2017

Comparative Viability of Spray Dried Lactobacilli Affected by Different Protective Agents and Storage Conditions

Vondel Vandeker Reyes Ortega
Louisiana State University and Agricultural and Mechanical College, vondelr@lsu.edu

COMPARATIVE VIABILITY OF SPRAY DRIED LACTOBACILLI AFFECTED BY DIFFERENT PROTECTIVE AGENTS AND STORAGE CONDITIONS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Nutrition and Food Sciences

by
Vondel Vandeker Reyes Ortega
B.S., Universidad Nacional de Agricultura, 2011
August 2017

ACKNOWLEDGMENTS

First, I would like to thank Dr. William B. Richardson, LSU Vice President for Agriculture, the Louisiana State University Agricultural Center, for providing assistantship through the Chancellor funds during my internship and throughout the completion of this program. My gratitude goes to Dr. Subramaniam Sathivel, my major professor, for giving me the opportunity to join his team and always encouraging me to be positive in any circumstance. His support has made me improve both personally and academically. I want to thank my committee members, Dr. Joseph Bankston and Dr. Michael Keenan for their helpful suggestions in this project. Special thanks to Dr. Arranee Chotiko for her invaluable insights in the conduction of this research. My appreciation goes to my former and current lab mates for their advice and friendship. Finally, I would like to thank my family who has supported me throughout my life with their unconditional love.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

A	cross-sectional area of the inlet or outlet air pipe (m^2)
AH_{aa}	absolute humidity of inlet ambient air (kg water/kg dry air)
AH_{ao}	absolute humidity of outlet air (kg water/kg dry solids)
c_{aa}	specific heat of inlet ambient dry air, kJ/(kg K)
c_p	specific heat of inlet ambient air, kJ/(kg K)
c_v	specific heat of water vapor, kJ/(kg K)
E_{vp}	evaporation rate calculated from the moisture content of the probiotic solution, powder collected from cyclone collector vessel and dust (kg water/h)
m_{aa}	dry air mass flow rate of inlet ambient air (kg dry air/h)
m_{ao}	dry air mass flow rate of outlet air (kg dry air/h)
m_d	dust flow rate (kg dry solids/h)
m_e	probiotic solution flow rate (kg dry solids/h)
mP	estimated powder production rate (kg dry solids/h)
p_v	saturation pressure of water vapor (kPa)
p_w	partial pressure exerted by water vapor (kPa)
Q	estimated energy used to spray dry the probiotic solutions, kJ/kg
RH	relative humidity (%)
T	temperature (K)
T_{aa}	temperature of inlet ambient air (K)
T_{ad}	temperature of inlet drying air (K)
ΔT	temperature difference between inlet ambient air and inlet drying air (K)
v	average velocity (m/s)
V	volumetric flow rate (m^3/h)
V'	specific volume (m^3/kg dry air).
w_d	moisture content (dry basis) of dust (kg water/kg dry solids)
w_e	moisture content (dry basis) of probiotic solution (kg water/kg dry solids)
w_p	moisture content (dry basis) of probiotic powder (kg water/kg dry solids)

ABBREVIATIONS

AAT	ambient air temperature
GA	gum arabic
HM	high maize starch
LA	*Lactobacillus acidophilus* NRRL B-4495
LAGA	*Lactobacillus acidophilus* NRRL B-4495 with gum arabic spray dried at 140°C
LAHM	*Lactobacillus acidophilus* NRRL B-4495 with high maize starch spray dried at 140°C
LAMD	*Lactobacillus acidophilus* NRRL B-4495 with maltodextrin spray dried at 140°C
LP	*Lactobacillus plantarum* NRRL B-4496
LPGA	*Lactobacillus plantarum* NRRL B-4496 with gum arabic spray dried at 140°C
LPHM	*Lactobacillus plantarum* NRRL B-4496 with high maize starch spray dried at 140°C
LPMD	*Lactobacillus plantarum* NRRL B-4496 with maltodextrin spray dried at 140°C
MD	maltodextrin

ABSTRACT

Because of their health benefits, probiotics are a significant part of the functional food industry. Spray drying is reported as the most common method used in the food industry to encapsulate probiotics. The objectives of this study were to investigate the effects of protective agents on the viability of lactobacilli after spray drying and during storage at different conditions and to evaluate spray drying conditions to produce these probiotic powders. *Lactobacillus plantarum* NRRL B-4496 (LP) and *Lactobacillus acidophilus* NRRL B-4495 (LA) were separately grown (~10^9 CFU/ml) and suspended in a 200 g/L solution of high maize starch (HM); maltodextrin (MD); or gum arabic (GA). The solutions were separately spray dried at 140 °C to obtain LP and LA-powders: LPHM, LPMD, LPGA, LAHM, LAMD, and LAGA. The powders were separately placed in aluminum bags and separately packed under 97% and 10% vacuum. The powders were stored at refrigerated (4 °C) or at room (23 °C) temperature for 60 days. Physicochemical properties, energy and mass balances, and cell viability during storage were determined. Triplicate experiments were conducted and data were statistically analyzed (α=0.05). The actual production rate of powders ranged from 0.091 to 0.105 (kg dry solids/h). The energy used during spray drying was not significantly different for any of the powders. After 60 days, LPHM powders packed under 10% and 97% vacuum and stored at 4 °C had significantly higher cell viability than the other powder samples. The study demonstrated significantly improved on the viability of LP at 10% vacuum stored at refrigerated temperature for the HM treatment compared to those treated with MD and GA. The data obtained showed that high maize starch can be used as a protective agent to maintain the viability of *L. plantarum* powder at recommended levels for up to 60 days of storage.

CHAPTER 1. INTRODUCTION

Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2001). They provide several beneficial effects to humans, such as protecting from bowel diseases, normalizing gut microbiota, improving lactose tolerance, and potentially reducing risk factors for colon cancer (Meng, Stanton, Fitzgerald, Daly, & Ross, 2008). These benefits are reached by different mechanisms including antimicrobial activity, immune, antimutagenic, and antigenotoxic effects, influence on enzyme activity, enzyme delivery, etc. (Sanders, 2000). Lactobacilli species, such as *L. acidophilus*, *L. rhamnosus*, *L. paracasei*, *L. plantarum*, are the most common probiotic microorganisms (Saad, Delattre, Urdaci, Schmitter, & Bressollier, 2013). Currently, the food industry is attempting to produce probiotic food products with high levels of viable cells because of rapid growth and expansion in the demand for healthy and nutritious foods (Soukoulis, Behboudi-Jobbehdar, Yonekura, Parmenter, & Fisk, 2014). However, probiotic viability in food products is negatively affected by several factors, including the presence of antimicrobial compounds, oxygen toxicity, post-acidification, and storage temperature (Vasiljevic & Shah, 2008). Poor cell viability during storage has been reported in many probiotic products as well as low survival after consumption (Lin, Hwang, Chen, & Tsen, 2006; Vinderola, Bailo, & Reinheimer, 2000; Vinderola, Mocchiutti, & Reinheimer, 2002).

Encapsulation is one of the approaches to assure probiotic viability. This technology can help protect cell viability and functionality during processing, storage, and delivery through the human gastrointestinal tract (de Vos, Faas, Spasojevic, & Sikkema, 2010). The cells are entrapped within an encapsulating agent, resulting in a reduction of cell injury and/or cell loss caused by adverse conditions (Shah, 2000). Spray drying is reported as the most common

method used in the food industry to encapsulate probiotics. This technique can produce good quality probiotic products with low production costs (Kailasapathy, 2002). Spray drying is a process in which a liquid feed is put in contact with a hot drying medium (100 to 300 °C), leading to the evaporation of the liquid, obtaining a dried product in form of powders, granules, or agglomerates (Solval, 2011). In order to protect probiotic cells during the microencapsulation process, different materials have been evaluated including gum arabic, alginate, gelatin, maltodextrin, pectin, skimmed milk, resistant starch, and chitosan (De Castro-Cislaghi, Carina Dos Reis, Fritzen-Freire, Lorenz, & Sant'Anna, 2012). Gum arabic is a polymer with excellent emulsification properties consisting of D-glucuronic acid, L-rhamnose, D-galactose, L-arabinose, and approximately 2% protein (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007). Gum arabic is also a source of dietary fiber that has been used in the probiotic food industry because of its health benefits (Desmond, Ross, O'callaghan, Fitzgerald, & Stanton, 2002). Maltodextrin is a hydrolyzed starch commonly used as wall material in microencapsulation of food ingredients. It has a relatively low cost, neutral aroma, and taste, low viscosity at high solids concentrations, and provides good protection against oxidation (Carneiro, Tonon, Grosso, & Hubinger, 2013). According to (Burgain, Gaiani, Linder, & Scher, 2011), resistant starch that is resistant to pancreatic enzymes in the small intestine is used as an encapsulating agent for targeted delivery of probiotic cells in the human colon. Due to its prebiotic and symbiotic functionality, resistant starch can be used by probiotic bacteria in the large intestine (Topping, Fukushima, & Bird, 2003). The aim of this investigation was to evaluate effects of different encapsulating agents: high maize starch, maltodextrin, and gum arabic on the viability of probiotics *Lactobacillus plantarum* NRRL B-4496 and *Lactobacillus acidophilus* NRRL B-4495 during spray drying and storage.

CHAPTER 2. LITERATURE REVIEW

2.1. Probiotics

The concept of probiotics progressed around 1900 when Henry Tissier, a French pediatrician observed that children with diarrhea had a low number of bacteria characterized by a peculiarly Y-shaped morphology in their stools. These Bifid bacteria were, on the contrary, abundant in healthy children (Gupta, 2015). The first observation of the positive role played by some selected bacteria is attributed to Eli Metchnikoff (1907), who suggested that "the dependence of the intestinal microbes on the food makes it possible to adopt measures to modify the flora in our bodies and to replace the harmful microbes by useful microbes" (FAO/WHO, 2001). He also pointed out that Bulgarian peasants had long and healthy lives because of the consumption of fermented milk products (Chuayana Jr, Ponce, Rivera, & Cabrera, 2003) which contained Lactobacillus that positively influenced the microflora of the gut, being able to establish and decrease the toxic microbial activity of the pathogenic bacterial population (Figueroa-González, Quijano, Ramírez, & Cruz-Guerrero, 2011).

The term “probiotic” originates from the Greek term “probios” meaning “for life” which is opposed to “antibiotic,” which means “against life” (Longdet, Kutshik, & Nwoyeocha, 2011). The term probiotic was coined in 1965 by Lilly and Stillwell to describe substances secreted by one microorganism which stimulates the growth of another (Seppo Salminen, Ouwehand, Benno, & Lee, 1999). Over the years probiotics have been defined in several ways, depending on their mechanisms of action, viability, and non-viability, effects on human health, etc. An early definition proposed by Parker (1974), they were described as “Organisms and substances which contribute to intestinal microbial balance”. Later, Fuller (1989) defined them as “A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. The author in this definition was focused on the importance of live cells in

order to have beneficial effects on the host and not include the antibiotic concept. The most recent and widely accepted definition is the one proposed by an expert panel of The Food and Agriculture Organization (FAO) and World Health Organization (WHO) that define probiotics as “live microorganisms which when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO, 2001). Although, there are reports which indicate that dead probiotic microorganisms or even bacterial DNA also show beneficial health effects on the host (Sarowska, Choroszy-Król, Regulska-Ilow, Frej-Madrzak, & Jama-Kmiecik, 2013).

The consumption of probiotic products is increasing worldwide because these organisms are regarded as GRAS (generally recognized as safe); which is a status used to address the problem of pathogen colonization in different ecosystems (Bouchard, Rault, Berkova, Le Loir, & Even, 2013). The optimal level of microorganisms required to observe a positive health benefit from consumption has not been established, but a daily recommended therapeutic dose should be between 10^9- 10^{11}CFU/ day (Mombelli & Gismondo, 2000). According to Kailasapathy and Chin (2000), the criteria for classifying a bacterial strain as a probiotic include: bacteria of human origin, stable against bile, acid, enzyme and oxygen, be able to colonize the human gastrointestinal tract, production of antimicrobial substances, and probable efficacy and safety. In Table 2.1 are shown some of the most important microorganisms considered as probiotics.

