrently, high DP inulin adsorbed on the surface of cells and formed viscous layers, helping lower the rate of ice growth by increasing the solution viscosity [119]. This would support our finding that the capsules with JA had better number of viable cells after freezing when compared to AM and controls.



Figures 4.1 Encapsulation efficiency of alginate encapsulated capsules containing LGG; AL= LGG capsule with only alginate, AM = LGG capsule with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG capsule with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

[a-b]Means±standard deviation with different letters were significantly different ($P \leq 0.05$).



Figures 4.2 Viability of LGG in alginate encapsulated capsules before and after freezing and after freeze drying; UC = unencapsulated cells, AL= LGG capsule with only alginate, AM = LGG capsule with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG capsule with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

[a-b]Means±standard deviation with different letters within the same treatment were significantly different ($P \leq 0.05$).

After freeze drying**,** the viability of LGG was continuously reduced. Our result suggested that AL had significant higher the number of viable cells than un-encapsulated cells. Removal of water from bacteria cells led to cell membrane transition and leakage [120]. The number of cell reduction in AL was 3.72 log cycles, while the fresh cell had 4.51 log reduction. Similarly to the result of cell viability after freezing, milk powder combined with JA could increase the cell survival after freeze drying. The number of viable cells in AM was reduced by 2.76 log reduction. AMJ10, AMJ15, and AMJ20 had cell reduction at 2.02, 2.01, and 1.9 log cycles, respectively. However, they were not significantly different. The protein in milk powder can accumulate within the cells,

resulting in reduction of the osmotic difference between the internal and external environments [121]. Combination of JA and milk powder helped improve cell protection from freeze drying damage more effectively than only milk powder. Sugars and some polysaccharides can function as a water replacer [120], helping stabilize the cell membrane during dehydration.

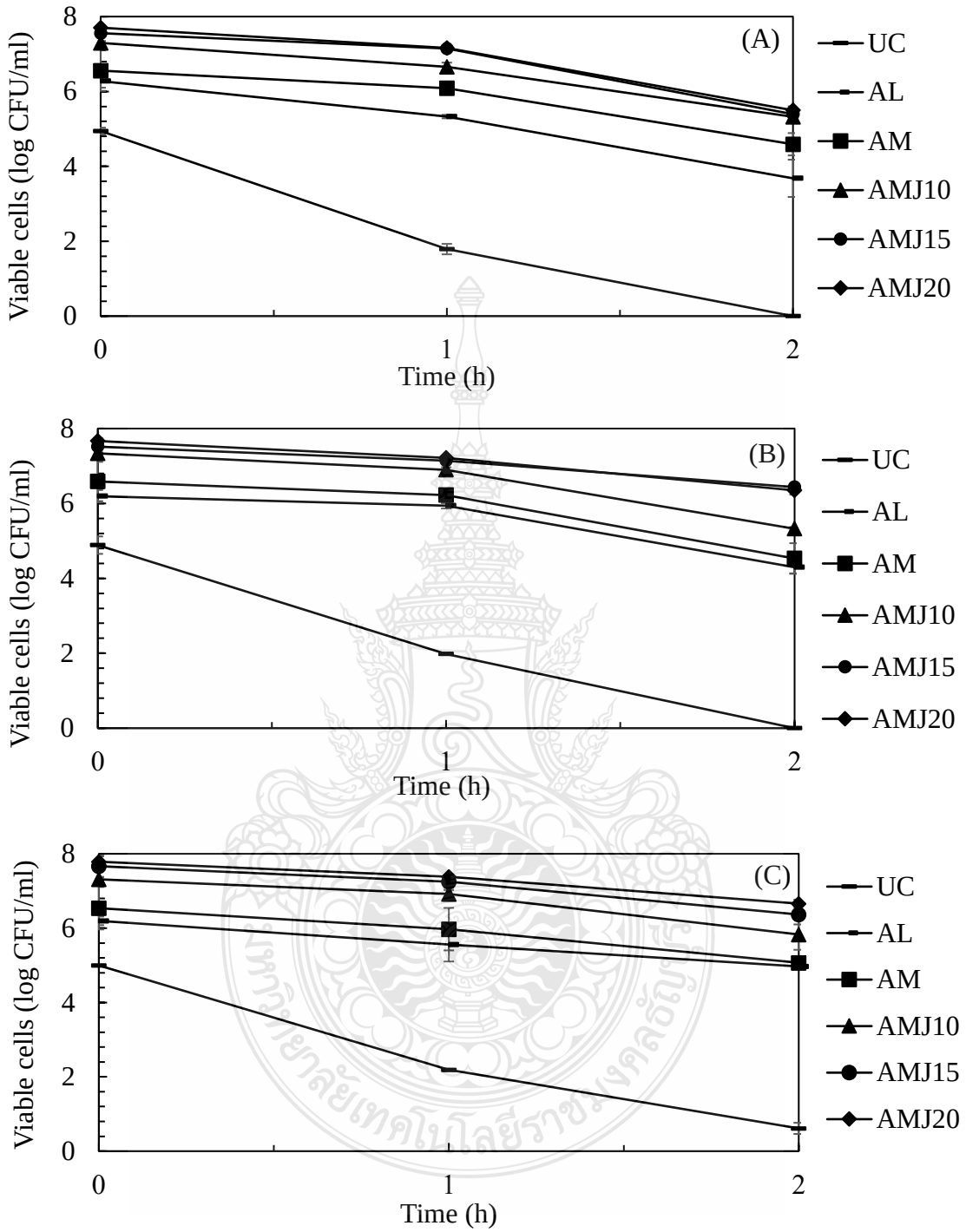
4.1.3 Viability of freeze dried alginate encapsulated LGG in simulated gastric conditions

The stomach is a crucial section for pH-sensitive components such as probiotic cells. In the fasted state, the stomach of healthy subjects has a range of pH from 1.3 to 2.5 [122] . Figure 4.3, showed the number of viable cells in freeze dried capsules after exposure to SGF. The viability of encapsulated LGG at pH 3.0 and 2.0 significantly less decreased than that at pH 1.5.

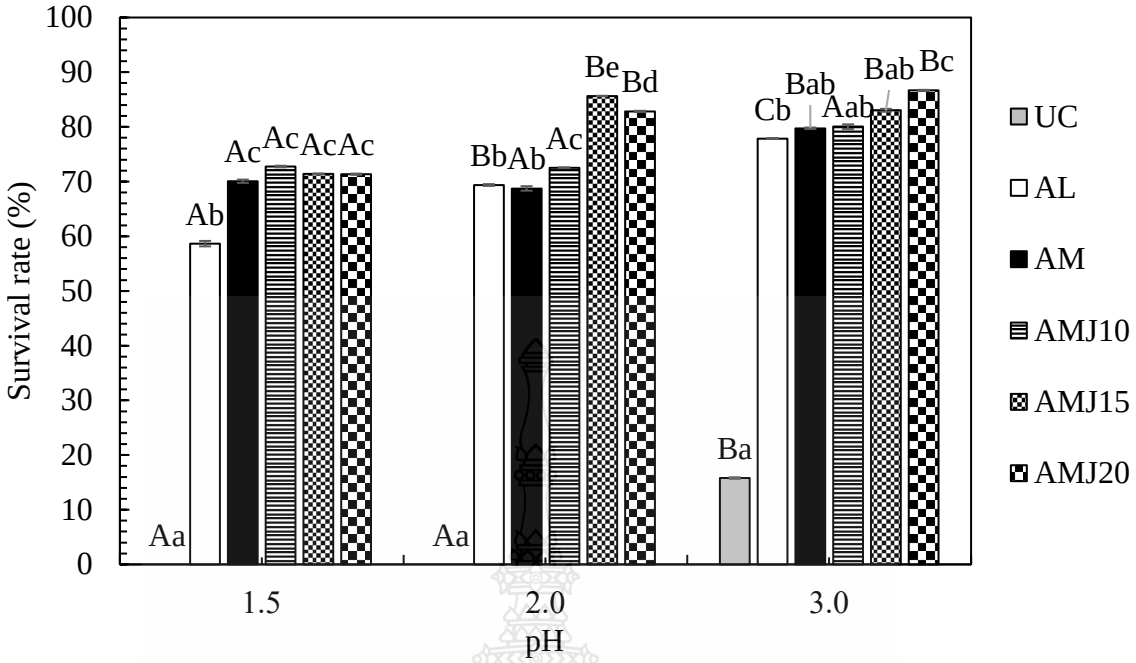
This showed that pH in the medium played an important role in cell viability by inhibiting bacterial growth and their activity by passage of undissociated acid forms through the cell membrane [123]. Additionally, the results indicated that after 2 h incubation in SGF, the cell viability was remarkably improved by alginate encapsulation, while no viable cells were detected in the un-encapsulated cells at pH 1.5 and 2.0. Regarding pH 2.0, it was found that JA helped increase LGG viability. AMJ15 had significantly greater survival rate than AMJ 20 and AMJ15, which were 85.65±0.03%, 82.82±0.12%, and 72.54±0.05%, respectively (Figure 4.4). Survival rates of AL and AM had no significant differences.

4.1.4 Viability of alginate encapsulated LGG in bile salt conditions

After incubating in bile salt conditions (3 mL/10 mL, 5 mL/100 mL and 10 mL/100 mL) for 3 h, high bile salt solution tolerance was observed for encapsulated cells when compared with un-encapsulated cells. Regardless to JA concentrations, AMJ showed the highest number of viable cells, followed by AM and AL, respectively, while fresh cells had no viable cells detected after incubating for 3 h (Figure 4.5). It was revealed that LGG were reduced by 4 log CFU/mL in 3% bile salt solution at 37 °C after 4 h incubation [124]. This resulted from that bile salt functions as an emulsifier and fat solubilizer, hydrolyzing plasma membranes of bacteria cells [125].



Figures 4.3 Viability of alginate encapsulated LGG (dried capsule) in stimulated gastric fluids (pH 1.5 (A), pH 2.0 (B), and pH 3.0 (C)); UC = unencapsulated cells, AL= LGG capsule with only alginate, AM = LGG capsule with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG capsule with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

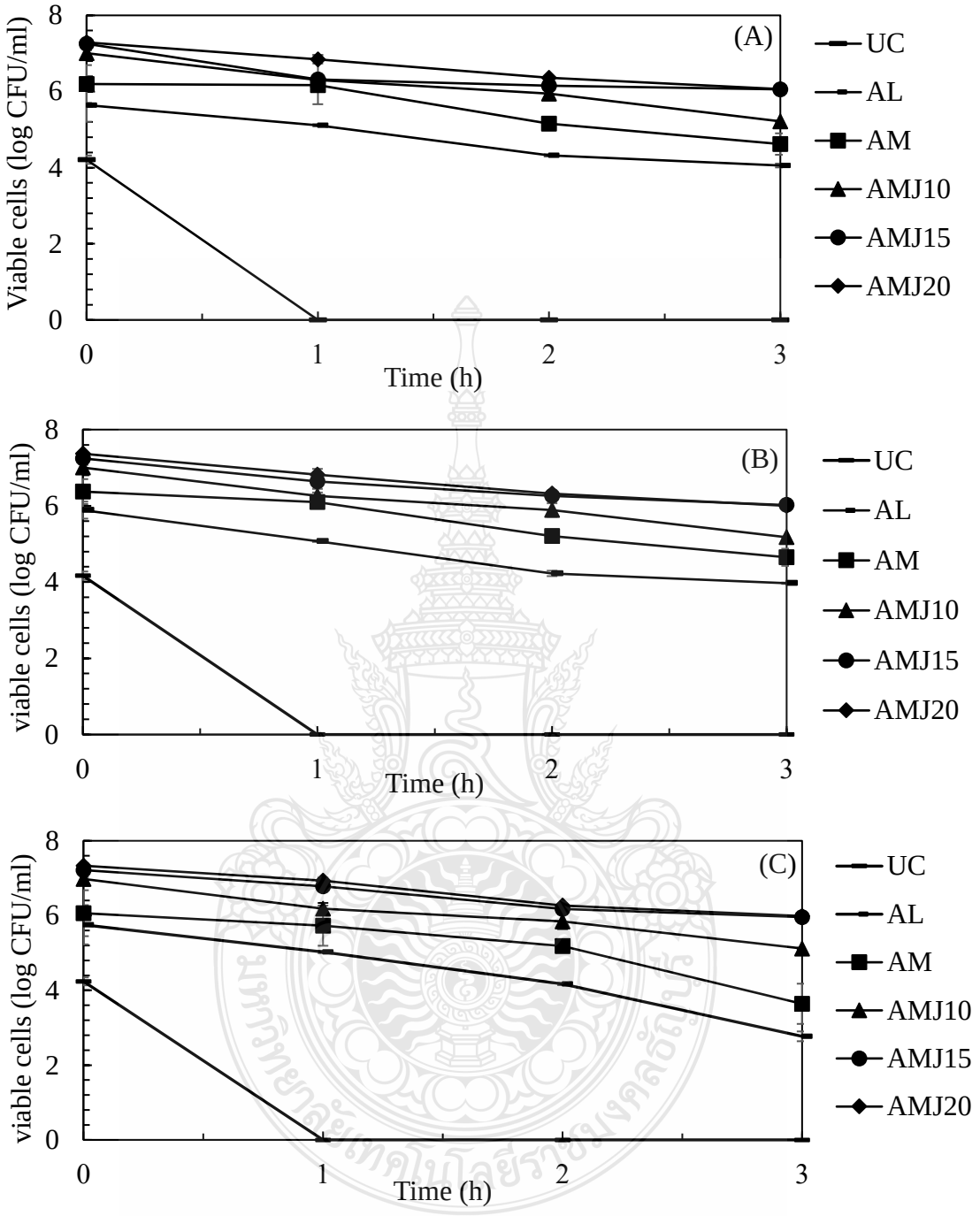


Figures 4.4 Survival rates of LGG after incubating in low pH conditions for 2 h; UC = unencapsulated cells, AL= LGG capsule with only alginate, AM = LGG capsule with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG capsule with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

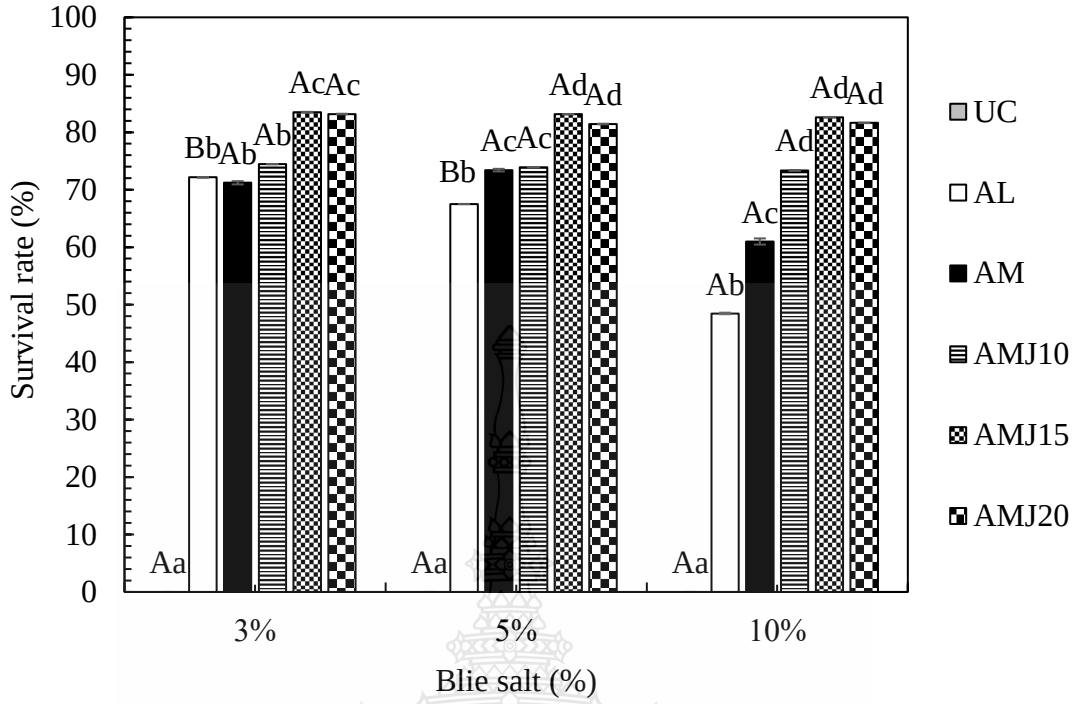
[a-b]Means±standard deviation with different letters within the same treatment were significantly different ($P \leq 0.05$).

[A,B]Means±standard deviation with different letters within the same color were significantly different ($P \leq 0.05$).

It was mentioned that the alginate beads could absorbed the bile salt, resulting in delay of bile salt permeability into the beads [93]. Besides, addition of JA in to the alginate encapsulated capsules could enhance the survival rates of LGG. At 10% bile salt, survival rates of LGG in AMJ10, AMJ15, and AMJ20 were 73.36±0.02%, 82.62±0.05%, and 81.63±00.04%, respectively, which were significantly higher than AM (60.99±0.15%) and AL (48.47±0.13%) (Figure 4.6). Regarding to 3% and 5% bile salts, survival rates of AMJ15 and AMJ20 was significant higher than AMJ10. This was probably that JA possibly provided the capsules with greater networks and diffusion path lengths, reducing penetration of bile solution and consequently decreasing cell losses.



Figures 4.5 Viability of alginate encapsulated LGG (dried capsule) in bile salt conditions (bile salt 3 mL/10 mL (A), bile salt 5 mL/100 mL (B), and bile salt 10 mL/100 mL (C)); UC = unencapsulated cells, AL = LGG capsule with only alginate, AM = LGG capsule with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG capsule with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.



Figures 4.6 Survival rates of LGG after incubating in bile salt conditions for 3 h; UC = unencapsulated cells, AL= LGG capsule with only alginate, AM = LGG capsule with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG capsule with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

$^{a-b}$Means±standard deviation with different letters within the same treatment were significantly different ($P \leq 0.05$).

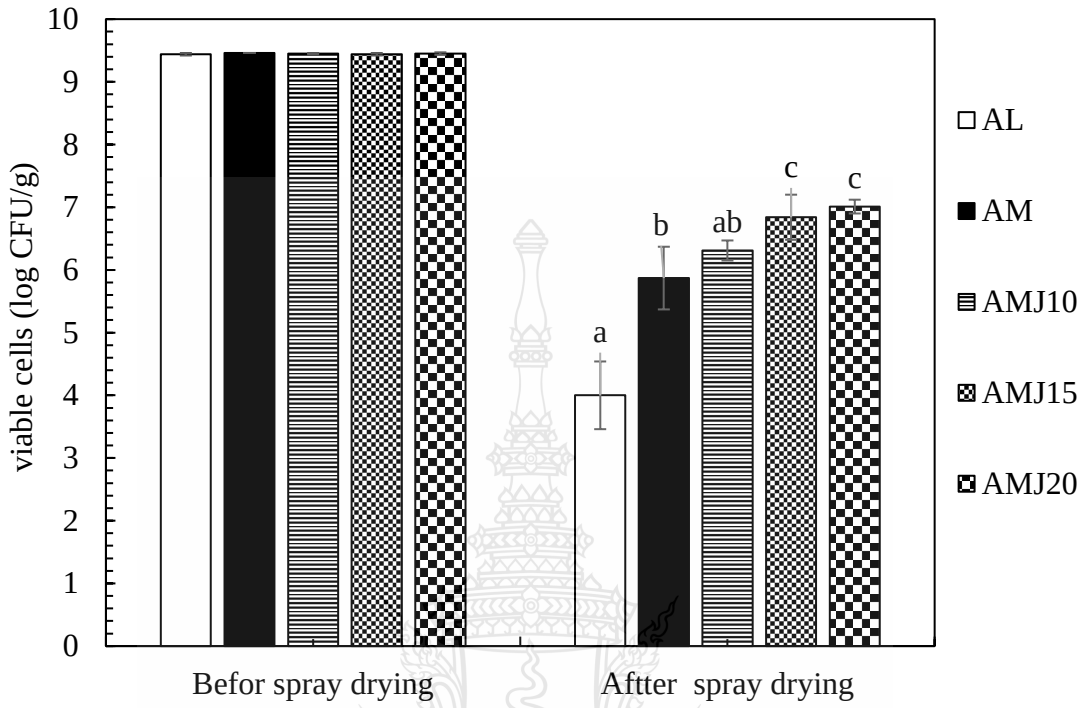
^{A,B}Means±standard deviation with different letters within the same color were significantly different ($P \leq 0.05$).

4.2 Effects of Jerusalem artichoke on viability of LGG after spray drying and in stimulated Nile tilapia gastrointestinal conditions

4.2.1 Production of spray dried LGG

After spray drying, the viability of LGG was obviously reduced. The number of viable cells in AL (4.00±0.54 log CFU/g) was significantly lower than that of AM (5.87±0.50 log CFU/g) and AMJ samples. The cell survival of AL and AM were 42.4±5.62% and 62.1±5.31%, respectively. The number of viable cells in AMJ samples were not significantly different, which were 7.01±0.11, 6.84±0.36, and 6.31±0.16 log CFU/g for AMJ20, AMJ15, and AMJ10, respectively, while the cell survival was

undergone for 74.3±1.11%. 72.4±3.86%, and 66.8±1.65% for AMJ20, AMJ15, and AMJ10, respectively (Figure 4.7).



Figures 4.7 Viability of LGG powders before and after spray drying; AL= LGG powder with only alginate, AM = LGG powder with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG powder with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

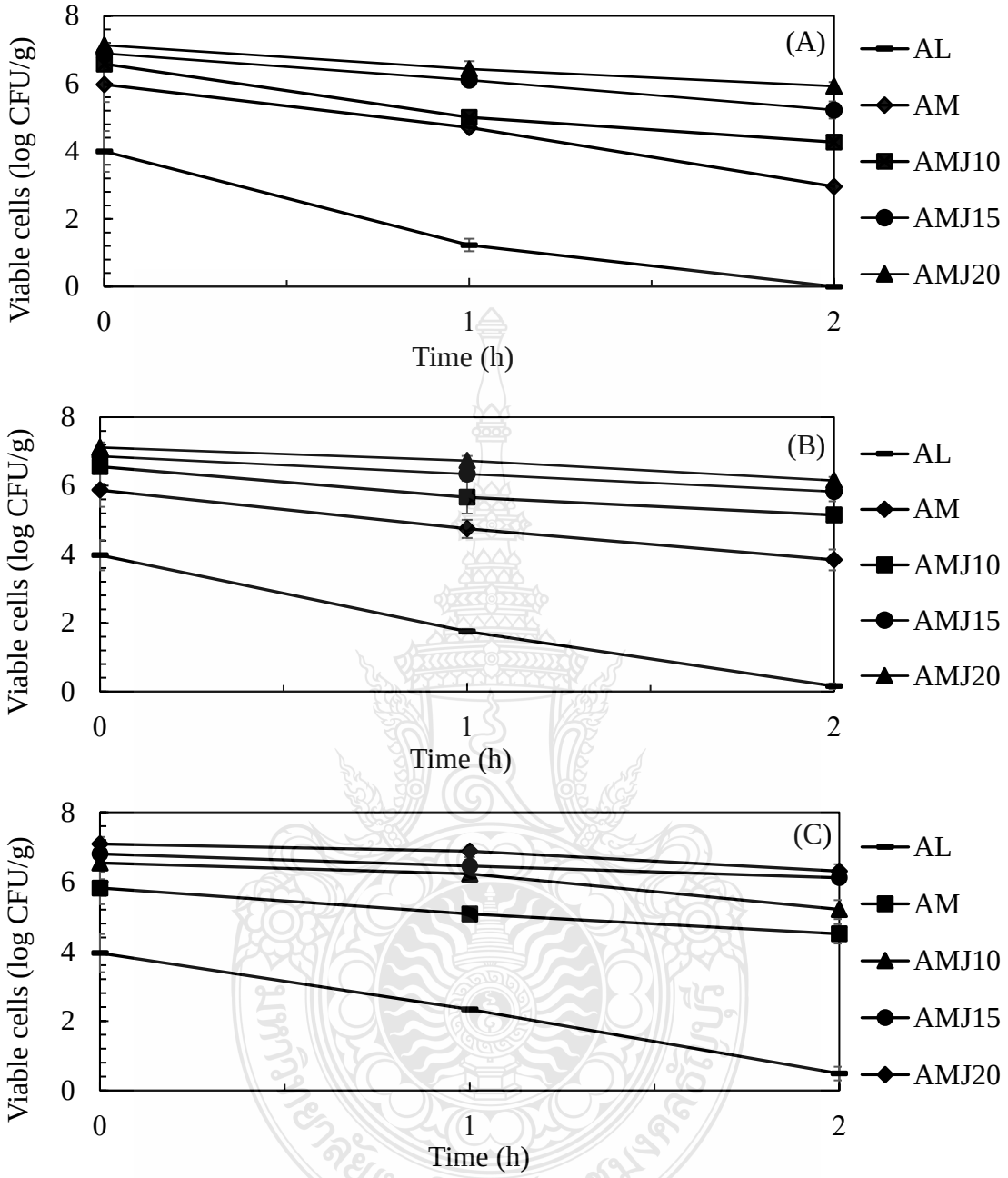
[a-b]Means±standard deviation with different letters within the same treatment were significantly different ($P \leq 0.05$).