Table 2.1. Microorganisms considered as probiotics.

Lactobacillus species	*Bifidobacterium* species	Other LAB	*Bacillus* species	Non LAB
L. acidophilus[ab]	*B. adolescentis*[ab]	[1]*Enterococcus faecalis*[ab]	[1]*B. cereus var. toyoi*[b]	*Clostridium butyricum*[a]
L. casei[ab]	*B. animalis*[ab]	*E. faecium*[ab]	*B. clausii*[a]	*Escherichia Coli* strain *nissle*[b]
L. crispatus[ab]	*B. animalis subsp. lactis*[ab]	[2]*Lactococcus lactis*[ab]	*B. coagulans*[a]	*Propionibacterium freudenreichii*[ab]
L. delbrueckii subsp. *delbrueckii*[a]	*B. bifidum*[ab]	*Leuconostoc mesenteroides*[ab]	*B. licheniformis*[a]	*Saccharomyces cerevisiae*[ab]
L. delbrueckii subsp. *bulgaricus*[a]	*B. breve*[ab]	[2]*Pediococcus acidilactici*[ab]	*B. mesentericus*[a]	*Saccharomyces cerevisiae* subsp. *boulardii*[ab]
L. delbrueckii subsp. *lactis*[a]	*B. infantis*[ab]	*P. pentosaceus*[a]	*B. subtillis*[a]	
L. gasseri[ab]	*B. longum*[ab]	*Streptococcus salivarius*[a]		
[1]*L. gallinarum*[ab]		*S. macedonicus*[a]		
L. helveticus[a]		*S. mitis*[a]		
L. fermentum[a]		*S. sanguis*[a]		
L. johnsonii[ab]		*S. thermophilus*[ab]		
L. paracasei[ab]				
L. plantarum[ab]				
L. reuteri[ab]				
L. rhamnosus[ab]				
L. sakei[a]				
L. salivarius[a]				

[1]Mainly used for animals
[2]Little is known about probiotic properties
Source: [a]Foligné, Daniel, and Pot (2013) and [b]Kechagia et al. (2013)

2.2. Health benefits of probiotics

Probiotics in the form of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in fermented milk have been ingested by humans for many years in the belief that they have health benefits. Due to the constant scientific evaluation in this area, there is now strong evidence that the use of probiotics helps in treating and preventing some human diseases (Boyle, Robins-Browne, & Tang, 2006). Probiotics provide a number of health benefits mainly through: a) maintenance of normal intestinal flora, protection against gastrointestinal and putrefactive pathogens (D'Aimmo, Modesto, & Biavati, 2007); b) enhancement of the immune system; c) reduction of serum cholesterol level and blood pressure; d) anti-carcinogenic activity; e) improved utilization of nutrients; f) improved nutritional value of food (Tripathi & Giri, 2014);

g) production of antimicrobial compounds; h) competition for mucosal binding sites (Marco, Pavan, & Kleerebezem, 2006); i) improvement in symptoms of lactose intolerance (Kechagia et al., 2013); j) respiratory and urinary tract infections have also been documented (Parvez, Malik, Ah Kang, & Kim, 2006). Some of the probiotic beneficial effects are described below:

2.2.1. Lactose intolerance

Lactose intolerance is the pathophysiological situation in which the small intestinal digestion and/or colonic fermentation is altered which leads to clinical symptoms due to the lack of the colonic bacterial enzyme β-galactosidase (Vonk, Reckman, Harmsen, & Priebe, 2012). When lactose intolerant people consume milk or lactose-containing products, the gastrointestinal symptoms include abdominal pain, flatulence, bloating, nausea, or diarrhea as a result from lactose maldigestion (Hertzler & Clancy, 2003). One approach that has been employed for overcoming the lactose intolerance problem is through the use of fermented dairy foods. For example, yogurt is well tolerated by lactose malabsorbers due to the reduced levels of lactose which is partially hydrolyzed during the fermentation process. Other factors that appear to be responsible for a better yogurt tolerance include; a) yogurt bacteria (i.e. *Str. thermophilus* and *Lb. delbrueckii ssp. bulgaricus)*, b) β-galactosidase produced by these bacteria and c) oro-caecal transit time (Chandan, White, Kilara, & Hui, 2008).

Kefir is another fermented dairy food for which claims of improved lactose digestion have been made. Kefir grains are small, irregularly shaped, yellow-white, hard granules that resemble miniature cauliflower blossoms which contain a complex mixture of bacteria, yeasts, polysaccharides, products of bacterial metabolism, and curds of milk protein (Hertzler & Clancy, 2003). Regular consumption of kefir in lactose intolerant individuals can help to relieve the gastrointestinal symptoms of lactose maldigestion due to the abundance of beneficial yeast and

bacteria which provide the enzyme lactase which consumes most of the lactose left after the culturing process (Otles & Cagindi, 2003). Rolfe (2000) and Seppo Salminen (2001) suggest the beneficial effects of probiotics and LAB on lactose intolerance can be explained by two mechanisms of action, namely, a) lower lactose concentration in the fermented foods due to the high lactase activity of bacteria used during production, and b) increase active lactase enzyme entering the small intestine with the fermented product or within the viable bacteria that survive gastric and bile conditions.

2.2.2. Prevention of diarrhea

It has been reported that a wide range of probiotic strains has been evaluated and help to relieve different types of diarrhea (i.e. infantile diarrhea, antibiotic-associated diarrhea, traveler's diarrhea, and relapsing *Clostridium difficile* colitis) (Tuohy, Probert, Smejkal, & Gibson, 2003). Disruption in the normal intestinal microflora structure can result in the proliferation of pathogens in the colon resulting in diarrhea. For instance, *Clostridium difficile* which is an indigenous colonic bacteria is the primary causative agent in pseudomembranous colitis. The proliferation of *C. difficile* is thought to occur after antibiotic treatment (Ziemer & Gibson, 1998). The treatment and prevention of infectious diarrhea are probably the best-documented health benefit of probiotic bacteria. Rotavirus is the most common cause of acute infantile diarrhea in the world and a significant cause of infant mortality, primarily in infants and young children (S. Salminen et al., 1998). However, the use of probiotic supplementation in infant formulas aid in the prevention of rotavirus infection.

Several microorganisms have been effective in reducing the severity and duration of acute diarrhea in children such as *L. reuteri*, *L. rhamnosus* GG, *L. casei*, and *S. boulardii* (Canani et al., 2007; Chmielewska, Ruszczynski, & Szajewska, 2008). Saxelin, Tynkkynen, Mattila-

Sandholm, and de Vos (2005) and Kechagia et al. (2013) state that the proposed mechanisms by which probiotics seem to help to relieve the effect of infectious diarrhea include competitive exclusion, enhancement of the immune response, and production of substances that directly inactivate the viral particles.

2.2.3. Protective role of probiotics on colon cancer

Colon cancer is one of the most important causes of cancer morbidity and mortality in Western cultures (Jemal et al., 2008; Landis, Murray, Bolden, & Wingo, 1998). Diets containing high animal proteins, fat, and low dietary fiber make an important contribution to the risk of colon cancer, while a diet rich in fruits and vegetables appears to have a protective effect. According to Brady, Gallaher, and Busta (2000), the development of colon cancer is a sequence of events occurring on definable steps. First, a metabolic activated precursor produces a carcinogen which causes an alteration in the DNA. In the next step, there is an overgrowth of colonic crypts, morphologically described as aberrant crypts (precancerous lesions) which will progress to polyps and eventually to tumors. Specific bacteria strains have been implicated in the pathogenesis of cancer, including *Streptococcus bovis, Bacteriodes, Clostridia and Helicobacter pylori* (Davis & Milner, 2009). There is evidence that suggests the consumption of probiotics may be able to play a preventive role in the onset of colorectal cancer (Uccello et al., 2012). Anticancer activity of probiotics is strain dependent; *L. acidophilus, L. reuteri, L. casei, B. longum,* and *B. breve* bacteria have been reported that may reduce the risk of developing colon cancer (Brady et al., 2000; Iyer et al., 2008; Wollowski, Rechkemmer, & Pool-Zobel, 2001).

The proposed mechanisms by which lactic acid bacteria may inhibit colon cancer include: enhancing the host's immune response to antimutagenic substances in the colon; binding, blocking, and degrading potential carcinogens and procarcinogens, reduction of the intestinal pH

that alters intestinal microflora activity linked to the production of carcinogens, alteration of physicochemical conditions in the colon; alteration of colonic motility and transit time, among others (Hirayama & Rafter, 1999; McIntosh, 1996; Uccello et al., 2012). Reid, Jass, Sebulsky, and McCormick (2003) report that lactobacilli and bifidobacteria bacteria modify the gut microbiota and reduce the risk of cancer due to their ability to decrease β-glucuronidase and carcinogenetic enzymes levels produced by colon microflora.

2.2.4. Modulation of immune response

Probiotics have been reported to enhance specific and nonspecific host immune responses without inducing a harmful inflammatory response (Kopp-Hoolihan, 2001). There is an established interaction between microbiota in the gastrointestinal tract and epithelial cells and immune cells. These bacteria recognize receptors on the epithelial cell surface and bind to them and as result, a cascade of immunological defense mechanisms such as the production of pro- and anti-inflammatory cytokines occur (Saxelin et al., 2005). Gill (1998) reported that lactic acid bacteria exert their immunity enhancing effects by augmenting both non-specific (e.g. phagocyte function, NK cell activity) and specific (e.g. antibody production, cytokine production, lymphocyte proliferation, delayed-type hypersensitivity) host immune responses. Dendritic cells which play a key role in the balance of T helper cells Th1, Th2 and Th3, initiate the local immune response in the intestinal mucosa (Marteau, Seksik, & Jian, 2002). Some of the proposed mechanisms by which probiotics might induce immunomodulatory beneficial effects in human diseases include: a) modulation and stabilization of microbiota composition; b) inhibition of inflammatory response of the intestinal immune system through inhibition of NF-κB activation; c) increase the activity of Natural Killer (NK) cells; and d) increasing the secretion of mucus; e) direct immunomodulatory action by inducing secretion of cytokines; f) inducing

dendritic cells maturation (Aureli et al., 2011). Erickson and Hubbard (2000), LeBlanc, Matar, Valdez, LeBlanc, and Perdigon (2002), and (Schultz et al., 2003) report that *L. acidophilus*, *Bifidobacterium longum*, *L. rhamnosus subsp. GG*, *Lactobacillus casei*, and *L. helveticus* are among probiotic bacteria that have been shown to enhance the immune response.

2.3. Probiotic mechanisms of action

The identification of the exact mechanisms by which probiotics exert their effect on the host is not completely understood (Harish & Varghese, 2006a). According to Hemaiswarya, Raja, Ravikumar, and Carvalho (2013), the effective performance of probiotics depends on their strong adherence and colonization of the human gut in order to improve the host immune system. It should be mentioned that probiotics differ significantly in their mechanism of action; also there is not a single probiotic able to exhibit all mechanisms of action for the prevention or therapy of different kinds of diseases (O'Hara & Shanahan, 2007; Oelschlaeger, 2010). Some of the proposed mechanisms of probiotic actions include: a) metabolic effects that exert an antimicrobial effect through the production of bacteriocins; b) lactic acid, and short-chain fatty acids which lower the gut lumen pH to avoid pathogens colonization; c) maintenance of the epithelial barrier by activation of tight junction proteins to avoid the development of a leaky intestine; d) inflammation prevention and apoptosis of the lining intestinal epithelial cells (Michail, 2005; Ng, Hart, Kamm, Stagg, & Knight, 2009; Sherman, Ossa, & Johnson-Henry, 2009). Also, Harish and Varghese (2006b) and Sarowska et al. (2013) report other proposed mechanisms such as modulation of the immune system, enhancement of microbial flora, and competition for nutrients.