However, AMJ20 and AMJ15 had significantly higher number of viable cells than AM ($P<0.05$). The results thus indicated that milk powder and JA played important roles in the cell viability during spray drying. It has been suggested that during drying water was removed from the bacteria cells, leading to cell membrane transition and leakage resulting in the cells death [120]. Milk protein is well-known for its ability to protect the cells from dehydration processes. The proteins in milk powder accumulate within the cells, helping stabilize cell membrane constituents and reducing the osmotic difference between the internal and external environments [121]. Lactose in milk also could interact with the polar head groups of phospholipids and proteins of the cell membrane,

consequently minimizing leakage of the membrane during spray drying [126]. In addition, it was recently reported that milk calcium possibly interacted with the bacterial cell wall to form a protective barrier [127]. The calcium in milk could cause milk protein aggregation during heat treatment, providing the protection of *L. rhamnosus* GG during thermal convective spray drying [128]. Sugars and some polysaccharides such as fructooligosaccharides (FOS) and inulin were reported to be able protect the cells from dehydration. JA typically contains 39 g/100 mL of inulin and 16 g/ 100 mL of fructo-oligosaccharide [112]. It was reported that FOS and inulin could function as a water replacer, helping stabilize the cell membrane during dehydration [120]. It was revealed that the amorphous structures of inulin could protect *L. plantarum* CIDCA 83114 during spray drying and storage and their cell stability directly, resulted from stabilization of inulin structures [120].

4.2.2 Viability of spray dried LGG powders in simulated gastrointestinal conditions (*In vitro* simulated gastric conditions)

As well-known, pH in the medium played an important role in inhibiting bacterial activity by passage of un-dissociated acid forms through the cell membrane, causing acidification of the cytoplasm and the cell death [129]. The maintenance of probiotic viability throughout the stomach is crucial. Figure 4.8 showed the number of viable cells in LGG powders after to simulated gastric fluids (SGF) at different pH for 2 h. LGG survived in the SGF at pH 3.0 and 2.0. The number of viable cells of all treatments at pH 3.0 and 2.0 were not significantly difference. However, remarkable decreases in the number of viable cells were significantly observed in SGF at pH 1.5. Regarding pH 1.5, the viable cell counts were enhanced by encapsulating LGG in milk powder and JA. The cell log reductions of AMJ20, AMJ15, and AMJ10 were 1.21, 1.67, and 2.31, respectively, while AM, having only milk powder, had 3.02 log reduction. No viable cells were detected in the control or in AL.



Figures 4.8 Viability of LGG powders in stimulated gastric fluids (pH 1.5 (A), pH 2.0 (B), and pH 3.0 (C)); AL= LGG powder with only alginate, AM = LGG powder with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG powder with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

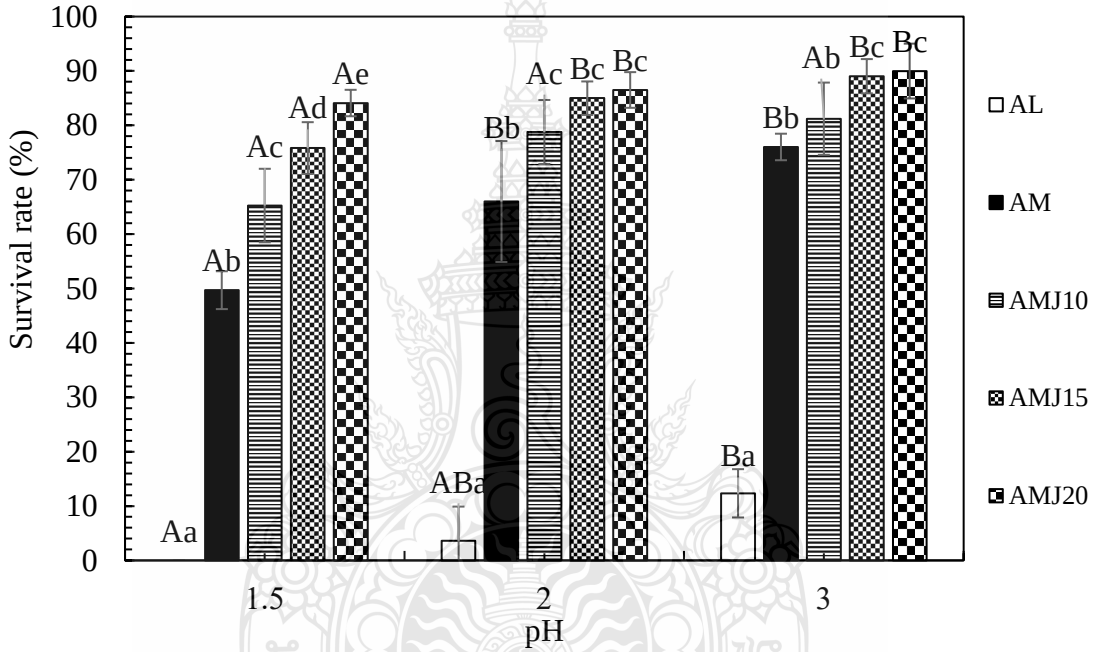
The result show that, AMJ20 and AMJ15 had survival rate at 84.10±2.43% and 75.82± 4.76%, respectively (Figure 4.9), which were significantly greater than that of AMJ10 (65.25±6.76%) and AM (49.69±3.48%). The results indicated that JA could protect LGG from high acid conditions. This was possibly attributed to chemical interactions between alginate and inulin of JA. It was reported that alginate was able to create interactions with inulin by the nucleophilic attack between the COO^- group of alginate and OH^- group of inulin, which leading to alginate-inulin matrix formation. The matrix probably improved the barrier protection to the bacterial cells by mitigating the migration of acid fluids to the cells. Moreover, it was mentioned that the presence of inulin in alginate matrix could decrease the porosity of the alginate when it was in gastric environment with low pH [131].

4.2.3 *In vitro* tilapia bile salt conditions

The viability of LGG powders after incubating in bile salt conditions (3 mL/100 mL, 5 mL/100 mL, and 10 mL/100 mL of fluids) for 3 h was shown in Figure 4.10 Bile salt is as an emulsifier and fat solubilizer that could hydrolyze plasma membranes of bacteria cells. This causes the cells to lose their cell wall integrity [132]. No viable cells were detected in the control (AL) for all bile salt conditions after exposure for 1 h. The cell losses were increased with the increasing of bile salt concentrations in all treatments and control. Regardless of bile salt concentrations, after incubating in bile salts for 3 h, AMJ20 had the lowest cell reductions, which were 0.86, 1.10, and 1.27 log reductions for 3 mL/100 mL, 5 mL/100 mL, and 10 mL/100 mL of tilapia bile salt, respectively, followed by AMJ15, AMJ10, and AM.

In regard to survival rates of LGG, at 3 mL/100 mL of fluids and 5 mL/100 mL of fluids, AMJ20 had significantly greater survival rates than AMJ15 and AMJ10, while AM had the lowest survival rate. Similarly, the LGG survival rate of AMJ 20 in bile salt at the concentration of 10 mL/100 mL of fluids was 80.52±0.46 %, which was significantly higher than AMJ15 (62.78 ± 7.52%) and AMJ10 (61.26± 1.39%) and followed by AM (44.93±2.74 %) (Figure 4.11). Therefore, this indicated that milk powder could protect LGG in the powders from bile salt effects. JA had ability to enhance the protection of the cells during bile salt conditions. The results are compatible with those of [133] mentioning that milk powder-alginate matrix could improve cell viability of

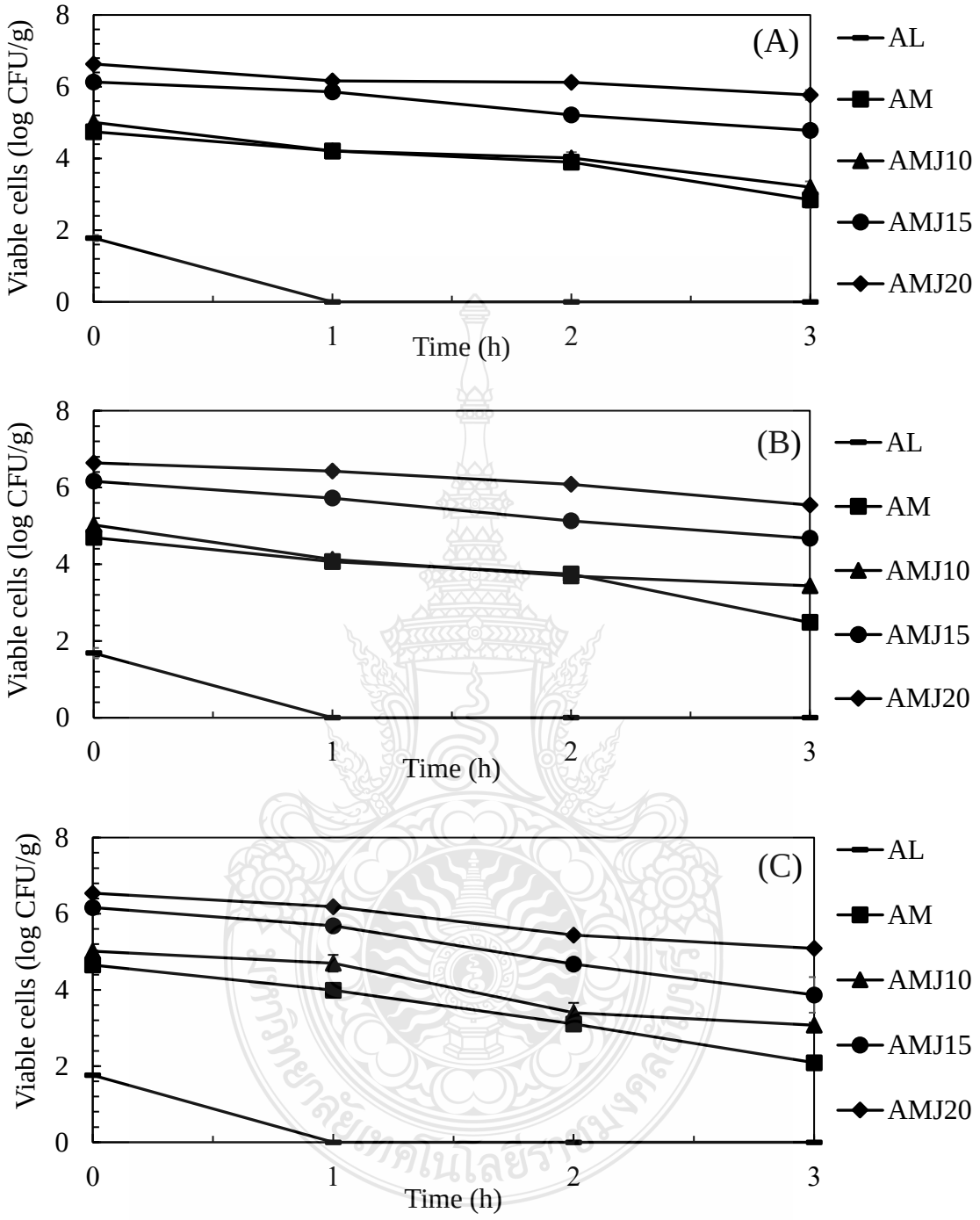
L. rhamnosus GG after being exposed in 10% tilapia bile when compared with only alginate matrix and fresh cells. This could be due to protein in skim milk possibly acting as an insoluble matrix protecting probiotics during gastrointestinal tract [134]. In addition, alginate and milk powder could structurally bind together increasing the matrix resistance to the effects of bile salt solutions. As mentioned earlier, JA was a rich source of inulin and FOS. *L. plantarum* that were encapsulated in milk powder coated inulin-alginate beads by an extrusion method had 1.21 log CFU/mL reduction after incubation in 1% bile salt solution treatment for 2 h [72].