2.4. Lactic acid bacteria

Lactic acid bacteria (LAB) have a long and safe history of application and consumption in the production of fermented foods and beverages. They cause rapid acidification of the raw food material through the production of lactic acid, acetic acid, ethanol, aroma compounds, bacteriocins, exopolysaccharides, and several enzymes for the production of fermented foods (Leroy & De Vuyst, 2004). Fermented foods' shelf life and safety are enhanced by the different antimicrobials produced by probiotics which inhibit pathogenic and spoilage microorganisms (Indira, Jayalakshmi, Gopalakrishnan, & Srinivasan, 2011). LAB are a group of Gram-positive, non-spore forming, non-aerobic, aero-tolerant, and acid tolerant (Agrawal, 2005), usually nonmotile, generally catalase-negative and usually devoid of cytochromes bacteria (Ringø & Gatesoupe, 1998). They are nutritionally fastidious, requiring rich media to grow (carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives, and vitamins) (Lebeer, Vanderleyden, & De Keersmaecker, 2008).

This bacterial group contains both rods (*Lactobacilli* and *Carnobacteria*) and cocci (*Streptococci*) (Kumar, 2011) that ferment carbohydrates and higher alcohols to form mainly lactic acid (Stiles & Holzapfel, 1997). LAB are commonly found in the gastrointestinal tract of various animals, dairy products, seafood products, soil, and on some plant surfaces (Ringø & Gatesoupe, 1998). The live bacteria present in probiotic products are LAB, including *Lactobacilli, Bifidobacteria,* and *Enterococci* (Agrawal, 2005). Based on the nature of carbohydrate fermentation, LAB are classified into homofermentative and heterofermentative bacteria. The homofermentative group consists of *Lactococcus, Pediococcus, Enterococcus, Streptococcus* and some lactobacilli that utilize the Embden-Meyerhof-Parnas (glycolytic) pathway to transform the carbon source mainly into lactic acid.

Heterofermentative bacteria, however, produce equimolar amounts of lactate, CO_2, ethanol or acetate from glucose by using phosphoketolase pathway. *Leuconostoc, Weisella* and some lactobacilli are members of this group (Vasiljevic & Shah, 2008).

2.4.1. Lactobacillus acidophilus

Lactobacillus acidophilus is a Gram-positive rod, with rounded ends, occurring in pairs or short chains. The typical size is 0.6-0.9 μm in length. It is non-flagellated, non-motile, non-spore forming, and intolerant to salt. It is also homofermentative producing mainly lactic acid (>85%) by the Embden-Meyerhof-Parnas pathway. Likewise, it is microaerophilic and capable of aerobic growth in static cultures. Anaerobic conditions (5% CO_2, 10% H, and 85% N) are preferable to stimulate its growth. Most strains of *L. acidophilus* can ferment amygdalin, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, salicin, sucrose, and esculin (Mosilhey, 2003; Selle, Klaenhammer, & Russell, 2014). *L. acidophilus* is probably the most explored lactobacilli, found in most probiotic foods in the market (Anekella, 2012) like milk, yogurt, toddler formula, dietary supplements, and traditional fermented foods (Bull, Plummer, Marchesi, & Mahenthiralingam, 2013).

L. acidophilus has been reported to be a beneficial probiotic microorganism with excellent therapeutic benefits. Rousseaux et al. (2007) found that *L. acidophilus* NCFM could alleviate abdominal pain in patients with irritable bowel syndrome by the expression of receptors on intestinal epithelial cells which have analgesic functions in the gut similar to that of morphine. Meanwhile, Simakachorn et al. (2000) report that an oral rehydration containing *L. acidophilus* LB reduced the duration of acute diarrhea in children compared to control group. Gilliland and Speck (1977) report that lactic acid and hydrogen peroxide produced by *L. acidophilus* inhibit growing of different enteric pathogens in associative liquid cultures. Most of the ability of

several *L. acidophilus* strains to inhibit microbes through the production of antimicrobial compounds has been demonstrated *in vitro*, but this pathogenic inhibitory activity has not been yet demonstrated in *in vivo* studies (Jose, 2015).

2.4.2. Lactobacillus plantarum

Lactobacillus plantarum is a Gram-positive, rod-shaped, and non-pathogenic bacterium. It is facultative heterofermentative meaning in aerobic conditions it produces acetic acid, while in anaerobic conditions it is able to undergo fermentation to produce lactic acid as a major product (Farnworth, 2008). This versatile bacterium can be found in different environments including dairy, meat, many vegetable fermentations, the human gastrointestinal tract, and human saliva.

Several *L. plantarum* strains have been tested for health effects due to their abundance, easy growth characteristics, and human origin (De Vries, Vaughan, Kleerebezem, & de Vos, 2006). Klarin et al. (2008) report that enteral administration of *L. plantarum* 299v reduced the incidence of colonization of *C. difficile* in critically ill patients treated with antibiotics, while 19% of the control group patients were positive for the pathogen. Bukowska, Pieczul-Mróz, Jastrzebska, Chelstowski, and Naruszewicz (1998) found that levels of total and LDL-cholesterol in men with moderately elevated blood cholesterol were reduced by 7.3 and 9.6%, respectively. Also, fibrinogen protein a coronary artery disease factor was reduced by 13.5% probably by modulation of the immune system response. Domingo (2017) used three *L. plantarum* strains in patients with irritable bowel syndrome (IBS) and he found that the DSM 9843 strain significantly reduced flatulence, whereas LPO 1, and 299v strains significantly reduced abdominal pain in the test group.

2.5. Encapsulation

Encapsulation was originally introduced in the area of biotechnology to make production processes more efficient as the matrix around the cells allows for rapid and efficient separation of the producer cells and the metabolites (de Vos et al., 2010). The technique, of significant interest for the pharmaceutical industry, is used for drug and vaccine delivery and also in the food industry for the protection of functional and bioactive compounds (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011). Encapsulation is a physicochemical or mechanical process to entrap a substance in a material in order to produce particles with diameters of a few nanometers or millimeters (Burgain et al., 2011). From a microbiological point of view, encapsulation is defined as the process of entrapment of cells by coating them with hydrocolloids in order to isolate them from the environment for an appropriate release in the gut (Jayalalitha, 2013) (Figure 2.1). The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is called a shell, coating, or membrane (Umer, Nigam, Tamboli, & Nainar, 2011). Encapsulation can be used to preserve and control flavor, color, texture, functional properties and to retain potential health benefits of functional ingredients and additives such as probiotics, enzymes, acids, vitamins, minerals, flavors, fatty acids, antioxidants, spices, etc. (Anekella, 2012; Mosilhey, 2003).

Applied to probiotics, the objective of encapsulation is to protect the cells against the host's natural barriers and mild heat treatment from food processing (Ding & Shah, 2007). Encapsulation also allows probiotics release in a viable and biologically active state in the gut (Picot & Lacroix, 2004), while improving taste, aroma, stability, nutritional value, and product appearance (Parra Huertas, 2010). Materials commonly used to encapsulate probiotic cells include different polysaccharides (agar, sodium alginate, carrageenan, gum arabic, chitosan,

starch, etc.), oligosaccharides (corn syrup, sucrose, and maltodextrin), proteins (gluten, casein, whey protein, and albumin), and lipids (waxes, paraffin, diglycerides, oils, fats, etc) (Das, Ray, Raychaudhuri, & Chakraborty, 2014; Serna-Cock & Vallejo-Castillo, 2013).

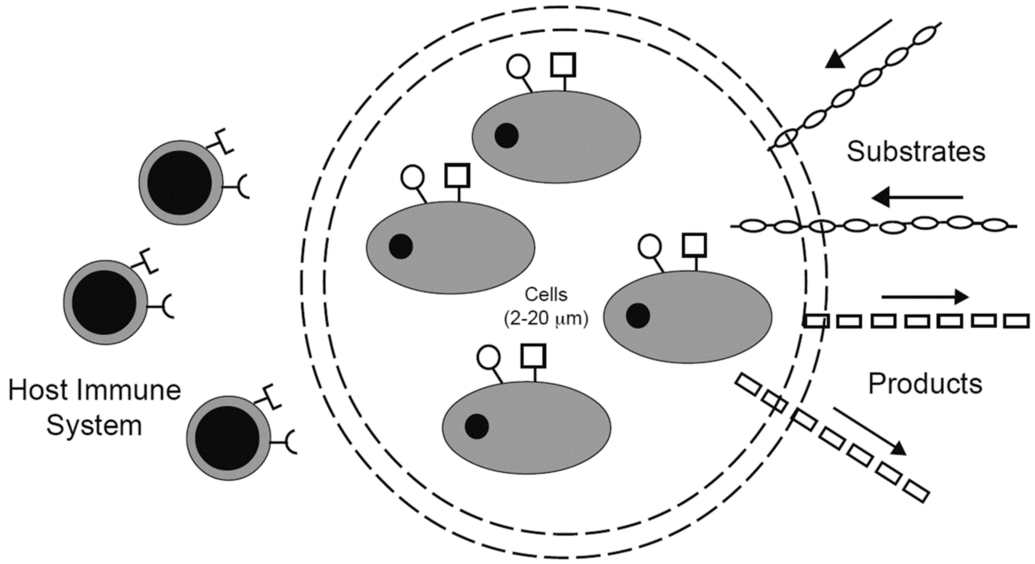

Figure 2.1. Protection of bacterial cells by microencapsulation.
Source: Kailasapathy (2002)

According to Umer et al. (2011) there are several reasons why substances may be encapsulated including a) protection of reactive substances from the environment; b) conversion of liquid active components into a dry solid system; c) to separate incompatible components for functional reasons; d) to mask undesired properties of the active components; e) to protect the immediate environment of the microcapsules from the active components; and f) to control release of the active components for either delayed or long-acting release. An exploration into the encapsulation process of microbial cells has utilized different techniques such as extrusion, coacervation, spray drying, and emulsification (Rathore, Desai, Liew, Chan, & Heng, 2013). However, spray drying is the most commonly used microencapsulation technology in food industries (Pu, Bankston, & Sathivel, 2011) because it is flexible, continuous, and a cost effective technique (Nedovic et al., 2011).

2.5.1. Spray drying

Spray drying is the transformation of a feeding solution, suspension, dispersion or emulsion into dried particulate form by spraying the feed into a hot drying medium. The dried product can be in the form of powders, granules or agglomerates depending upon the physical and chemical properties of the feed, the dryer design, and desired powder properties (Patel, Patel, & Suthar, 2009). Spray drying is widely used in the food industry for the encapsulation of vitamins, minerals, flavor compounds, antioxidants (Murugesan & Orsat, 2012), natural food colorants, lipids, probiotics, antimicrobials, polyphenols, anti-oxidants, etc. (Kandansamy & Somasundaram, 2012). The process of microencapsulation by spray drying (Figure 2.1) involves the formation of an emulsion or suspension of coating and core material, atomization of the emulsion into a drying chamber with circulating hot dry air, and evaporation of droplets moisture while the coating material entraps the core (Mosilhey, 2003).

During the drying process the temperature-time profile of the droplets can be divided into two periods: a) constant rate period (beginning of drying), where the temperature of spray-dried particles and heat inactivation are limited to the wet bulb temperature by the evaporative cooling effect, and b) falling rate period, where the temperature of the spray-dried particles increases but generally does not reach inlet air temperature (Santivarangkna, Kulozik, & Foerst, 2007). The most important factors which optimize the spray drying conditions include feed flow rate, air inlet/outlet temperature, and feed temperature (Kandansamy & Somasundaram, 2012). Other critical parameters of spray drying process include viscosity of the feed, feed solid content, the surface tension of the feed, volatility of solvent, and nozzle material (More Swati & Wagh, 2014). According to Patel et al. (2009), the critical elements of a spray drying system includes the atomizer, the air flow, and the spray drying chamber.

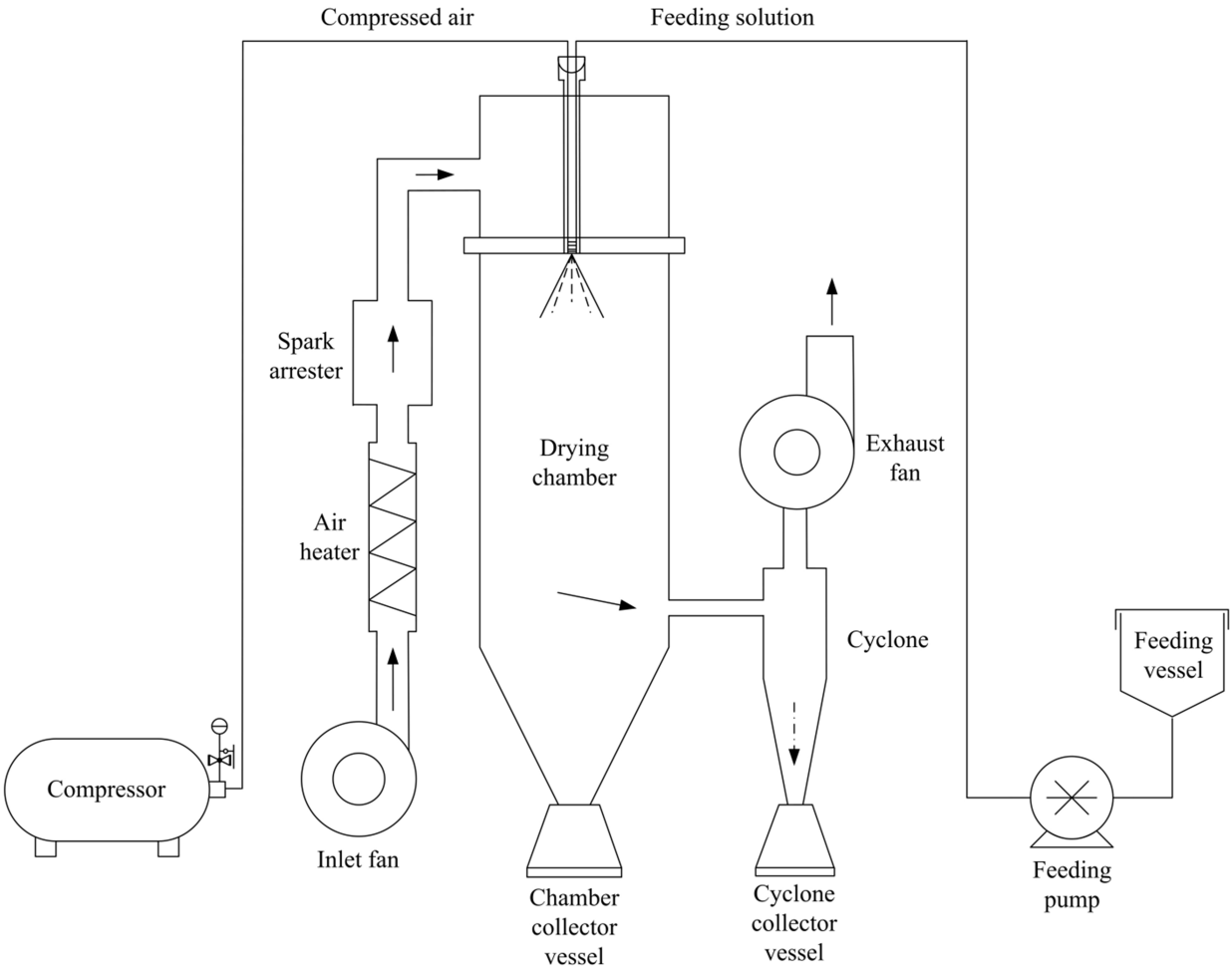

Figure 2.2. Schematic representation of the pilot scale FT80 Tall Form Spray Dryer Armfield Limited®

2.5.1.1. Atomizer

The atomizer is regarded as the “heart” of any spray drying system. According to Kandansamy and Somasundaram (2012), the main purpose of the atomization process is to maximize the heat transfer surface between the liquid and the dry air in the drying chamber. More Swati and Wagh (2014) state that the atomizer includes three main functions. One function is the dispersion of the feed material into small droplets in order to distribute and mix it thoroughly with the hot gas in the drying chamber. Secondly, the droplets produced must not be so large that they are incompletely dried, nor so small that product recovery is difficult. Lastly,

the atomizer must act as a metering device, controlling the rate at which the material is fed into the drying chamber. There are several types of atomizers which are classified according to the type of energy used including rotary nozzles (centrifugal energy), two-fluid or pneumatic nozzles (kinetic energy), pressure nozzles (pressure energy), and ultrasonic nozzles (acoustic energy) (Miller & Gil, 2012).

2.5.1.2. Drying air flow pattern

Regarding the liquid spray direction, the large industrial dryers can have a variety of air flow patterns including co-current, counter-current, and mixed flow pattern (Figure 2.2).

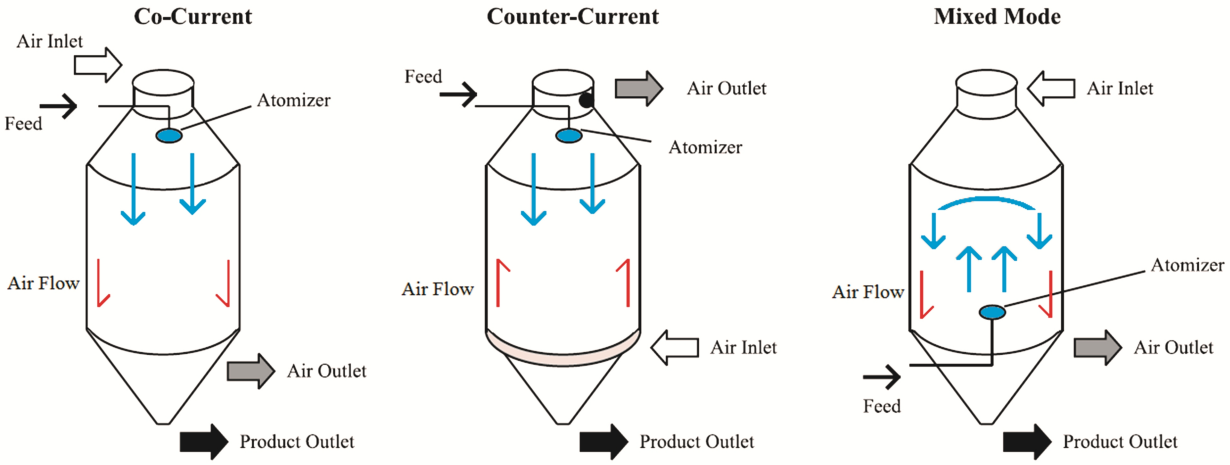

Figure 2.3. Different spray dryer configurations according to product and air flow patterns. Source: Vega-Mercado, Góngora-Nieto, and Barbosa-Cánovas (2001).

Most of the dryers used are of the co-current air flow and mixed flow pattern (Wisniewski, 2015). Patel et al. (2009) describe these three different spray drying air flow patterns:

a) Co-current flow: in this configuration, the spray is directed into the hot air entering the dryer and both pass through the chamber in the same direction.

b) Counter-current flow: in this dryer design, the spray and the air are introduced at opposite ends of the dryer, with the atomizer positioned at the top and the air entering at the bottom.

c) Mixed flow: dryers of this type combine both co-current and counter current flow. In a mixed flow dryer, the air enters at the top and the atomizer is located at the bottom.

2.5.1.3. Spray drying chamber

The drying chamber is an important component of a spray dryer where the atomized droplets are contacted with the hot air and the drying process begins. Before entering the drying chamber, the air is heated by the heating element to a predefined temperature depending upon the characteristics of the feed fluid (S. Singh & Dixit, 2014). Air circulating within the chamber keeps a flow pattern; this prevents the deposition of partially dried product on the wall or atomizer. Air movement and temperature of inlet air influence the type of final product (Deis, 1997). According to Mujumdar (1995), the drying chamber design depends on a) the type of atomizer used because the sprayed angle will determine the droplets trajectory and therefore the diameter and height of the chamber, and b) the air-fluid contact system selected. Spray dryers based on the position of the drying chamber can be either horizontal or vertical (Aundhia et al., 2011).

2.5.2. Spray drying of probiotics

Among all possible drying techniques used to preserve probiotic bacteria, spray drying is one of the most predominant in the dairy industry (Huang et al., 2017). This technique is carried out by dispersing the cells in a polymer solution which is atomized in the drying chamber leading to evaporation of the solvent and consequently to the formation of microcapsules (De Castro-Cislaghi et al., 2012). Nowadays, most efforts have focused on the drying of *Lactobacillus*, *Lactococcus* and various *Bifidobacteria* species (Huang et al., 2017).

In spray drying, bacterial cultures are exposed to osmotic, heat, and oxidative stress due to the high temperature used for product dehydration, which causes thermal inactivation of cells

(Paéz et al., 2012). The cytoplasmic membrane, cell wall, DNA, and RNA of cells are mostly affected during spray drying, which results in a reduction of their metabolic activity (Perdana et al., 2013; Tripathi & Giri, 2014). Peighambardoust et al. (2011) state that inlet air temperature has a small effect on bacterial inactivation, while the combination of temperature and time played a major role in cells inactivation. The outlet air temperature is believed to be the major drying parameter affecting the viability of spray dried bacterial cultures (Santivarangkna, Kulozik, & Foerst, 2008). This parameter depends on the inlet air temperature, air flow rate, product feed rate, and atomized droplet size (Santivarangkna et al., 2007). Also, many investigators have reported that low outlet air temperature increases survival of cells (Ananta, Volkert, & Knorr, 2005; Desmond et al., 2002).

Cultures may need to be spray dried using co-current conditions because the high inlet air temperature will have less effect on cells due to the evaporative cooling effect (Santivarangkna et al., 2008). A big particle size of atomized cells suspension is another factor that greatly affects cells viability since drying time increases due to the higher contact time between cells and hot air (Riveros, Ferrer, & Borquez, 2009). Fu and Chen (2011) report that the atomization process *per se* has a negligible effect on cell inactivation during the drying process. Viability of probiotics is also greatly affected by different factors during storage period including composition of the food, types of packaging material, and storage conditions such as storage temperature, moisture content, relative humidity, oxygen content, exposure to light, etc. (Meng et al., 2008; Tripathi & Giri, 2014).

A number of studies have been conducted on spray drying of *L. plantarum* and *L. acidophilus* and reported on their performance under different drying conditions. Perdana et al. (2013) compared a single droplet drying to spray and freeze drying and found that dehydration

and thermal shock affect the viability of *L. plantarum* WCFS1 during drying. These cell inactivation mechanisms were dependent on the moisture content, temperature, and drying time. Golowczyc, Silva, Abraham, De Antoni, and Teixeira (2010) investigated the survival of three microorganisms isolated from kefir during spray drying at different outlet temperatures (70, 75, 80 and 85^{o}C) and storage in different conditions (20 and 6^{o}C). *L. plantarum* CIDCA 83114 showed a reduction of 1 log CFU/ml after drying and also the highest survival rate for all the tested outlet air temperatures when stored at 6^{o}C. Iaconelli et al. (2015) investigated the impact of freeze-drying, air-drying, and spray-drying on viability and functionality of three bacterial species without the use of protective agents. Cultivability of *L. plantarum* CNRZ 1997 was the least affected by the three drying methods (0 and 1 log reduction). Immunomodulatory properties of *L. plantarum* were not affected by drying processes may be due to its heat tolerance. They conclude that drying processes can positively or negatively affect probiotic viability and functionality and that sensitivity to each method is bacterial strain-specific. J. Barbosa et al. (2015) obtained probiotic orange powders by spray, freeze, and hot air drying techniques to investigate the survival of two lactic acid bacteria during storage. There was no decrease in *L. plantarum 299v* during spray and freeze drying, but a reduction of almost 2 log cycles occurred for convective drying. During storage at 4 °C, no differences in the viability of *L. plantarum* were observed between the drying methods. Freeze dried *L. plantarum* stored at room temperature without light showed a low reduction in viability.