Figures 4.9 Survival rates of LGG powder after incubating in low pH conditions for 2 h; AL = LGG powders with only alginate, AM = LGG powder with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG powder with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

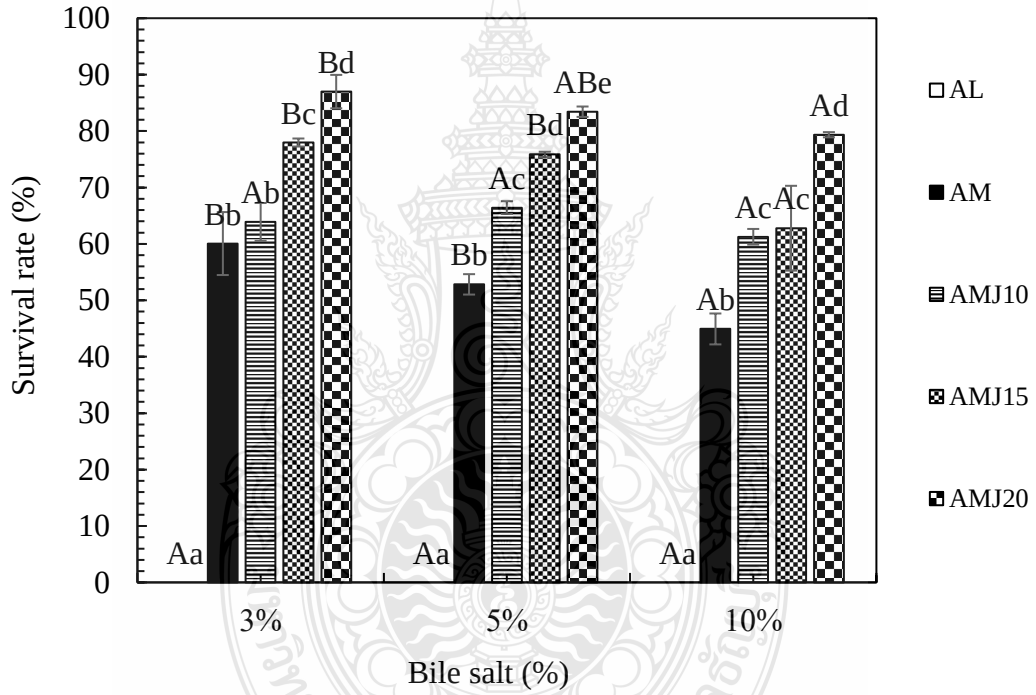
$^{a-b}$Means±standard deviation with different letters within the same treatment were significantly different ($P \leq 0.05$).

^{A,B}Means±standard deviation with different letters within the same color were significantly different ($P \leq 0.05$).



Figures 4.10 Viability of LGG powders in bile salt conditions (bile salt 3 mL/10 mL (A), bile salt 5 mL/100 mL (B), and bile salt 10 mL/100 mL (C)); AL = LGG powder with only alginate, AM = LGG powder with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG powder with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

Addition of inulin could enhance the acid and bile tolerance of encapsulated *L. acidophilus* and *L. casei* powders, resulting in higher number of cells than cultured cells after they were put into simulated gastric juice (pH 1.55) for 2 h, followed by intestinal juice containing 0.6% bile salt for 2.5 h [93]. Spray drying of *L. acidophilus* La-5 with 10% of inulin and 6% of FOS as coating agents could significantly reduce the number of cell reduction when they were in simulated intestinal phases. Moreover, [135] noted that protection provided by the coating agents was further elevated by food products. This indicates another possible advantage of incorporating the probiotic into the fish feed.



Figures 4.11 Survival rates of LGG powder after incubating in bile salt conditions for 3 h; AL = LGG powder with only alginate, AM = LGG powder with alginate and milk powder, AMJ10, AMJ15, and AMJ20 = LGG powder with alginate, milk powder, and JA at 100, 150, and 200 g/L respectively.

[a-b]Means±standard deviation with different letters within the same treatment were significantly different ($P \leq 0.05$).

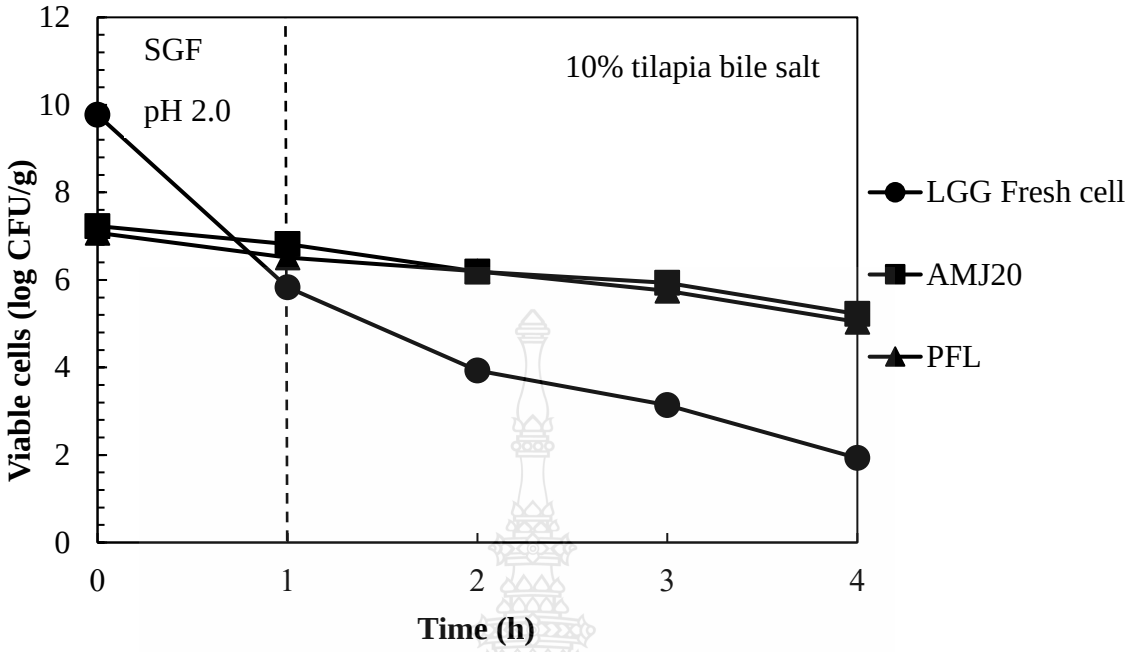
[A,B]Means±standard deviation with different letters within the same color were significantly different ($P \leq 0.05$).

4.3 Development of Nile Tilapia Pelleted Feed Containing LGG and JA

4.3.1 Cell Viability in Nile tilapia pelleted feeds containing LGG

Nile tilapia pelleted feeds containing LGG (PFL) were composed of 55.35 g of carbohydrate/100 g dry feed matter, 24.54 g of protein /100 g dry feed matter, 8.66 g of fat /100g dry feed matter, and 11.13 g of ash /100 g dry feed matter. The moisture content of PFL was 2.71 g/100 g of PFL after drying at 50 °C for 8 h. It was recommended that crude protein levels of tilapia feeds should not under 20 % due to poor palatability. Feed containing approximately 25% of crude protein were commonly used in Thailand as it was cost effective [136]. After pelleting and drying, the final cell concentration of LGG in PFL was 7.08±0.04 log CFU/g, which was not significantly different with the spray dried LGG powder (7.23±0.06 log CFU/g). This indicated that the pelleting and drying processes did not affect the cell viability of LGG powder. The number of viable cells in probiotic products to provide benefits to the host was recommended to be at least 10^6 CFU/g [137].

In the simulated gastrointestinal tract, after incubating the PFL in a low pH condition (pH 2.0) for 1 h prior to expose to bile salt solution at 10 mL/100 mL for 3 h as shown in Figure 4.12, the cell viability in PFL was reduced by 0.57 log CFU/g, which was not significantly different form the spray dried LGG (0.41 log reduction), while rapid loss of LGG fresh cells was detected (3.94 log reduction). The number of viable cells after 1 h incubation in SGF at pH 2.0 were 6.51±0.02, 6.82±0.02, and 5.83±0.04 log CFU/g for PFL, spray dried LGG, and fresh cells, respectively. Regarding bile salt tolerance, the results showed that the number of viable cells in PFL was 5.04±0.03 log CFU/g, followed by the spray dried LGG (5.22±0.04 log CFU/g), which had undergone 2.04 and 2.01 log reduction, respectively. The results were not significantly different. On the contrary, there was a massive decrease in viable cells of the fresh cell, which was 7.84 log cycles. Only 1.93±0.02 log CFU/g survived. The survival rates after 3 h in the bile salt solution were 77.42±0.43%, 76.53±0.44%, and 33.1±0.05% for PFL, spray dried LGG, and fresh cells, respectively. Addition of spray dried LGG into the pelleted feed could potentially provide not only high nutritional and functional values, but also convenience of use and well as decrease of feed preparation times and labor.



Figures 4.12 Viability of LGG in Nile tilapia pelleted feeds during exposure to stimulated gastrointestinal condition; Fresh cell = LGG grown in MRS broth for 24 h, AMJ20 = LGG powder with alginate, milk powder, and JA at 200 g/L, and PLF = pelleted feed containing LGG

4.4 Effects of Nile Tilapia pelleted feed containing *Lactobacillus rhamnosus* GG and Jerusalem articoke on growth performance, blood chemistry and intestines of Nile tilapia

4.4.1 Growth performance

Probiotics are considered as a good alternative to promote fish growth performance by increasing digestibility and nutrient utilization, inhibiting some pathogenic bacteria, and resisting some diseases. Table 4.1 showed the growth performance of tilapias fed with pelleted feeds containing LGG and JA (PFL), including weight gain (%), specific growth rate, feed conversion ratio (FCR), and survival rate (%). Probiotic (LGG), prebiotic (JA), and synbiotics (LGG+JA) in PFL could increase weight gain and specific growth rate of Nile tilapia, while reduced feed conversion ratio when compared to the control group. PFL had significantly greater weight gain (79.86±25.08%) and specific growth rate (1.940±0.466) than other groups. Control had significantly higher feed conversion ratio (2.393±0.376) than other treatments, while that of PFL

(1.976±0.621) was significantly lower than JA (1.963±0.313) but higher than LLG (1.963±0.313). The result indicated that combination of LGG and JA could enhance the growth of Nile tilapia remarkably.

Table 4.1 Growth performance of Nile tilapia after feeding pelleted feed containing LGG and JA for 30 days

Treatments	Initial mean body weight (g)	Mean body weight at 30 days (g)	Weight gain at 30 days (%)	Specific growth rate at 30 days	Feed conversion ratio at 30 days
Control	30.37±2.40	49.48±1.78	63.47±9.95^{a}	1.635±0.202^{a}	2.393±0.376^{e}
AL+MP	28.19±4.75	49.64±4.69	78.32±18.97^{d}	1.919±0.356^{d}	1.973±0.478^{b}
LGG	35.27±2.47	62.35±1.31	77.41±12.35^{c}	1.907±0.232^{c}	1.963±0.313^{a}
JA	32.28±4.44	54.60±3.22	71.00±19.15^{b}	1.778±0.374^{b}	2.192±0.591^{d}
PFL	35.64±7.64	62.74±7.41	79.86±25.08^{e}	1.940±0.466^{e}	1.976±0.621^{c}

Means±standard deviation values in the same column with different letter indicate were significantly different ($P \leq 0.05$).