Su, Lin, and Chen (2007) encapsulated both *L. acidophilus* BCRC 14079 and culture filtrates from lao-chao, a yogurt-like product, by spray drying at various outlet air temperatures and evaluate probiotic viability during storage. It was found that the culture filtrates provided good protection for both milk-clotting enzymes and probiotics. Also, the culture filtrates

improved microencapsulation efficiency and lowered water activity, but a reduction in the survival of *L. acidophilus* occurred when outlet air temperature was increased.

The viability of *L. acidophilus* was maintained above 10^7 CFU/g, representing >92% survival percentage during storage. Zhao, Sun, Torley, Wang, and Niu (2008) spray dried *L. acidophilus* XH1 with a mixture of β-cyclodextrin and acacia gum at different concentrations. They concluded that 20% (w/v) was the optimal wall material concentration to achieve a uniform capsule size (avg. 22.153 µm). After 8 weeks of storage at 4^oC, the number of live bacteria was hiher than 10^7 CFU/ml. Yonekura, Sun, Soukoulis, and Fisk (2014) investigated *L. acidophilus* NCIMB 701748 spray dried with sodium alginate, chitosan and hydroxypropyl methylcellulose (HPMC) as coencapsulants to evaluate cell viability after drying and during storage. *L. acidophilus* with chitosan powders had a low viable count (5.312 log CFU/g) during drying may be due to the positively charged chitosan amino groups and drying stress. Chitosan significantly enhanced the storage stability of spray-dried *L. acidophilus* powders that showed a low inactivation rate (0.007 ± 0.001/day) and significantly higher viable counts in chitosan powders (4.72 log CFU/g) when compared with other treatments during 35 days of storage at 25°C. They state that chitosan may be used as a shelf-life enhancer of anhydrobiotics.

2.6. Wall materials

The selection of a coating material for microencapsulation by spray drying is very important to attain an efficient encapsulation and microcapsule stability. Wall materials selection is based on the physicochemical properties such as solubility, molecular weight, glass/melting transition, crystallinity, diffusibility, film forming, and emulsifying properties (Gharsallaoui et al., 2007). The wall material offers protection to the core and allows a targeted release at the functional site interacting with the prevailing conditions in the release medium

(Anandharamakrishnan & Ishwarya, 2015). According to Agnihotri, Mishra, Goda, and Arora (2012), the selection of appropriate wall material decides the physical and chemical properties of the resultant microcapsules. Different materials such as gum arabic, alginate, gelatine, maltodextrin, pectin, skim milk, starch, and chitosan, among others, have been used to microencapsulate probiotics (De Castro-Cislaghi et al., 2012). Maltodextrin, gum arabic, and starch are commonly used as carriers to increase the glass transition temperature (Tg) of spray-dried products and to bring the liquid solution to an economically spray-dryable range (Mestry, Mujumdar, & Thorat, 2011).

2.6.1. Resistant starch

Resistant starch is the small fraction of starch that is not digested by pancreatic amylases in the small intestine. It reaches the large intestine where it will be fermented by the colonic flora to produce short-chain fatty acids (Sajilata, Singhal, & Kulkarni, 2006) and lower the pH in the lumen (Jayalalitha, 2013). Resistant starch can be used to ensure the viability of probiotic populations because it offers an ideal surface for cells adherence to the starch granule during processing, storage, and transit through the upper gastrointestinal tract, providing robustness and resilience to environmental stresses (Crittenden et al., 2001). Resistant starch has been classified into four general subtypes called RS1, RS2, RS3, RS4, and RS5 (Raigond, Ezekiel, & Raigond, 2015). The RS2 type is made of ungelatinized resistant granules with type B crystallinity which is slowly hydrolyzed by α-amylase. Food sources include raw potatoes, green bananas, and high-amylose (high-amylose corn) starches (Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010). In relation to probiotics encapsulation, de Araújo Etchepare et al. (2016) state that resistant starch is a technological support that helps to control the release of bioactive molecules, provide thermal stability, and increased the shelf life of sensitive

compounds. Several studies report the protective effects of resistant starch on probiotic cultures viability when exposed to different adverse conditions and during storage (de Araújo Etchepare et al., 2016; Fahimdanesh et al., 2013; Homayouni, Azizi, Ehsani, Yarmand, & Razavi, 2008).

2.6.2. Maltodextrin

Maltodextrin is a polysaccharide produced from the acidic or enzymatic hydrolysis of starch. It is a polymer of D-glucose chains linked by glycosidic α-(1-4) and α-(1-6) bonds that are formed by amylose and amylopectin with different equivalents of dextrose (Saavedra-Leos, Leyva-Porras, Araujo-Díaz, Toxqui-Terán, & Borrás-Enríquez, 2015). The dextrose equivalency (DE) is the parameter used to classify this polysaccharide (Kurozawa, Park, & Hubinger, 2009). Maltodextrin offers advantages such as relatively low cost, neutral aroma and taste, low viscosity at high solids concentrations, and good protection against oxidation. However, the main problem when it is used as wall material during spray drying is its low emulsifying capacity (Carneiro et al., 2013). Due to its high glass transition temperature (Tg), maltodextrin reduces stickiness in food products with low Tg and low molecular weight sugars during the spray drying process (De Oliveira et al., 2009). The use of maltodextrin with different DE and combined with other encapsulating agents have been used to encapsulate probiotic bacteria through spray drying. Protective effects on probiotic cells have been reported mainly due to the ability of maltodextrin to form a high viscous glassy matrix during drying (Joana Barbosa, Borges, & Teixeira, 2016; Semyonov, Ramon, & Shimoni, 2011; Shokri, Fazeli, Ardjmand, Mousavi, & Gilani, 2015)

2.6.3. Gum arabic

Gum arabic is a polymer consisting of D-glucuronic acid, L-rhamnose, D-galactose, and L-arabinose, with approximately 2% protein that gives it emulsification properties (Dickinson, 2003). Gum arabic has a unique combination of excellent emulsifying properties, low solution

viscosity, and high water solubility which makes it an effective encapsulation agent (Dauqan & Abdullah, 2013). Due to these and other properties, it is the most widely used encapsulating material in microencapsulation by spray drying (da Silva et al., 2013). However, the use of this polymer is difficult due to its limited supply and high cost (Estevinho, Rocha, Santos, & Alves, 2013). Besides its technological benefits when used as an encapsulating agent, gum arabic also has the added advantage of providing the health benefits associated with dietary fiber for use in the probiotic food industry (Chun, Kim, & Cho, 2014; Desmond et al., 2002).

CHAPTER 3. MATERIALS AND METHODS

3.1. Microorganisms

Lactobacillus plantarum NRRL B-4496 (LP) and *Lactobacillus acidophilus* NRRL B-4495 (LA) were provided by ARS Culture Collection (Washington DC, US). The frozen bacteria were separately activated twice in deMan Rogosa Sharpe (MRS) broth (Neogen Corporation, Lansing, MI, USA). Seventy-five mL of each strain were inoculated in MRS broth (1500 mL) and incubated at 37 °C for 16 h to reach stationary phase. The LP and LA cell cultures were harvested and washed with sterile distilled water by centrifugation at 10000 x *g* for 10 min at 4 °C (Beckman J2-HC, Beckman Coulter, Inc., Brea, CA, USA).

3.2. Preparation of probiotics solutions

Three wall material solutions were separately prepared by suspending 200 g of wall material per liter of distilled water. High maize starch (Hi-maize ® 260, Ingredion Incorporated, NJ, USA), maltodextrin (Dextrose Equivalent (DE) of 9-13, Now Foods Company, Bloomingdale, IL, USA), and gum arabic (Frontier Co-op, Norway, IA, USA) were dissolved in distilled water and autoclaved at 121 °C for 15 min . The wall material solutions were then separately mixed with the LP and LA cell cultures (~10^9 CFU/mL) to produce LP and LA solutions, respectively. The cell solutions were analyzed for moisture content by a microwave-type moisture analyzer (Model 907875, CEM Corporation, Inc., Matthews, NC, USA) as well as cell viability before spray drying was determined.

3.3. Spray drying of probiotic solutions

LP and LA probiotic solutions mixed with wall materials were separately fed into a pilot-scale spray dryer (FT80/81 Tall Form Spray Dryer Armfield Inc., Ringwood, UK) under co-current drying conditions. The spray drying process started by blowing the ambient air into the

air heating chamber by the inlet fan where the ambient air was heated at 140°C by an electric resistance heater. The heated air was then blown into the top of the drying chamber. At the same time, a feeding pump delivered the probiotic solutions mixed with wall materials through a two-fluid type spray nozzles where they were atomized with an atomizing air pressure of 14.5 psig and sprayed into the main dryer chamber. The probiotic solutions mixed with wall materials droplets were dried yielding dried powder and dust. Then the dried powder, dust, and air were pulled out of the drying chamber to the cyclone by the exhaust fan where the powder and dust were separated in the cyclone due to density differences. The powder was collected in the cyclone walls and powder collector. The dust was trapped in the filter bag and the exhaust air was released through filter bag to the ambient. During the drying process the air velocity and temperature of inlet ambient air; the relative humidity, temperature, and air velocity that passed through the exhaust fan were measured using a hot-wire anemometer (Anemomaster Model 6162, Kanomax Inc. Japan). The relative humidity of inlet ambient air was measured using an Omega 4-in-1 multifunctional anemometer (Omega Engineering, Stamford, CT, USA). Also, the internal diameter of both ambient air intake pipe and exhaust air pipe were measured. In total, six different spray dried probiotic powders were produced, namely, *L. plantarum* with high maize starch (LPHM), *L. plantarum* with maltodextrin (LPMD), *L. plantarum* with gum arabic (LPGA), *L. acidophilus* with high maize starch (LAHM), *L. acidophilus* with maltodextrin (LAMD), and *L. acidophilus* with gum arabic (LAGA). Samples of approximately 1.5 g of the six spray dried powders were separately placed in 4” x 6” aluminum bags and packed either under 97% or 10% vacuum (Koch Ultravac UV550 Double-Chamber Vacuum-Packaging, Kansas City, USA). The packed powders were stored separately at refrigerated (4 °C) and at room (23 °C) temperatures for up to 60 days in order to analyze the cell viability after spray

drying and during storage. The powder production rate and actual powder production rate were determined. The powder production rate was the mass of the powder collected from the powder collector divided by the time of production; the actual powder production rate was the dry solids mass of powder recovered from the powder collector divided by the time of production. The estimated production rate was the sum of the actual production rate and the rate at which powder was retained within the internal spray dryer walls.

3.3.1. Estimation of production rate of probiotic powders

The mass balances expressed as average mass flow rates of dry solids entering and leaving the spray dryer system (Figure 3.1.) is described by Eq. (1).

$$m_e = mP + m_d \quad (1)$$

The production rate of powder (*mP*) was obtained using the Eq. (2).

$$mP = m_e - m_d \quad (2)$$

Where: m_e is the probiotic solution flow rate (kg dry solids/h); m_d is the dust flow rate (kg dry solids/h); and *mP* is the estimated powder production rate (kg dry solids/h) which included both the actual production flow rate (m_p) for the powder collected from the cyclone collector vessel and product retained in the spray dryer. The physical properties of the product retained in the spray dryer were assumed to be the same as the powder collected in cyclone collector vessel (Pu et al., 2011).