Similarly, LGG and/or JA separately mixed with red Nile basal tilapia diets for 30 days and determined for their growth performance. It was reported that JA and LGG could improve the weight gain and specific growth rate of red Nile tilapia when they were compared with basal diets but they were not significantly different with symbiotic JA+LGG [111]. This mean that corporation JA and LGG into the pelleted feed could improve the fish growth performance more effectively than separately mixing them with diets. Moreover, milk powder may play an important role in improving fish growth performance due to lactoferrin, a milk glycoprotein that showed inhibitory activity against bacterial pathogens [138]. In addition, it was reported that JA had positive effects on growth performance in Nile tilapia that were comparable to those of inulin. The growth performances (including final weight, specific growth rate, and feed conversion ratio) of Nile tilapia fed the JA diets were superior to those of fish fed the inulin diets. Moreover,

JA also contains various minerals and vitamins including iron, calcium, potassium, vitamin B complex, vitamin C, and vitamin A [85]. These micronutrients in JA also may have had addition al positive effects on growth response and feed utilization in the Nile tilapia.

4.4.2 Blood chemistry

Blood chemical parameters are able to indicate the nutritional and health status of animals. Table 4.2 demonstrated blood chemical parameters of Nile tilapia fed with different diets for 30 days. The result showed that the fish fed with LGG had the higher total glucose but was significantly different with JA and synbiotics (LGG+JA) in PFL as well as AL+MP. The control group showed the lowest total glucose contents. This indicated that LGG could contribute to digestibility improvement enzyme activities such as amylase, protease and lipase which was related to energy contribution and growth performance of the Nile tilapia [111]. However, glucose content of the fish fed with PFL was lower than that of LGG. This could be because PFL contained spray-dried LGG, which might partially damage from the thermal process of spray drying, consequently affecting the activity of the bacteria. However, it was not significantly different.

Fish from the control group had higher T-bilirubin, D-bilirubin and AST. This may indicate that synbiotics (LGG+JA) in PFL had no significantly different with the control, indicating LGG and JA was safe for the fish. ALT and AST are enzyme indicators, which are frequently used in the diagnosis of damage caused by pollutants in various tissues, such as liver, muscle, and gills [141]. It is generally accepted that increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of even minor cellular damage [142]. It was found that blood levels of ALT and AST may increase because of cellular damage in the liver and that high levels of these enzymes in serum are usually indicative of disease and necrosis in the liver of animals. The increased ALT and AST activity in the serum is caused mainly by leakage of these enzymes from liver cytosol into the bloodstream as a result of liver damage [143]. Tissue protein content has been suggested as an indicator of xenobiotic-induced stress in aquatic organisms [144], while total serum protein, the majority of serum proteins synthesized in the liver, is used as an indicator of liver impairment [145].

In addition, the result showed that PFL group had the lower total protein significantly different from the control group but no significant differences in triglyceride, total bilirubin and direct bilirubin. Similarly, BUN of fish fed the control diet and those fed the synbiotics (LGG+JA) showed no significant difference. This demonstrated that LGG, JA and the diet had no effect on the majority of blood serum biochemical parameters in the fish. The LGG+JA would not have any negative effects on blood of Nile tilapia. However, the blood chemistry depends on other factors such as water quality, food quality, cultivation condition and crowding stress. Changes brought about by a stressor could be metabolic in nature, affecting molecular and cellular components such as enzymes or impairing functions such as metabolism, immune response, osmoregulation, and hormonal regulation [146].

4.4.3 Villous height

After 30 days on the treatment diets, all treatments had higher villi height in the proximal intestine than the distal part (Table 4.3 and 4.4). The fish fed with synbiotics (LGG+JA) in PFL showed the highest villi height in proximal part which was 933.36±199.371μm, followed by JA (876.5±165.4μm) and LGG (876.5±165.4μm). This indicated that combination of LGG with JA could enhance the intestinal morphology. The results were confirmed with the study of [109]. The villi functions are associated with nutrient absorption, contributing to the fish growth. The higher the villi were, the greater chance more nutrients were absorbed [109,145] Moreover, it was possible that JA as prebiotics could be fermented by the fish gut microbiota, resulting in the release of short chain fatty acids for intestinal epithelium cells to be used as an energy sources [146,147]. It was reported that direct supplementation with JA exhibited better effects than the purified inulin at the equivalent inulin levels [108].

Table 4.2 Blood chemistry of Nile tilapia fed experimental diets for 30 days

Parameters	Experimental groups					
	Range from reference	Control	AL+SM	LGG	JA	PFL
ALT (SGPT) (U/L)	9.50-42.23	6.85±2.41[b]	6.00±2.16[ab]	5.28±1.38[ab]	4.57±2.07[a]	6.60±2.29[b]
AST (SGOT) (U/L)	34.52-278.75	33.57±12.30	30.00±25.65	26.42±7.23	27.00±11.59	25.60±13.02
BUN (mg/dL)	1.80-18.01	2.00±0.82	2.00±1.15	2.14±0.38	1.42±0.53	2.00±1.10
Glucose (mg/dL)	45.17-51.85	51.85±25.80[a]	72.28±14.94[ab]	81.00±12.01[b]	72.71±19.23[ab]	66.85±21.57 [ab]
Total protein (g/dL)	2.60±6.56	3.04±0.24[b]	2.75±0.18[a]	2.74±0.13[a]	2.78±0.26[a]	2.57±0.08[a]
Triglyceride (mg/dL)	200.00-403.90	392.85±164.12	410.57±172.82	373.42±179.35	287.14±75.14	271.71±237.67
Cholesterol (mg/dL)	161.33-219.23	224.42±48.16[b]	171.71±17.89[a]	185.00±33.95[a]	179.71±24.11[a]	149.42±35.20[a]
Total Bilirubin (mg/dL)	0.031-0.050	0.031±0.012[b]	0.025±0.010[a]	0.030±0.012[b]	0.025±0.010[a]	0.026±0.011[ab]
Direct Bilirubin (mg/dL)	0.015-0.270	0.016±0.014[b]	0.008±0.011[a]	0.011±0.001[a]	0.014±0.001[b]	0.010±0.001[a]

Means±standard deviation values in the same row with different letter indicate were significantly different ($P \leq 0.05$).

SGPT = serum glutamic pyruvic transaminase; SGOT = serum glutamic oxaloacetic transaminase; BUN = blood urea nitrogen.

Range from reference depending on diet formulation [[111,148–151]]

Table 4.3 Morphology villus height of the intestine of fish after feeding pellets feed containing JA and LGG for 30 days.

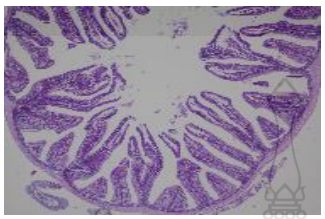
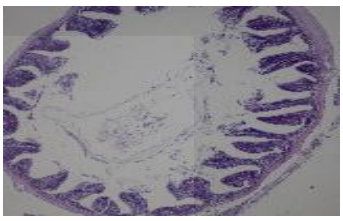
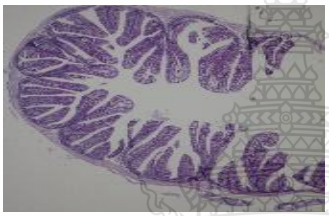
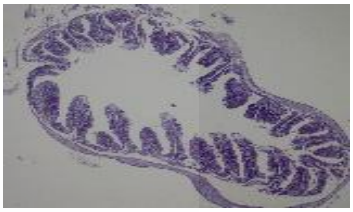
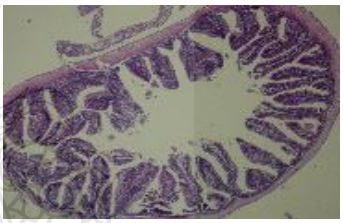
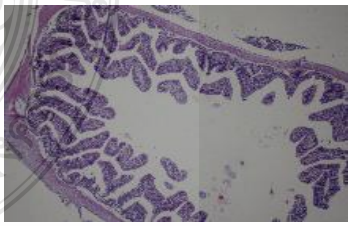
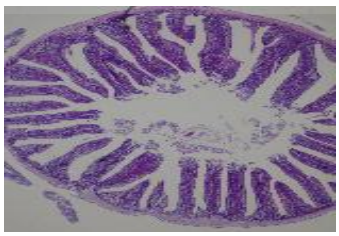
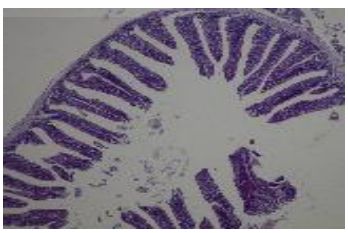
Treatments	Proximal	Distal
Control		
AL+MP		
LGG		
JA		
PFL		

Table 4.4 Average villus height of the intestine of fish after feeding pellets feed containing JA and LGG for 30 days

Treatments	Villous height (μm)	
	Proximal	Distal
Control	611.1±68.4[a]	415.8±45.7[b]
AL+MP	673.0±102.5[ab]	394.5±59.5 [a]
LGG	681.5±86.1[b]	653.6±72.4 [d]
JA	876.5±165.4[c]	687.2±352.9[e]
PFL	933.3±199.3[c]	528.6±56.9[c]

Means±standard deviation values in the same column with different letter indicate were significantly different ($P \leq 0.05$).

CHAPTER 5

CONCLUSION

The data from the experiment indicated that applications of microencapsulation by either gel extrusion or spray drying methods by using alginate-skim milk matrix could protect and improve viability of *L. rhamnosus* GG from freezing and drying and under stimulated gastrointestinal tracts of Nile tilapia. Kantawan or Jerusalem Artichoke (JA) could enhance the cell viability in both capsule and spray dried powder forms. After processing, survival rates of LGG in both capsules and powders were not significantly different. However, addition of JA at 20g/100 mL significantly yielded the highest cell viability when the LGG exposed to tilapia bile salt at 10mL/100mL. Spray dried LGG with JA powders had cell viability higher than LGG capsules with JA. Production of Nile tilapia pelleted feed containing LGG and JA (PFL) by pelleting at ambient temperature and drying at 50°C had no negative effects on cell viability. After pelleting and drying the number of viable cell was over 10^7 CFU/g as it was recommended for probiotic products. Corporation of spray dried LGG with JA powders into the pelleted feeds did not enhance cell viability during exposure to simulated gastrointestinal tract. However, PFL could yield higher weight gain and specific grow rate of the fish and improve the villi height after feeding for 30 days. LGG increased glucose levels in the fish blood as well as the LGG with JA (PFL) group and JA alone. However, PFL and JA groups had no significantly different in glucose levels when compared with that of the fish fed with basal diet. In summary JA could be used as a potential protective agent in for LGG. Corporation of JA and LGG into pelleted feed could be practical nutritional supplement in Nile tilapia.

List of Bibliography

[1] C. Martin, D. P. Morgavi, M. Dorea, "Methane mitigation in ruminants: from microbe to the farm scale," *Animal*., vol. 4, pp. 351–65, 2010.

[2] D. J. Macintosh, S. S. De Silva, "The influence of stocking density and food ration on fry survival and growth in *Oreochromis mossambicus* and O. *niloticus* female × O. *aureus* male hybrids reared in a closed circulated system," *Aquaculture*., vol. 41, pp. 345–58, 1984.

[3] R. Harikrishnan, C. Balasundaram, M. S. Heo," *Lactobacillus sakei* BK19 enriched diet enhances the immunity status and disease resistance to *streptococcosis* infection in kelp grouper, *Epinephelus bruneus*," *Fish Shellfish Immunol*., vol. 29, pp. 1037–43, 2010.

[4] H. C. P. Figueiredo, P. H. Klesius, C. R. Arias, J. Evans, C. A. Shoemaker, D. J. Pereira, "Isolation and characterization of strains of *Flavobacterium columnare* from Brazil," *J. Fish Dis*., vol. 28, pp. 199–204, 2005.

[5] K. Jeffery, D. Stone, S. Feist, S. J. Verner, "An outbreak of disease caused by *Francisella sp*. in Nile tilapia *Oreochromis niloticus* at a recirculation fish farm in the UK. *Dis Aquat Organ.*, vol. 91, pp. 161–5, 2010.

[6] E. Soto, M. Griffin, M. Arauz, A. Riofrio, A. Martinez, M. E. Cabrejos, "*Edwardsiella ictaluri* as the Causative Agent of Mortality in Cultured Nile Tilapia," *J. Aquat Anim Health*., vol. 24, pp. 81–90, 2012.

[7] Y. Li, S. H. Cai, "Identification and Pathogenicity of *Aeromonas sobria* on Tail-rot Disease in Juvenile Tilapia *Oreochromis niloticus*," *Curr Microbiol*., vol. 62(2), pp. 623-627, 2011.