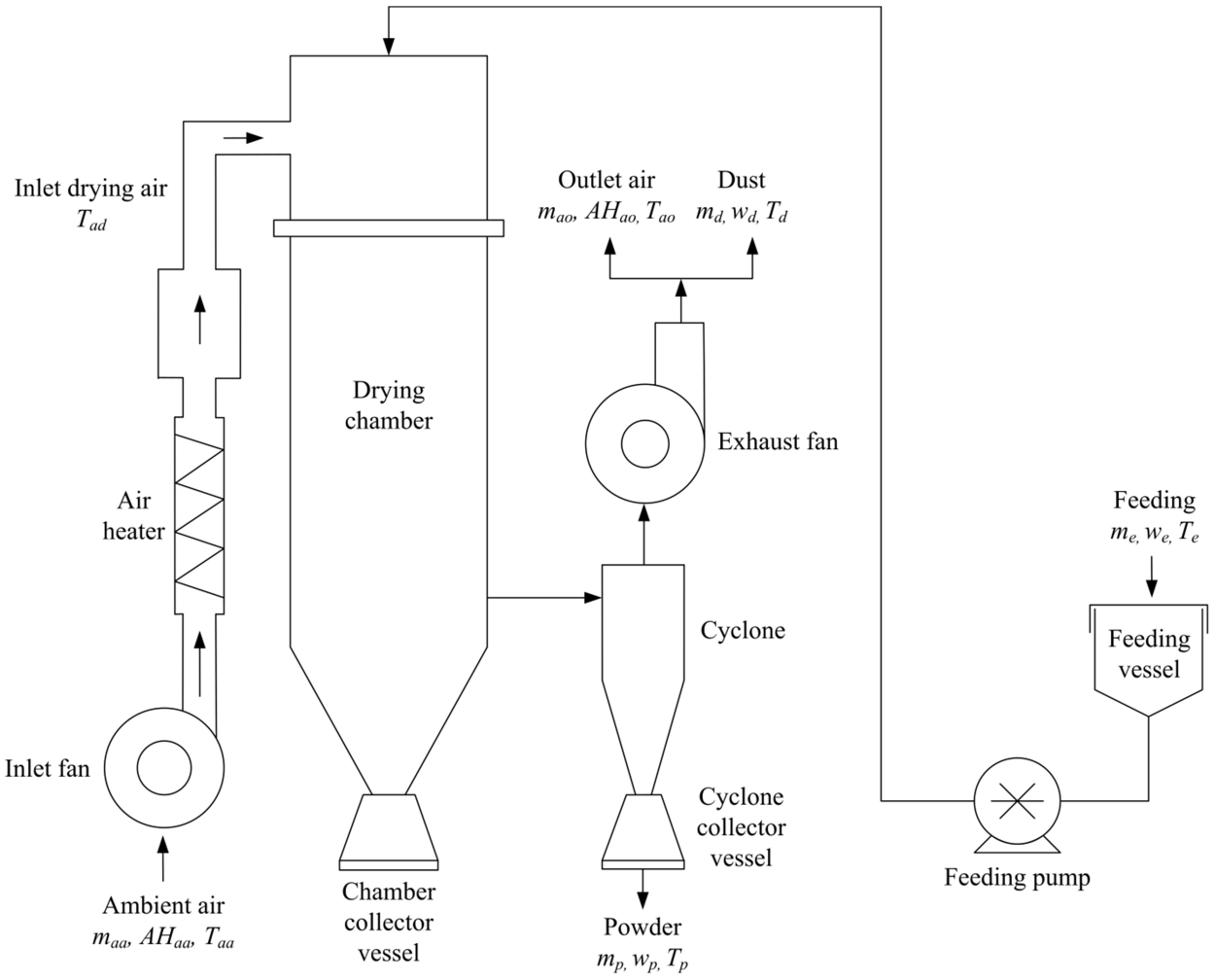

Figure 3.1. Material balances for the spray drying system.

3.3.2. Estimation of evaporation rate for drying probiotic solutions

The overall moisture balance expressed as water entering and leaving the spray dryer is described by Eq. (3).

$$m_{aa}AH_{aa} + m_e w_e = m_{ao}AH_{ao} + m_d w_d + mP w_p \quad (3)$$

Where: m_{aa} is the dry air mass flow rate of inlet ambient air (kg dry air/h); m_{ao} is the dry air mass flow rate of outlet air (kg dry air/h); m_e is the mass flow rate of probiotic solution (kg dry solids/h); m_d is the mass flow rate of dust (kg dry solids/h); mP is the estimated powder production rate (kg dry solids/h) which included both the product flow rate (m_p) for the powder

recovered from cyclone collector vessel and product retained in the spray dryer; AH_{aa} is the absolute humidity of inlet ambient air (kg water/kg dry air); AH_{ao} is the absolute humidity of outlet air (kg water/kg dry solids); w_e is the dry basis moisture content of probiotic solution (kg water/kg dry solids); w_d is the dry basis moisture content of dust (kg water/kg dry solids); and w_p is the dry basis moisture content of product (kg water/kg dry solids). It has been assumed that the powder retained in the spray dryer had essentially the same moisture content as the collected powder and that the encapsulation effectively removes that moisture from the air stream (Solval, Sundararajan, Alfaro, & Sathivel, 2012). The evaporation rate (Ev_p) based on the moisture content of the probiotic solution; powder recovered from the cyclone collector vessel; and dust was calculated using Eq. (4).

$$E_{vp} = m_e w_e - m_d w_d - mPw_p \quad (4)$$

The dry air mass flow rate of inlet ambient or outlet air were estimated following the method described by Solval (2011) using Eq. (5).

$$m = \frac{V}{V'} \quad (5)$$

Where: m is the dry air mass flow rate (kg dry air/h); V is the volumetric flow rate of inlet or outlet air (m^3/h), and V' is the specific volume of inlet or outlet dry air (m^3/kg dry air).

The volumetric flow rate of inlet ambient or outlet air was calculated using Eq. (6).

$$V = v \times A \quad (6)$$

Where: v is the average velocity of the inlet or outlet air (m/s) and A is the cross-sectional area of the inlet or outlet air pipe (m^2).

The specific volume of inlet or outlet dry air was calculated using Eq. (7).

$$V' = (0.082T + 22.4)\left(\frac{1}{29} + \frac{AH}{18}\right) \quad (7)$$

Where: T is the temperature of inlet ambient or outlet air (K) and AH is the absolute humidity of inlet ambient or outlet air (kg water/kg dry air).

The absolute humidity of the inlet ambient or the outlet air was calculated using Eq. (8).

$$AH = 0.622 \times \frac{p_w}{101.325 - p_w} \quad (8)$$

Where: AH is the absolute humidity of the inlet ambient or outlet air (kg water/kg dry air) and p_w is the partial pressure exerted by water vapor (kPa).

The partial pressure exerted by water vapor is estimated using Eq. (9).

$$p_w = p_v \times RH \quad (9)$$

Where: p_w is the partial pressure exerted by water vapor (kPa); p_v is the saturation pressure of water vapor (kPa), and RH is the relative humidity (%).

3.3.3. Estimation of energy used to dry the probiotic solutions

The estimation of the energy used to heat the air to spray dry the probiotic solutions mixed with wall materials was estimated using Eq. (5) as described by (Singh & Heldman, 2001).

$$Q = m_{aa} c_p \Delta T = m_{aa}(c_{aa} + c_v AH_{aa})(T_{ad} - T_{aa}) \quad (5)$$

Where: m_{aa} is dry air mass flow rate of inlet ambient air (kg dry air/h); C_p is specific heat of inlet ambient air (kJ/kg K); c_{aa} is specific heat of inlet ambient dry air (kJ/kg K); c_v is the specific heat of water vapor (kJ/kg K); AH_{aa} is the absolute humidity of inlet ambient air (kg water/kg dry air); ΔT is the temperature difference between inlet ambient air and inlet drying air (K); T_{ad} is the temperature of inlet drying air (K); and T_{aa} is the temperature of inlet ambient air (K).

3.3.4. Physicochemical properties of the probiotic powders

The probiotic powders were analyzed for water activity (a_w), moisture content, and color. The water activity was measured using an AquaLab Pawkit (Decagon Devices, Inc., Pullman, WA, USA). The moisture content was determined using a microwave-type moisture analyzer (Model 907875, CEM Corporation, Inc., Matthews, NC, USA). The color of the probiotic powders was determined using the chroma meter LabScan XE (Hunterlab, VA, USA). The data was reported in CIELAB color scales *L**, *a**, and *b**. In the CIELAB color scale, the *L** parameter ranges from 0 to 100, that defines the lightness level; *a** denotes the variation from red (+*a**) to green (−*a**); while *b** denotes the variation from yellow (+*b**) to blue (−*b**). Chroma and hue angle values were calculated with Eq. (6) and (7), respectively. All the samples were analyzed in triplicate.

$$Chroma = \left[a^* + b^* \right]^{1/2} \quad (6)$$

$$Hue = \tan^{-1}\left(\frac{b^*}{a^*} \right) \quad (7)$$

3.4. Determination of probiotics viability after spray drying and during storage

Probiotic solutions mixed with wall materials and powders were tested for cell viability by separately suspending and homogenizing with a vortex mixer the probiotic solutions (1 g) and probiotic powders (1 g) in 9 mL of 0.85 g/100 mL sterile saline solution, respectively. Serial dilutions and the pour plating method using MRS agar (Neogen Corporation, Lansing, MI, USA) with 0.6 g $CaCO_3$/100 mL (Sigma-Aldrich, St. Louis, MO, USA) was performed in triplicate. The plates were incubated at 37 °C, enumerated after 48 h and results expressed as colony forming units per gram sample (CFU/g).

3.5. Scanning electron microscopy (SEM)

The morphology of probiotic powders was observed in a scanning electron microscope (JSM-6610LV, JEOL Ltd. Japan). Samples were mounted on aluminum SEM stubs and then coated with platinum in an Edwards S150 sputter coater (Edwards High Vacuum International, Wilmington, MA, USA) for 4 minutes prior to observation at both 1000X and 3000X magnification.

3.6. Statistical analysis

The data were analyzed using SAS (Statistical Analysis System) software version 9.4 (SAS Institute Inc., Cary, NC, USA). Experiments were performed in triplicate and the data were reported as means±standard deviation. Tukey's test at an alpha of 0.05 was carried out to determine significant differences among the treatments.

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Physicochemical properties of the probiotic powders

4.1.1. Water activity of LP and LA powders

In this study, water activity (a_w) of LP and LA powders was not affected either by type of wall material or bacterial strain. As shown in Table 4.1, a_w values of both LP and LA powders ranged from 0.26 to 0.36 and were not significantly different. The a_w indicates free water (water not bound to molecules) which allows biochemical reactions to proceed. The a_w of probiotic powders or products has an impact on maintaining cell viability. According to Viernstein, Raffalt, and Polheim (2005), lower a_w (0.2-0.3) caused better cell viability during storage. However, membrane lipids could be oxidized, leading to viability reduction, if a_w is lower than 0.1.

4.1.2. Moisture content of LP and LA powders

Moisture contents (wet basis) of LP and LA powders varied between 5.63% and 8.98% (Table 4.1). Regardless of probiotic strain, the maltodextrin (LPMD = 5.89% and LAMD = 5.63%) powders had lower moisture content than gum arabic (LPGA = 7.84% and LAGA = 8.94%) and high maize starch (LPHM = 8.90% and LAHM = 8.98%) treatments. Similar to our results Tonon, Brabet, Pallet, Brat, and Hubinger (2009) reported that spray dried açai powder produced with maltodextrin (10 DE) had a lower moisture content than those produced with gum arabic. Encapsulating agents play an important role in moisture content of powders after spray drying, which is related to their glass transition temperature (Tg). Tg is specific to each amorphous material and is affected by molecular weight, chemical structure and moisture content of the material. de Barros Fernandes, Borges, and Botrel (2014) defined Tg as the temperature at which a state of polymeric material changes from a glassy amorphous state to a

rubbery state. Tg has an influence on duration of particle crust formation during the drying process. Aghbashlo, Mobli, Madadlou, and Rafiee (2012) stated that when the crust is formed, water is enclosed within the particle, causing the interior moisture to be difficult to evaporate. Droplets containing wall materials with lower Tg form the crust before the droplets containing materials with higher Tg (Pourashouri et al., 2014). Therefore, it is possible that crust formation of droplets containing MD occurred later than that of droplets with GA and HM. As a result, LPMD and LAMD powders had lower moisture content than LP and LA powders containing HM or GA. Kurozawa et al. (2009) reported that spray dried chicken breast protein hydrolysate powders with 20% (w/w) maltodextrin ($9 \leq DE \leq 12$) had a higher Tg value compared to those produced with 20% (w/w) gum arabic. Meanwhile, Freire, Fertig, Podczeck, Veiga, and Sousa (2009) report no Tg detection for Hylon V, a high amylose (56%) maize starch, claiming that the glass transition temperature can only be identified in these type of samples with a moisture content above 13%.