[8] P. Srisapoome, N. Areechon, "Efficacy of viable *Bacillus pumilus* isolated from farmed fish on immune responses and increased disease resistance in Nile tilapia (*Oreochromis niloticus*): Laboratory and on-farm trials," *Fish Shellfish Immunol*., vol. 67, pp. 199–210, 2017.

[9] A. K. Siwicki, D. P. Anderson, O. W. Dixon, "In vitro immunostimulation of rainbow trout (*Oncorhynchus mykiss*) spleen cells with levamisole," *Dev Comp Immunol*., vol. 14, pp. 231–237, 1990.

List of Bibliography (Continued)

[10] D. P. Weston, "Environmental considerations in the use of antibacterial drugs in aquaculture," *Aquaculture*., vol. 149, pp. 331-346, 1996.

[11] FAO/WHO, "Report on Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria," pp. 172, 2001.

[12] D. Roy, "Technological aspects related to the use of *bifidobacteria* in dairy products," *Le Lait.*, vol. 85, pp. 39–56, 2005.

[13] M. Sakai, "Current research status of fish immunostimulants," *Aquaculture*., vol. 172, pp. 63–92, 1999.

[14] M. Sakai, T. Otubo, S. Atsuta, M. Kobayashi, "Enhancement of resistance to bacterial infection in rainbow trout *Oncorhynchus mykiss* (Walbaum) by oral administration of *bovine lactoferrin*," *J. Fish Dis*., vol. 16, pp. 239–47, 1993.

[15] M. Samanya, K. Yamauchi, "Histological alterations of intestinal villi in chickens fed dried *Bacillus subtilis*," *Comp Biochem Physiol Mol Integr Physiol*., vol. 133, pp. 95–104, 2002.

[16] J. L. Stafford, E.C. Wilson, M. Belosevic, "Recombinant transferrin induces nitric oxide response in goldfish and murine macrophages., *Fish Shellfish Immunol*., vol. 17, pp. 171–85, 2004.

[17] S. Misra, N. P. Sahu, A. K. Pal, B. Xavier, S. Kumar, S. c. Mukherjee, "Pre and post-challenge immuno-haematological changes in *Labeo rohita* juveniles fed gelatinised or non-gelatinised carbohydrate with n-3 PUFA," *Fish Shellfish Immunol*., vol. 21, pp. 346–356, 2006.

[18] B. K. Das, C. Debnath, P. Patnaik, D. K. Swain, K. Kumar, B. K. Misrhra, " Effect of β-glucan on immunity and survival of early stage of *Anabas testudineus* (Bloch). *Fish Shellfish Immunol*., vol. 27, pp. 678–683, 2009.

[19] X. Geng, X. H. Dong, B. P. Tan, Q. H. Yang, S. Y. Chi, H. Y. Liu, "Effects of dietary chitosan and *Bacillus subtilis* on the growth performance, non-specific immunity and disease resistance of cobia *Rachycentron canadum*." *Fish Shellfish Immunol.*, vol. 31, pp. 400–406, 2011.

List of Bibliography (Continued)

[20] R. Harikrishnan, M. C. Kim, J. S. Kim, C. Balasundaram, M. S. Heo, "Probiotics and herbal mixtures enhance the growth, blood constituents, and nonspecific immune response in *Paralichthys olivaceus* against *Streptococcus parauberis*," *Fish Shellfish Immunol.*, vol. 31, pp. 310–317, 2011.

[21] M. A. Esteban, A. Cuesta, J. Ortuño, J. Meseguer, "Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata L*.) innate immune system," *Fish Shellfish Immunol*., vol. 11, pp. 303–315, 2011.

[22] S. E. Gilliland, "Acidophilus Milk Products: A Review of Potential Benefits to Consumers," *Journal of Dairy Science*., vol.72, pp. 2483-2494, 1989.

[23] M. K. Tripathi, S. K. Giri, "Probiotic functional foods: Survival of probiotics during processing and storage," *J. Funct Foods*., vol. 9, pp. 225–241, 2014.

[24] A. L. Hattingh, B. C. Viljoen, "Yogurt as probiotic carrier food," *Int Dairy J*., vol. 11, pp. 1–17, 2001.

[25] E. L. Abbot, W. D. Smith, G. P. S. Siou, C. Chiriboga, R. J. Smith, J. A. Wilson, "Pili mediate specific adhesion of *Streptococcus pyogenes* to human tonsil and skin," *Cell Microbiol*., vol. 9, pp. 1822–1833, 2007.

[26] A. C. Ouwehand, "Antimicrobial components from lactic acid bacteria," *Lact Acid Bact*., vol. 5, pp. 139–159, 1998.

[27] M. Ng, D. Y. Lin, S. T. Waller, "Optimal Long-Term Infrastructure Maintenance Planning Accounting for Traffic Dynamics," *Comput-Aided Civ Infrastruct Eng*., vol. 24, pp. 459–469, 2009.

[28] D. Mack, J. Riedewald, H. Rohde, T. Magnus, H. H. Feucht, H.A. Elsner, "Essential functional role of the polysaccharide intercellular adhesin of *Staphylococcus epidermidis* in hemagglutination," *Infect Immun*., vol. 67, pp. 1004–1008, 1999.

[29] S. Caballero, F. Trujillo, J. A. Vianna, H. B. Garrido, M. G. Montiel, S. B. Pedreros, "Taxonomic status of the genus sotalia: species level ranking ranking for "Tucuxi" (*Sotalia fluviatilis*) AND "Costero" (*Sotalia guianensis*) dolphins. *Mar Mammal Sci*., vol. 23, pp. 358–386, 2007.

List of Bibliography (Continued)

[30] M. Montalto, N. Maggiano, R. Ricci, V. Curigliano, L. Santoro, F.D. Nicuolo, *"Lactobacillus acidophilus* protects tight junctions from aspirin damage in HT-29 cells," *Digestion*., vol. 69, pp. 225–228, 2004.

[31] C. G. Llorente, S. Muñoz, A. Gil, "Role of Toll-like receptors in the development of immunotolerance mediated by probiotics," *Proc Nutr Soc*., vol. 69, pp. 381–389, 2010.

[32] F. Yan, H. Cao, T. L. Cover, R. Whitehead, M. K. Washington, D. B. Polk, "Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth," *Gastroenterology*., vol. 132, pp. 562–575, 2007.

[33] E. M. Tuomola, A. C. Ouwehand, S. J. Salminen, "The effect of probiotic bacteria on the adhesion of pathogens to human intestinal mucus," *FEMS Immunol Med Microbiol*., vol. 26, pp. 137–142, 1999.

[34] J. Hirn, E. Nurmi, T. Johansson, L. Nuotio, "Long-term experience with competitive exclusion and salmonellas in Finland," *Int J Food Microbiol.*, vol. 15, pp. 281–285, 1992.

[35] M. Hassan, M. Kjos, I. F. Nes, "Natural antimicrobial peptides from bacteria: characteristics and potential applications to fight against antibiotic resistance," *J Appl Microbiol*., vol. 113, pp. 723–736, 2012.

[36] E. J. Schiffrin, D. Brassart, A. L. Servin, F. Rochat, A. D. Hughes, "Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection," *Am J Clin Nutr*., vol. 66, pp. 515-520, 1997.

[37] J. Hirano, T. Yoshida, T. Sugiyama, N. Koide, I. Mori, T. Yokochi,"The Effect of *Lactobacillus rhamnosus* on Enterohemorrhagic *Escherichia coli* Infection of Human Intestinal Cells In Vitro," *Microbiol Immunol*., vol. 47, pp. 405–409, 2003.

[38] I. G. Rodríguez, B. Sánchez, L. Ruiz, F. Turroni, M. Ventura, P. R. Madiedo, "Role of extracellular transaldolase from *Bifidobacterium bifidum* in mucin adhesion and aggregation," *Appl Environ Microbiol*., vol. 78, pp. 3992–3998, 2012.

List of Bibliography (Continued)

[39] A. C. Ouwehand, S. Salminen, S. Tölkkö, P. Roberts, J. Ovaska, E. Salminen, " Resected human colonic tissue: new model for characterizing adhesion of lactic acid bacteria," *Clin Diagn Lab Immunol*., vol. 9, pp. 184–186, 2002.

[40] M. L. V. Tassell, M. J. Miller, "*Lactobacillus adhesion* to mucus," *Nutrients.*, vol. 3, pp. 613–636, 2011.

[41] B. L. Buck, E. Altermann, T. Svingerud, T. R. Klaenhammer, "Functional analysis of putative adhesion factors in *Lactobacillus acidophilus* NCFM," *Appl Environ Microbiol*., vol. 71, pp. 344–351, 2005.

[42] U. Hynönen, B. W. Westerlund, A. Palva, T. K. Korhonen, "Identification by flagellum display of an epithelial cell and fibronectin binding function in the SlpA surface protein of *Lactobacillus brevis*," *J Bacteriol*., vol. 184, pp. 3360–3367, 2002.

[43] S. Hekmat, D. J. McMahon, "Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice cream for use as a probiotic food," *J Dairy Sci*., vol. 75, pp. 1415–1422, 1992.

[44] D. Granato, G. F. Branco, F. Nazzaro, A. G. Cruz, J. A. F. Faria, "Functional Foods and Nondairy Probiotic Food Development: Trends, Concepts, and Products," *Compr Rev Food Sci Food Saf*., vol. 9, pp. 292–302, 2010.

[45] F. A. M. Klaver, F. Kingme, A.H. Weerkamp, "Growth and survival of bifidobacteria in milk," *Neth Milk Dairy J*., vol. 47, pp. 151-154, 1993.

[46] R. I. Dave, N. P. Shah, "Effectiveness of ascorbic acid as an oxygen scavenger in improving viability of probiotic bacteria in yoghurts made with commercial starter cultures," *Int Dairy J*., vol. 7, pp. 435–443, 1997.

[47] L. J. Marnett, H. K. Hurd, M. C. Hollstein, D. E. Levin, H. Esterbauer, B. N. Ames, "Naturally occurring carbonyl compounds are mutagens in *Salmonella tester* strain TA104," *Mutat Res*., vol. 148, pp. 25–34, 1985.

[48] D. Charalampopoulos, R. A. Rastall, "Prebiotics and Probiotics Science and Technology," *Springer Science & Business Media*., vol. 33, pp. 43–57, 2009.

List of Bibliography (Continued)

[49] K. Kailasapathy, K. Sultana, "Survival and [beta]-D-galactosidase activity of encapsulated and free *Lactobacillus acidophilus* and *Bifidobacterium lactis* in ice-cream," *Aust J Dairy Technol*., vol. 9, pp. 45–62, 2003.

[50] R. R. Watson, V. R. Preedy, "Probiotics, Prebiotics, and Synbiotics: Bioactive Foods in Health Promotion," *Academic Press*., vol. 17, pp. 35–44, 2015.

[51] J. G. Galvão, V. F. Silva, S. G. Ferreira, F. R. M. França, D. A. Santos, Freitas, "β-cyclodextrin inclusion complexes containing *Citrus sinensis* (L.) Osbeck essential oil: An alternative to control Aedes aegypti larvae," *Thermochim Acta*., vol. 608, pp. 14–19, 2015.

[52] R. Pathania, H. Khan, R. Kaushik, M. Khan, "Essential oil nanoemulsions and their antimicrobial and food applications," *Curr Res Nutr Food Sci J*., vol. 6, pp. 626–643, 2018.

[53] G. Özkan, S. E. Bilek, "Microencapsulation of Natural Food Colourants," *Int J Nutr Food Sci.*, vol. 3, pp. 145, 2014.

[54] W. K. Ding, N. Shah, "Survival of Free and Microencapsulated Probiotic Bacteria in Orange and Apple Juices," *Int Food Res J*., vol. 15, pp. 219–232, 2008.

[55] T. C. Pimentel, G. S. Madrona, S. Garcia, S. H. Prudencio, "Probiotic viability, physicochemical characteristics and acceptability during refrigerated storage of clarified apple juice supplemented with *Lactobacillus paracasei ssp*. paracasei and oligofructose in different package type," *LWT - Food Sci Technol.*, vol. 1, pp. 415–22, 2015.

[56] H. Tuorila, A. Cardello, "Consumer Responses to an Off-Flavor in Juice in the Presence of Specific Health Claims," *Food Qual Prefer*., vol. 13, pp. 561–569, 2002.