Table 4.1. Water activity and moisture content (dry basis) of probiotic powders.

Wall Material	Water activity		Moisture content (dry basis, kg water/kg dry solids)	
	LP	LA	LP	LA
HM	0.36 ± 0.04^{Aa}	0.35 ± 0.01^{Aa}	0.10 ± 0.00^{Aa}	0.09 ± 0.00^{Aa}
MD	0.29 ± 0.02^{Aa}	0.26 ± 0.00^{Aa}	0.06 ± 0.00^{Ba}	0.06 ± 0.00^{Aa}
GA	0.28 ± 0.01^{Aa}	0.31 ± 0.05^{Aa}	0.08 ± 0.00^{Ba}	0.09 ± 0.02^{Aa}

Values are means ± SD of triplicate determinations. ^{A,B}Means with the same letter in a column are not significantly different ($P \leq 0.05$). ^{a}Means with the same letter in a row within a parameter are not significantly different ($P \leq 0.05$). LP = *L. plantarum* NRRL B-4496, LA = *L. acidophilus* NRRL B-4495, HM = high maize starch, MD = maltodextrin, and GA = gum arabic.

4.1.3. Color of LP and LA powders

The color values of probiotic powders with different wall materials are reported in Table 4.2. The color of LP and LA powders is attributed to the carrier color (da Silva et al., 2013).

Regardless of bacteria strain, the powders with MD and HM had significantly greater lightness (*L**) than GA. This was because the colors of MD and HM were white, while GA used in this experiment was light brown. The color of spray dried powders can be affected by the concentrations (Comunian et al., 2011) and types (Fritzen-Freire et al., 2012) of wall materials.

Table 4.2. Color values of probiotic powders.

		HM	MD	GA
*L**	LP	91.83 ± 0.67^{Aa}	93.58 ± 0.76^{Aa}	88.77 ± 0.36^{Ba}
	LA	91.02 ± 0.96^{Aa}	93.67 ± 1.09^{Aa}	87.03 ± 0.32^{Bb}
*a**	LP	0.25 ± 0.03^{Aa}	-0.45 ± 0.01^{Cb}	0.17 ± 0.01^{Ba}
	LA	-0.04 ± 0.01^{Ab}	-0.39 ± 0.01^{Ba}	0.11 ± 0.11^{Aa}
*b**	LP	7.21 ± 0.13^{Aa}	3.23 ± 0.27^{Ca}	5.04 ± 0.23^{Ba}
	LA	5.55 ± 0.29^{Ab}	2.47 ± 0.63^{Ba}	3.16 ± 0.71^{ABa}
Hue	LP	88.02 ± 0.26^{Bb}	97.95 ± 0.41^{Aa}	88.12 ± 0.17^{Ba}
	LA	90.42 ± 0.17^{Ba}	99.19 ± 2.47^{Aa}	88.27 ± 1.53^{Ba}
Chroma	LP	7.22 ± 0.12^{Aa}	3.26 ± 0.27^{Ca}	5.04 ± 0.23^{Ba}
	LA	5.55 ± 0.29^{Ab}	2.50 ± 0.62^{Ba}	3.16 ± 0.71^{Ba}

*L**, *a**, and *b** are the degree of lightness to darkness, redness to greenness, and yellowness to blueness, respectively. Values are means ± SD of triplicate determination. ^{ABC}Means with the same letter in a row are not significantly different ($P \leq 0.05$). ^{ab}Means with the same letter in a column within a parameter are not significantly different ($P \leq 0.05$). LP = *L. plantarum* NRRL B-4496, LA = *L. acidophilus* NRRL B-4495, HM = high maize starch, MD = maltodextrin, and GA = gum arabic.

4.2. Spray drying of probiotic solutions

The estimated evaporation rates calculated based on the moisture content of the probiotic solutions mixed with wall materials, powder recovered from cyclone collector vessel, and dust ranged between 0.758 and 0.830 kg water/h (Table 4.3). The water evaporation during spray drying is affected by the inlet temperature (Gharsallaoui et al., 2007), the dryness of the air (Goula & Adamopoulos, 2005), and feeding flow rate (Garg, Sharma, Jayaprakashan, & Subramanian, 2009). In this study, probiotic solutions mixed with wall materials had different mass flow rates (kg/h) which produced as a result different evaporation rate values for the

production of the probiotic powders. The energy required to spray dry the probiotic solutions varied between wall materials (Table 4. 3). According to Kajiyama and Park (2011), the energy required to heat the ambient air during spray drying is affected by the feeding flow rate, feeding initial and final moisture content. The required power was calculated as 2.09, 2.04, and 2.06 kW for spray drying LPHM, LPMD, and LPGA, respectively. Meanwhile, the required power for spray drying LAHM, LAMD, and LAGA, was calculated as 2.09, 2.10, and 2.16 kW, respectively. The heat energy for heating the inlet ambient air was generated by an electric heater with a power of 4.5kW. In this study, the power requirements were within the available power of the electric heater of the FT80/81 Tall Form Spray Dryer (Spray Dryer Manual, Armfield, Ringwood, UK).

Table 4.3. Estimated evaporation rates and energy required to spray dry the probiotic solutions.

Wall Material	Evaporation rate (kg water/h)[1]		Energy used to spray dry probiotic powders (kJ/kg)	
	LP	LA	LP	LA
HM	0.758 ± 0.02^{Aa}	0.766 ± 0.01^{Ba}	8255.89 ± 268.92^{Aa}	8266.44 ± 123.00^{Aa}
MD	0.773 ± 0.04^{Aa}	0.775 ± 0.01^{Ba}	7557.63 ± 10.85^{Ba}	7789.16 ± 288.95^{Aa}
GA	0.814 ± 0.00^{Aa}	0.830 ± 0.01^{Aa}	7532.73 ± 34.58^{Bb}	7774.37 ± 13.91^{Aa}

Values are means ± SD of triplicate determination. [1]Calculated based on the moisture content of the probiotic solution mixed with wall material, powder recovered from cyclone collector vessel, and dust (kg water/h). [AB]Means with the same letter in a column are not significantly different ($P \leq 0.05$). [a]Means with the same letter in a row within a parameter are not significantly different ($P \leq 0.05$). LP = *L. plantarum* NRRL B-4496, LA = *L. acidophilus* NRRL B-4495, HM = high maize starch, MD = maltodextrin, and GA = gum arabic.

As shown in Table 4.4, the actual production rates of LP and LA powders ranged from 0.091 to 0.105 (kg dry solids/h) and were significantly ($P \geq 0.05$) lower than the estimated production rates that ranged from 0.132 to 0.147 (kg dry solids/h). These results were expected because all the produced powders were not recovered due to adherence of the powder particles in

the different internal parts of the spray dryer. The summary of the inlet and outlet air conditions for spray drying of LP and LA powders are shown in Table 4.5 and 4.6, respectively. As Table 4.6 shows, the outlet air temperatures varied between 336.31±0.62 and 342.76±0.16 K. According to Peighambardoust et al. (2011), outlet air temperatures are affected by the inlet air temperature, air flow rate, product feed rate, medium composition, and atomized droplet size. The dry air mass flow rate of the inlet (Table 4.5) and outlet (Table 4.6) air values were similar; this means that the measurements carried out to determine these values were accurate.

Table 4.4. Data for the estimation of the production rate of probiotic powders.

Batch		Mass flow rate powder ($x10^{-3}$ kg/h)	Actual production rate (dry basis, $x10^{-3}$ kg dry solids/h)	Estimated production rate (dry basis, $x10^{-3}$ kg dry solids/h)
LPHM	Powder	104.92 ± 7.22^{a}	95.56 ± 6.36^{Aa}	138.31 ± 3.79^{Ba}
	Dust	0.96 ± 0.65^{a}	0.87 ± 0.59^{a}	
LPMD	Powder	111.65 ± 6.38^{a}	105.13 ± 5.79^{Aa}	147.19 ± 3.18^{Ba}
	Dust	2.47 ± 0.19^{a}	2.32 ± 0.18^{a}	
LPGA	Powder	101.62 ± 0.70^{a}	93.63 ± 0.07^{Aa}	133.19 ± 14.49^{Aa}
	Dust	2.25 ± 0.31^{a}	2.07 ± 0.30^{a}	
LAHM	Powder	101.02 ± 6.71^{a}	91.92 ± 5.60^{Aa}	132.59 ± 1.06^{Ba}
	Dust	2.38 ± 0.38^{a}	2.16 ± 0.35^{a}	
LAMD	Powder	106.12 ± 0.48^{a}	100.13 ± 0.43^{Aa}	144.36 ± 0.12^{Ba}
	Dust	2.09 ± 0.33^{a}	1.97 ± 0.32^{a}	
LAGA	Powder	108.97 ± 7.10^{a}	99.27 ± 8.30^{Aa}	147.24 ± 2.96^{Ba}
	Dust	1.35 ± 1.13^{a}	1.22 ± 1.01^{a}	

Values are means ± SD of triplicate determination. The estimated powder production rate included both powder recovered from cyclone collector vessel and product retained inside the spray dryer. ^{AB}Means with the same letter in a row are not significantly different ($P \leq 0.05$). ^{a}Means with the same letter in a column are not significantly different ($P \leq 0.05$). LP=*L. plantarum* NRRL B-4496, LA=*L. acidophilus* NRRL B-4495. HM = high maize starch, MD = maltodextrin, and GA = gum arabic. LPHM=LP with high maize starch spray dried at 140°C, LPMD=LP with maltodextrin spray dried at 140°C, LPGA=LP with gum arabic spray dried at 140°C, LAHM=LA with high maize starch spray dried at 140°C, LAMD=LA with maltodextrin spray dried at 140°C, and LAGA=LA with gum arabic spray dried at 140°C.

Table 4.5. Summary of inlet air conditions for spray drying the probiotic solutions.

Condition	LPHM	LPMD	LPGA	LAHM	LAMD	LAGA
Ambient air temperature (AAT) (K)	306.46±1.53	307.65±0.89	308.03±2.43	306.42±0.47	305.73±1.62	303.48±0.84
Inlet air velocity (m/s)	18.32±0.21	18.15±0.11	18.32±0.16	18.39±0.12	18.32±0.26	18.37±0.19
Internal pipe diameter (m)	0.034	0.034	0.034	0.034	0.034	0.034
Volumetric flow rate of inlet air (m^3/h)	59.87±0.69	59.33±0.39	59.87±0.54	60.09±0.39	59.87±0.85	60.04±0.62
Relative humidity of inlet air (%)	54.73±2.02	52.14±0.66	53.23±0.85	61.85±1.67	52.80±8.24	49.19±8.32
Partial pressure exerted by water vapor at the inlet point (kPa)	2.81±0.14	2.87±0.11	2.99±0.35	3.17±0.18	2.59±0.17	2.15±0.46
Saturation pressure of water vapor at the inlet point (kPa)*	5.15	5.49	5.62	5.12	4.94	4.34
Absolute humidity of inlet air ($x10^{-3}$ kg water/kg dry air)	17.75±0.90	18.07±0.69	18.92±2.34	20.06±1.13	16.31±1.10	13.46±2.98
Specific volume of inlet air (m^3/kg dry air)	0.90±0.01	0.90±0.01	0.90±0.01	0.90±0.01	0.89±0.00	0.88±0.01
Mass flow rate of inlet air (kg dry air/h)	67.17±1.21	66.26±0.17	66.70±0.17	67.18±0.66	67.48±1.20	68.47±0.19
Specific heat of dry air at AAT (kJ/kg K)*	1.0133	1.0133	1.0133	1.0133	1.0133	1.0133
Specific heat of water vapor at AAT (kJ/kg K)**	1.88	1.88	1.88	1.88	1.88	1.88
Temperature of inlet drying air (K)	413.15±0.21	413.13±0.66	413.13±0.99	413.80±0.28	413.83±0.24	413.50±0.26

Values are means ± SD of triplicate determination. *Obtained from Appendix A 4.2 and A 4.4, respectively (Singh & Heldman, 2001). **Selected as 1.88 kJ/(kg K) according to Singh & Heldman, 2001. LP=*L. plantarum* NRRL B-4496, LA=*L. acidophilus* NRRL B-4495. HM = high maize starch, MD = maltodextrin, and GA = gum arabic. LPHM=LP with high maize starch spray dried at 140°C, LPMD=LP with maltodextrin spray dried at 140°C, LPGA=LP with gum arabic spray dried at 140°C, LAHM=LA with high maize starch spray dried at 140°C, LAMD=LA with maltodextrin spray dried at 140°C, and LAGA=LA with gum arabic spray dried at 140°C.