[57] A. A. Zyrek, C. Cichon, S. Helms, C. Enders, U. Sonnenborn, M. A. Schmidt MA, "Molecular mechanisms underlying the probiotic effects of *Escherichia coli* Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair," *Cell Microbiol.*, vol. 9, pp. 804–816, 2007.

List of Bibliography (Continued)

[58] A. M. Mortazavian, M. EHSANI, M. Mousavi, K. Rezaei, S. Sohrabvandi, J. Reinheimer, "Effect of refrigerated storage temperature on the viability of probiotic micro-organisms in yogurt," *Int J Dairy Technol*., vol. 60, pp. 123–127, 2007.

[59] Y . H. Park, F. Hamidon, C. Rajangan, K. P. Soh, C. Y. Gan, T. S. Lim, "Application of Probiotics for the Production of Safe and High-quality Poultry Meat," *Food Sci Anim Resour*., vol. 36, pp. 567–576, 2016.

[60] B. V. Kumar, S. V. N. Vijayendra, O. V. S. Reddy, "Trends in dairy and non-dairy probiotic products - a review," *J Food Sci Technol*., vol. 52, pp. 6112–6124, 2015.

[61] A. Y. Tamime, "Probiotic Dairy Products," *John Wiley & Sons*., vol. 6, pp. 877–883, 2008.

[62] P. ger, C. Santivarangkna, "Advances in probiotic technology," *CRC Press*., vol. 4, pp. 19–26, 2015.

[63] FAO/WHO, "The state of food and agriculture," *Food & Agriculture Org*., vol.88, 2001.

[64] N. Fu, X. D. Chen, "Towards a maximal cell survival in convective thermal drying processes," *Food Res Int*., vol. 44, pp. 1127–1149, 2011.

[65] Y. Ma, Q. Wang, Y. Xu, X. Huang, A. Korenevsky A, "Microencapsulation of Bacteriophage Felix O1 into Chitosan-Alginate Microspheres for Oral Delivery," *Appl Environ Microbiol*., vol. 74, pp. 4799–4805, 2008.

[66] A. M. Urbanska, J. Bhathena, S. Prakash, "Live encapsulated *Lactobacillus acidophilus* cells in yogurt for therapeutic oral delivery: preparation and in vitro analysis of alginate-chitosan microcapsules," *Can J Physiol Pharmacol*., vol. 85, pp. 884–893, 2007.

[67] A. Talwalkar, K. Kailasapathy, "The role of oxygen in the viability of probiotic bacteria with reference to *L. acidophilus* and *Bifidobacterium spp*. *Curr Issues Intest Microbiol*., vol. 5, pp. 1–8, 2001.

[68] K. Sultana, G. Godward, K. Kailasapathy, "Encapsulation of probiotic bacteria with alginate-starch and evaluation of survival in simulated gastrointestinal conditions and in yoghurt," *Int J Food Microbiol*., vol. 62, pp. 47–55, 2000.

List of Bibliography (Continued)

[69] L. Cui, G. Pan, L. Li, J. Yan, A. Zhang, R. Bian, "The reduction of wheat Cd uptake in contaminated soil via biochar amendment:a two-year field experiment," *Bioresources.*, vol. 7, pp. 5666–5676, 2012.

[70] C. Soukoulis, I. Fisk, "Innovative Ingredients and Emerging Technologies for Controlling Ice Recrystallization, Texture, and Structure Stability in Frozen Dairy Desserts: A Review," *Crit Rev Food Sci Nutr*., vol. 56, pp. 2543–59, 2016.

[71] A. Gani, F. A. Masoodi, U. Shah, S. Asima, "Food Hydrocolloids as Encapsulating Agents in Delivery Systems," *CRC Press*., vol. 4, pp. 34–46, 2019.

[72] L. Wang, X. Yu, H. Xu, Z. Aguilar, H. Wei, "Effect of skim milk coated inulin-alginate encapsulation beads on viability and gene expression of *Lactobacillus plantarum* during freeze-drying," *LWT - Food Sci Technol*., vol. 68, pp. 109–117, 2015.

[73] M. Z. Shabbir, M. M. Nazir, A. Maqbool, M. Lateef, M. A. B. Shabbir, A. Ahmad A, "Seroprevalence of *Neospora caninum* and *Brucella abortus* in dairy cattle herds with high abortion rates," *J Parasitol*., vol. 97, pp. 740–742, 2011.

[74] A. Chotiko, S. Sathivel, "Three protective agents for pectin-rice bran capsules for encapsulating *Lactobacillus plantarum*," *Food Biosci*., vol. 16, pp. 56–65, 2016.

[75] H. Kaplan, R. W. Hutkins, "Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria," *Appl Environ Microbiol.*, vol. 66, pp. 2682–2684, 2000.

[76] N. Tiengtam, P. Paengkoum, S. Sirivoharn, K. Phonsiri, S. Boonanuntanasarn, " The effects of dietary inulin and Jerusalem artichoke (*Helianthus tuberosus*) tuber on the growth performance, haematological, blood chemical and immune parameters of Nile tilapia (*Oreochromis niloticus*) fingerlings," *Aquac Res*., vol. 48, pp. 5280–5288, 2017.

[77] T. Heidebach, P. Först, U. Kulozik, "Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins," *Food Hydrocoll.*, vol. 23, pp. 1670–1677, 2009.

List of Bibliography (Continued)

[78] M. Alander, J. Mättö, W. Kneifel, M. Johansson, B. Kögler, R. Crittenden, "Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of *Bifidobacterium lactis* Bb-12 in the gastrointestinal tract," *Int Dairy J.*, vol. 11, pp. 817–825, 2001.

[79] M. Macfarlane, "Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics," *Journal of Applied Microbiology*., vol. 17, April 2020.

[80] J. Saavedra, A. Tschernia, "Human studies with probiotics and prebiotics: Clinical implications," *Br J Nutr*., vol. 87, pp. 241-246, 2002.

[81] V. Sangwan, S. K. Tomar, B. Ali, R. R. B. Singh, A. K. Singh, S. Mandal, "Galactooligosaccharides Purification Using Microbial Fermentation and Assessment of Its Prebiotic Potential by In Vitro Method," *Phys. rev.*, vol. 13, pp. 777-780, 2014.

[82] M. J. Playne, R. G. Crittenden, "Galacto-oligosaccharides and Other Products Derived from Lactose," *In: McSweeney P, Fox PF, editors. Adv. Dairy Chem.,* Vol. 3, p. 121–201, 2009.

[83] A. Böhm, I. Kaiser, A. Trebstein, T. Henle, "Heat-induced degradation of inulin," *Eur Food Res Technol*., vol. 220, pp. 466–471, 2005.

[84] N. J. Zuidam, V. A. Nedović, "Encapsulation Technologies for Active Food Ingredients and Food Processing," pp. 327-343, 2013.

[85] A. S. Akalin, D. Erişir, "Effects of inulin and oligofructose on the rheological characteristics and probiotic culture survival in low-fat probiotic ice cream," *J Food Sci*., vol.73, pp. 184-188, 2008.

[86] A. V. Rao, N. Shiwnarain, I. Maharaj, "Survival of Microencapsulated *Bifidobacterium pseudolongum* in Simulated Gastric and Intestinal Juices," *Can Inst Food Sci Technol J.,* 1989;22:345–9. vol.22, pp. 345–349, 1989.

[87] G. K. Gbassi, T. Vandamme, "Probiotic encapsulation technology: from microencapsulation to release into the gut," *Pharmaceutics*., vol.4, pp. 149–163, 2012.

List of Bibliography (Continued)

[88] W. P. Voo, P. Ravindra, B. T. Tey, E. S. Chan,"Comparison of alginate and pectin based beads for production of poultry probiotic cells," *J Biosci Bioeng*., vol.111, pp. 294–299, 2011.

[89] J. Burgain, C. Gaiani, C. C. Grimal, C. Jeandel, J. Scher, "Encapsulation of *Lactobacillus rhamnosus* GG in microparticles: Influence of casein to whey protein ratio on bacterial survival during digestion," *Innov Food Sci Emerg Technol.*, vol.19, pp. 233–242, 2013.

[90] N. J. Zuidam, V. Nedovic, "Encapsulation Technologies for Active Food Ingredients and Food Processing," *Springer Science & Business Media;* pp. 269-302, 2009.

[91] S. B. Doherty, M. Auty, C. Stanton, R. P. Ross, G. F. Fitzgerald, A. Brodkorb, "Survival of entrapped *Lactobacillus rhamnosus* GG in whey protein micro-beads during simulated ex vivo gastro-intestinal transit," *Int Dairy J*., vol.22, pp. 31–43, 2012.

[92] J. A. Rowley, G. Madlambayan, D. J. Mooney, "Alginate hydrogels as synthetic extracellular matrix materials," *Biomaterials*., vol. 20, pp. 45–53, 1999.

[93] W. Krasaekoopt, S. Watcharapoka, "Effect of addition of inulin and galacto-oligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice," *LWT - Food Sci Technol*., vol. 57, pp. 761–766, 2014.

[94] S. Gouin, "Microencapsulation: Industrial appraisal of existing technologies and trends," *Trends Food Sci Technol*., vol. 15, pp. 330–347, 2004.

[95] W. Krasaekoopt, B. Bhandari, H. Deeth, "Evaluation of encapsulation techniques of probiotics for yoghurt," *Int Dairy J*., vol. 13, pp. 3–13, 2003.

[96] Z. Lou, H. Wang, D. Wang, Y. Zhang, "Preparation of inulin and phenols-rich dietary fibre powder from burdock root," *Carbohydr Polym*., vol. 78, pp. 666–671, 2009.

[97] J. Choi, S. Y. Lee, "Economic considerations in the production of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) by bacterial fermentation," *Appl Microbiol Biotechnol*., vol. 53, pp. 646–649, 2000.

List of Bibliography (Continued)

[98] M. Bekhit, L. S. González, G. B. Messaoud, S. Desobry, "Encapsulation of *Lactococcus lactis* subsp. lactis on alginate/pectin composite microbeads: Effect of matrix composition on bacterial survival and nisin release," *J Food Eng*., vol. 180, pp. 1–9, 2016.

[99] T. Bilenler, I. Karabulut, K. Candogan, "Effects of encapsulated starter cultures on microbial and physicochemical properties of traditionally produced and heat treated sausages (sucuks)," *LWT*., vol. 75, pp. 425–433, 2017.

[100] H. Gandomi, S. Abbaszadeh, A. Misaghi, S. Bokaie, N. Noori, "Effect of chitosan-alginate encapsulation with inulin on survival of *Lactobacillus rhamnosus* GG during apple juice storage and under simulated gastrointestinal conditions," *LWT - Food Sci Technol*., vol. 69, pp. 365–371, 2016.

101] L. Wang, X. Yu, H. Xu, Z. P. Aguilar, H. Wei, "Effect of skim milk coated inulin-alginate encapsulation beads on viability and gene expression of *Lactobacillus plantarum* during freeze-drying," *LWT - Food Sci Technol*., vol. 68, pp. 8–13, 2016.

[102] C. Anandharamakrishnan, "Spray Drying Techniques for Food Ingredient Encapsulation," *John Wiley & Sons*., vol. 4, pp. 19–24, 2015.

[103] C. Arpagaus, P. John, A. Collenberg, D. Rütti, "10 - Nanocapsules formation by nano spray drying," *Nanoencapsulation Technol. Food Nutraceutical Ind.*, pp. 346–40, 2017.

[104] A. Gharsallaoui, G. Roudaut, O. Chambin, A. Voilley, R. Saurel, "Applications of spray-drying in microencapsulation of food ingredients: An overview," *Food Res Int*., vol. 40, pp. 1107–1121, 2007.

[105] F. P. D. C. Cislaghi, C. D. R. E. Silva, C. B. F. Freire, J. G. Lorenz, S. E. S. Anna, "Bifidobacterium Bb-12 microencapsulated by spray drying with whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage," *J Food Eng*., vol. 113, pp. 186–193, 2012.

List of Bibliography (Continued)

[106] K. Judprasong, S. Tanjor, P. Puwastien, P. Sungpuag, "Investigation of Thai plants for potential sources of inulin-type fructans," *J Food Compos Anal*., vol. 24, pp. 642–649, 2011.

[107] M. Nyman, "Fermentation and bulking capacity of indigestible carbohydrates: the case of inulin and oligofructose," *Br J Nutr*., vol. 87, pp. 163–168, 2002.

[108] S. Boonanuntanasarn, N. Tiengtam, T. Pitaksong, P. Piromyou, N. Teaumroon, "Effects of dietary inulin and Jerusalem artichoke (*Helianthus tuberosus*) on intestinal microbiota community and morphology of Nile tilapia (*Oreochromis niloticus*) fingerlings," *Aquac Nutr*., vol. 24, pp. 712–722, 2018.

[109] N. Pirarat, K. Pinpimai, M. Endo, T. Katagiri, A. Ponpornpisit, N. Chansue N, "Modulation of intestinal morphology and immunity in nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG," *Res Vet Sci*., vol. 91, pp. 92–97, 2011.

[110] P. Nopadon, P. Komkiew, R. Channarong, C. Nantarika, E. O. Lin, K. Takayuki, "Viability and morphological evaluation of alginate-encapsulated *Lactobacillus rhamnosus* GG under simulated tilapia gastrointestinal conditions and its effect on growth performance, intestinal morphology and protection against *Streptococcus agalactiae*," *Anim Feed Sci Technol*., vol. 207, pp. 93–103, 2015.

[111] M. Sewaka, C. Trullas, A. Chotiko, C. Rodkhum, N. Chansue, S. Boonanuntanasarn, "Efficacy of synbiotic Jerusalem artichoke and *Lactobacillus rhamnosus* GG-supplemented diets on growth performance, serum biochemical parameters, intestinal morphology, immune parameters and protection against *Aeromonas veronii* in juvenile red tilapia (*Oreochromis spp*.)," *Fish Shellfish Immunol*., vol. 86, pp. 260–268, 2019.

[112] K. Khuenpet, W. Jittanit, S. Sirisansaneeyakul, W. Srichamnong, "Inulin Powder Production from Jerusalem Artichoke (*Helianthus tuberosus L*.) Tuber Powder and Its Application to Commercial Food Products," *J Food Process Preserv*., vol. 41, pp. 130-197, 2017.

List of Bibliography (Continued)

[113] W. Yanbo, X. Zirong, "Effect of probiotics for common carp (Cyprinus carpio) based on growth performance and digestive enzyme activities," *Anim Feed Sci Technol*., vol. 127, pp. 283–292, 2006.

[114] W. R. Gombotz, S. Wee,"Protein release from alginate matrices," *Adv Drug Deliv Rev*., vol. 31, pp. 267–285, 1998.

[115] A. A. Idoruine, A. Weerkamp, C. W. Weber, "In vitro mineral binding capacity of three fiber sources for Ca, Mg, Cu and Zn by two different methods," *International Journal of Food Sciences and Nutrition*., vol. 47, pp. 4, 1996.

[116] X. C. Meng, C. Stanton, G. F. Fitzgerald, C. Daly, R. P. Ross, "Anhydrobiotics: The challenges of drying probiotic cultures," *Food Chem*., vol. 106, pp. 1406–1416, 2008.

[117] F. Gaggia, B. Biavati, "The role of protective and probiotic cultures in food and feed and their impact in food safety," *Elsevier Enhanced Reader*., pp.1024-1033, 2011.

[118] W. Lingyun, W. Jianhua, Z. Xiaodong, T. Da, Y. Yalin, C. Chenggang C, "Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers," *J Food Eng*., vol. 79, pp. 1087–1093, 2007.

[119] K. Judprasong, S. Tanjor, P. Puwastien, P. Sungpuag, "Investigation of Thai plants for potential sources of inulin-type fructans," *J Food Compos Anal*., vol. 24, pp. 642–649, 2011.

[120] S. B. Doherty, V. L. Gee, R. P. Ross, C. Stanton, G. F. Fitzgerald, A. Brodkorb, "Development and characterisation of whey protein micro-beads as potential matrices for probiotic protection," *Food Hydrocoll*., vol. 25, pp. 1604–1617, 2011.

[121] Y. Mille, J. P. Obert, L. Beney, P. Gervais, "New drying process for lactic bacteria based on their dehydration behavior in liquid medium," *Biotechnol Bioeng*., vol. 88, pp. 71–6, 2004.

List of Bibliography (Continued)

[122] C. Nemeth, A. A. M. Andersson, R. Andersson, E. Mangelsen, C. Sun, and P. Åman, "Relationship of Grain Fructan Content to Degree of Polymerisation in Different Barleys," *Agricultural Science*., vol.14, 2020.

[123] D. Luo, W. Xu, J. Liu, and S. Liu, "Comparison of Hot Air and Superheated Steam Drying of Jerusalem Artichoke (*Helianthus tuberosus L*.) Tubers and Inulin Powder Production 2015. *American Society of Agricultural and Biological Engineers*., vol. 14, 2020.

[124] W. K. Ding, N. P. Shah, "Acid, Bile, and Heat Tolerance of Free and Microencapsulated Probiotic Bacteria," J Food Sci., vol. 72, pp. 446–450, 2007.

[125] M. Begley, C. G. M. Gahan, C. Hill, "The interaction between bacteria and bile," *FEMS Microbiol Rev*., vol. 29, pp. 625–651, 2005.

[126] G. M. Maciel, K. S. Chaves, C. R. F. Grosso, M. L. Gigante, "Microencapsulation of *Lactobacillus acidophilus* La-5 by spray-drying using sweet whey and skim milk as encapsulating materials," *J Dairy Sci*., vol. 97, pp. 1991–1998, 2014.

[127] X. Zheng, N. Fu, S. Huang, R. Jeantet, X. D. Chen, "Exploring the protective effects of calcium-containing carrier against drying-induced cellular injuries of probiotics using single droplet drying technique," *Food Res Int*., vol. 90, pp. 226–234, 2016.

[128] J. Wang, S. Huang, N. Fu, R. Jeantet, X. D. Chen, "Thermal Aggregation of Calcium-Fortified Skim Milk Enhances Probiotic Protection during Convective Droplet Drying," *J Agric Food Chem*., vol. 64, pp. 6003–6010, 2016.

[129] P. Lund, A. Tramont, D. D. Biase, "Coping with low pH: molecular strategies in neutralophilic bacteria," *FEMS Microbiol Rev*., vol. 38, pp. 1091–1125, 2014.

[130] P. Lund, A. Tramonti, D. B. Daniela, "Coping with low pH: molecular strategies in neutralophilic bacteria," *FEMS Microbiology Reviews, Oxford Academic*., vol.38, pp. 1091-1125, 2020.

[131] A. Atia, A. Gomaa, I. Fliss, E. Beyssac, G. Garrait, M. Subirade, "A prebiotic matrix for encapsulation of probiotics: physicochemical and microbiological study," *J Microencapsul*., vol. 33, pp. 89–101, 2016.

List of Bibliography (Continued)

[132] A. Y. Bustos, G. F. D. Valdez, S. Fadda, M. P. Taranto, "New insights into bacterial bile resistance mechanisms: the role of bile salt hydrolase and its impact on human health," *Food Res Int*., vol. 112, pp. 250–262, 2018.

[133] N. Pirarat, K. Pinpimai, C. Rodkhum, N. Chansue, E. L. Ooi, T. Katagiri T, "Viability and morphological evaluation of alginate-encapsulated *Lactobacillus rhamnosus* GG under simulated tilapia gastrointestinal conditions and its effect on growth performance, intestinal morphology and protection against *Streptococcus agalactiae*," *Anim Feed Sci Technol*., vol. 207, pp. 93–103, 2015.

[134] D. Ying, S. Schwander, R. Weerakkody, L. Sanguansri, C. G. Demarchi, M. A. Augustin, "Microencapsulated *Lactobacillus rhamnosus* GG in whey protein and resistant starch matrices: Probiotic survival in fruit juice," *J Funct Foods*., vol. 5, pp. 98–105, 2013.

[135] D. X. D. Santos, A. A. Casazza, B. Aliakbarian, R. Bedani, S. M. I. Saad, P. Perego, "Improved probiotic survival to in vitro gastrointestinal stress in a mousse containing *Lactobacillus acidophilus* La-5 microencapsulated with inulin by spray drying," *LWT*., vol. 99, pp. 404–410, 2019.

[136] R. C. Bhujel, "On-farm feed management practices for Nile tilapia (*Oreochromis niloticus*) in Thailand," vol. 13, pp. 119–130, 2014.

[137] A. K. Anal, S. Singh, "Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery," *Trends Food Sci Technol*., vol. 18, pp. 240–251, 2007.

[138] P. P. Ward, O. M. Conneely, "Lactoferrin: Role in iron homeostasis and host defense against microbial infection. *Biometals*., vol. 17, pp. 203–208, 2004.

[139] T. L. Frde, "Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium," *Environ Pollut Barking Essex*., vol. 109, pp. 277–282, 2000.

List of Bibliography (Continued)

[140] V. Palanivelu, S. Balasubramanian, M. Balasubramanian, "Influence of insecticidal derivative (*Cartap Hydrochloride*) from the marine polychaete on certain enzyme systems of the fresh water fish *Oreochromis mossambicus*," *J Environ Biol Acad Environ Biol India*., vol. 26, pp. 191–195, 2005.

[141] R. B. Harvey, L. F. Kubena, M. H. Elissalde, "Influence of vitamin E on aflatoxicosis in growing swine," *Am J Vet Res*., vol. 55, pp. 572–577, 1994.

[142] R. K. Singh, B. Sharma, "Carbofuran-induced biochemical changes in Clarias batrachus," *Pestic Sci*., vol. 53, pp. 285–290, 1998.

[143] J. L. Yang, H. C. Chen, "Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology," *Chemosphere*., vol. 53, pp. 877–882, 2003.

[144] B. A. Barton, G. K. Iwama, "Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids," *Annu Rev Fish Dis*., vol. 1, pp. 3-26, 1991.

[145] W. F. Caspary, "Physiology and pathophysiology of intestinal absorption," *Am J Clin Nutr*., vol. 55, pp. 299-308, 1992.

[146] H. M. Blottiere, J. P. Galmiche, C. Cherbut, "Molecular analysis of the effect of short-chain fatty acids on intestinal cell proliferation," *Proc Nutr Soc*., vol. 62, pp. 101–106, 2003.

[147] E. R. L. Pelicano, P. A. Souza, H. B. A. Souza, D. F. Figueiredo, M. M. Boiago, R. S. Carvalho, "Intestinal mucosa development in broiler chickens fed natural growth promoters," *Braz J Poult Sci*., vol. 7, pp. 221–229, 2005.

[148] M. S. Hassaan, E. Y. Mohammady, M. R. Soaudy, J. Palma, E. E. Shawer, E. I. Earoun, "The effect of dietary sericite on growth performance, digestive enzymes activity, gut microbiota and haematological parameters of Nile tilapia, *Oreochromis niloticus* (*L*.) fingerlings," *Anim Feed Sci Technol*., vol. 262, pp. 114-400, 2020.

List of Bibliography (Continued)

[149] N. Tiengtam, S. Khempaka, P. Paengkoum, S. Boonanuntanasarn, "Effects of inulin and Jerusalem artichoke (*Helianthus tuberosus*) as prebiotic ingredients in the diet of juvenile Nile tilapia (*Oreochromis niloticus*)," *Anim Feed Sci Technol*., vol. 207, pp. 120–129, 2015.

[150] J. Ragaza, S. Velasquez, G. Maliwat, M. Chan, R. Abisado, R. Traifalgar, "Dietary Spirulina (*Arthrospira platensis*) enhances performance of juvenile Nile tilapia (*Oreochromis niloticus*)," *J Appl Phycol*., vol. 28, 2015.

[151] S. Boonanuntanasarn, A. Jangprai, S. Kumkhong, J. E. Plagnes, V. Veron, V. Burel, "Adaptation of Nile tilapia (*Oreochromis niloticus*) to different levels of dietary carbohydrates: New insights from a long term nutritional study," *Aquaculture*., vol. 496, pp. 58–65, 2018.

Biography

Name – Surname	Miss Unchaleeporn Sribounoy
Date of Birth	22 October 1994
Address	19 / 24 Village No. 3 Lane China mother 2 Road Phaholyothin 54 / 1 Sub – area Khlong road Area Saimai Province Bangkok Postal Code 10220
Education	Master of science program in applied biology faculty of science and technology
Experiences Number	-
Telephone Number	088-8511-222
Email Address	unchaleeporn_s@mail.rmutt.ac.th