Table 4.6. Summary of outlet air conditions for spray drying the probiotic solutions.

Condition	LPHM	LPMD	LPGA	LAHM	LAMD	LAGA
Outlet air temperature (K)	341.86±1.15	341.31±0.85	342.76±0.16	341.13±0.78	339.38±1.46	336.31±0.62
Outlet air velocity (m/s)	4.63±0.11	4.59±0.16	4.65±0.10	4.64±0.08	4.59±0.12	4.50±0.04
Internal pipe diameter (m)	0.072	0.072	0.072	0.072	0.072	0.072
Volumetric flow rate of outlet air (m^3/h)	67.79±1.56	65.55±0.18	68.16±1.32	67.86±1.12	67.08±1.87	65.81±0.62
Relative humidity of outlet air (%)	10.24±0.52	12.00±0.14	11.25±0.49	12.45±0.78	12.00±0.28	11.40±0.14
Partial pressure exerted by water vapor at the outlet point (kPa)	3.03±0.01	3.47±0.07	3.46±0.17	3.57±0.10	3.19±0.30	2.65±0.11
Saturation pressure of water vapor at the outlet point (kPa)*	29.61	28.92	30.72	28.70	26.55	23.17
Absolute humidity of outlet air ($x10^{-3}$ kg water/kg dry air)	19.16±0.06	22.07±0.47	22.03±1.10	22.74±0.67	20.24±2.00	16.68±0.71
Specific volume of outlet air (m^3/kg dry air)	1.00±0.01	1.00±0.00	1.01±0.01	1.00±0.00	1.00±0.01	0.98±0.01
Mass flow rate of outlet air (kg dry air/h)	67.56±1.13	66.62±1.43	67.93±1.46	67.86±1.21	67.83±1.17	67.39±0.43

Values are means ± SD of triplicate determination. *Obtained from Appendix A 4.2 (Singh & Heldman, 2001). LP=*L. plantarum* NRRL B-4496, LA=*L. acidophilus* NRRL B-4495. HM = high maize starch, MD = maltodextrin, and GA = gum arabic. LPHM=LP with high maize starch spray dried at 140°C, LPMD=LP with maltodextrin spray dried at 140°C, LPGA=LP with gum arabic spray dried at 140°C, LAHM=LA with high maize starch spray dried at 140°C, LAMD=LA with maltodextrin spray dried at 140°C, and LAGA=LA with gum arabic spray dried at 140°C.

4.3. Effect of wall materials on the viability of spray-dried probiotic powders

The viability of LP and LA powders after spray drying with different wall materials is illustrated in Figures 4.1 and 4.2, respectively. Initial cell counts in LPHM, LAHM, LPMD, LAMD, LPGA, and LAGA probiotic solutions before spray drying were 9.48, 9.06, 9.25, 9.05, 9.06, and 9.24 log CFU/g, respectively. The number of viable cells of both LP and LA powders decreased by less than 1.25 log CFU/g after spray drying, except for LPGA powders which had a 0.26 log CFU/g reduction. After spray drying, the number of viable cells in LPGA, LPHM, and LPMD powders was 8.80, 8.31, and 8.09 log CFU/g, respectively. Meanwhile, LAGA had a number of viable cells of 8.10 log CFU/g, followed by LAHM with 7.90 log CFU/g, and 7.82 log CFU/g for LAMD. These results showed that GA was the best protective agent of cells after the spray drying process. Regarding the storage temperatures, the results showed that the cell viability of LP and LA powders was more stable at refrigerated temperature (4 °C) than those stored at ambient temperature (23 °C). These results are in agreement with other studies that used different bacterial strains and encapsulating agents (Gardiner et al., 2000; Hamsupo et al., 2005; Soukoulis et al., 2014). According to Chávez and Ledeboer (2007) and Santivarangkna et al. (2007), the survival of probiotic bacteria is inversely related to the temperature during storage conditions. Furthermore, De Castro-Cislaghi et al. (2012) state that the encapsulating agent also has a direct effect on the stability of the microencapsulated cells. Regarding the vacuum conditions, both strains showed a higher survival at 97% vacuum than at 10% vacuum when they were kept at 4 °C and at 23 °C. This suggests that lower levels of oxygen improved cell viability during storage (Chávez & Ledeboer, 2007). According to (Champagne, Gardner, & Roy, 2005), oxygen affects probiotic cells due to the intracellular production of hydrogen peroxide. In

addition, Tripathi and Giri (2014) revealed that the production of free radicals from the oxidation of cellular fats can be toxic to probiotic cells. A detailed discussion of each condition follows:

4.3.1. Viability of spray-dried L. plantarum NRRL B-4496 powders during storage at refrigerated (4 °C) temperature

At 60 days of storage under 4 °C (Figure 4.1a), the results demonstrated that LPHM powders yielded the highest viability of LP, followed by LPGA and LPMD. At 60 days of storage, LPHM powders stored at 97% vacuum had a cell viability of 8.17 log CFU/g powder representing a 0.14 log reduction in viability. Regarding LPHM powder stored at 10% vacuum, the number of viable cells after 60 days was 8.08 log CFU/g which corresponds to a 0.23 log reduction in viability. Bandyopadhyay and Mandal (2014) and Anal and Singh (2007) state that resistant starch offers a surface for adherence of the bacterial cells during processing, storage, and transit through the upper gastrointestinal tract, providing robustness and resilience to environmental stresses. Probably this is the reason why LP had a higher viability during refrigerated storage at both vacuum conditions compared to those with MD and GA. Goderska and Czarnecki (2008) reported that the use of high maize starch (Hylon VII) as an encapsulating agent kept the viability of spray dried *Bifidobacterium bifidum* DSM 20239 cells stable during four months of storage at 4 °C, stating that may be the adhesion of cells to the starch is responsible for the improvement on the cells viability. The viability of LPGA powders was not significantly different ($P \leq 0.05$) than HM powders at both 97% and 10% vacuum conditions, having at the 60^{th} day of storage a viability of 7.94 and 7.85 log CFU/g, respectively. Regarding LPMD powders, both reported viability of more than 7 log CFU/g at the 60^{th} day of storage time. The number of viable cells was 7.85 log CFU/g for LPMD at 97% vacuum and 7.34 log CFU/g for LPMD at 10% vacuum. The numbers of viable cells in all samples in the present study met the recommended levels in a probiotic product to confer health benefits to humans (Meng et al.,

2008; Reddy, Madhu, & Prapulla, 2009; Su, Lin, & Chen, 2007; Ying, Sun, Sanguansri, Weerakkody, & Augustin, 2012). According to the results, the three wall materials can be used to preserve *L. plantarum* up to 60 days of storage at refrigerated (4 °C) conditions. However, other factors such as powder yields, costs, and physicochemical properties need to be considered to select a wall material for a specific application.

4.3.2. Viability of spray-dried L. plantarum NRRL B-4496 powders during storage at room (23 °C) temperature

When *L. plantarum* powders were stored at room temperature (Figure 4.1b), a rapid decrease in cell viability was observed in all powder samples. GA was more effective to preserve the cells viability than HM and MD after 60 days of storage. However, the number of viable cells in GA powders at 97% and 10% vacuum had decreased significantly ($P \geq 0.05$) after 5 days of storage from 8.80 to 7.87 log CFU/g. At the 60^{th} day, the highest number of live bacteria was found in LPGA-vac and LPGA-air powders with a cell viability of 6.97 and 6.33 log CFU/g respectively. Also, LPHM-air and LPMD-vac powders reported a cell viability of 6.11, and 6.02 log CFU/g, respectively. It was obvious that the viability of LP powders stored at 23 °C was significantly lower at the end of the storage period than LP powders stored at 4 °C. These results are supported by other studies that found the same downward trend in viability when *L. plantarum* was stored at high temperatures (Bucio, Hartemink, Schrama, Verreth, & Rombouts, 2005; Coghetto, Flores, Brinques, & Ayub, 2016; Lapsiri, Bhandari, & Wanchaitanawong, 2012). Fu and Chen (2011) and Teixeira, Castro, and Kirby (1996) claim this downward trend in viability is likely because during long storage periods at high temperatures the bacteria undergo a natural degradation of life-essential macromolecules such as lipids and proteins due to oxidation and denaturation processes.

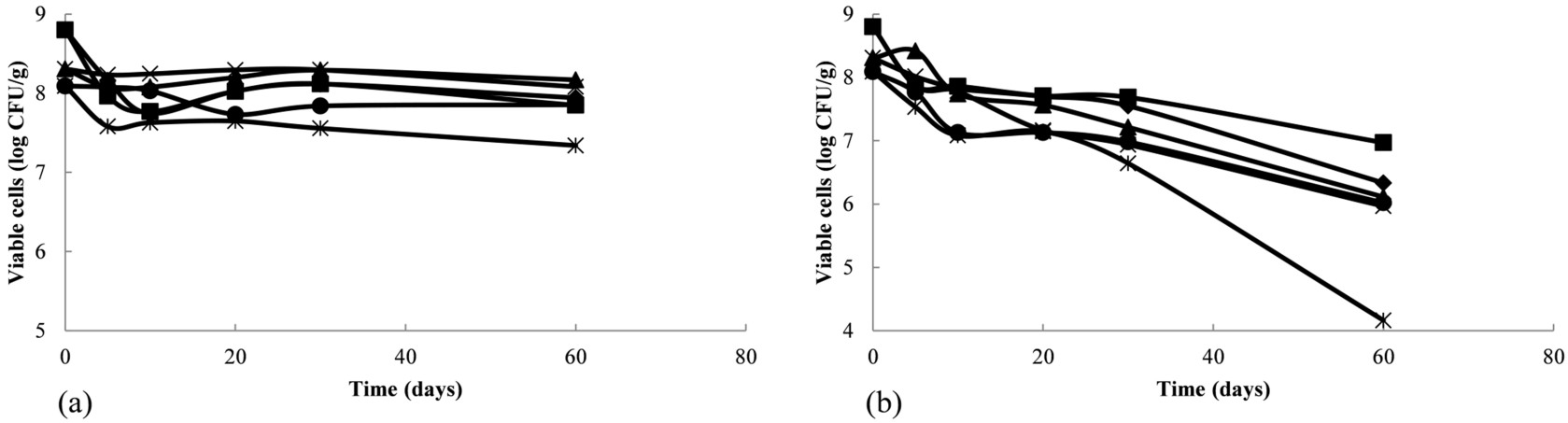

Figure 4.1. Effect of different wall materials on the survival of spray-dried *L. plantarum* NRRL B-4496 microcapsules stored under 97 and 10% vacuum at (a) refrigerated (4 °C) and (b) room (23 °C) temperature. HM = high maize starch, MD = maltodextrin, GA = gum arabic, Vac = 97% vacuum, Air = 10% vacuum, and LP = *L. plantarum* NRRL B-4496. GA-Vac (—◆—), and GA-Air (—■—) = spray-dried LP with GA stored under 97 and 10% vacuum, respectively; HM-Vac (—▲—) and HM-Air (—✕—) = spray-dried LP with HM stored under 97 and 10% vacuum, respectively; and MD-Vac (—✳—) and MD-Air (—●—) = spray-dried LP with MD stored under 97 and 10% vacuum, respectively.

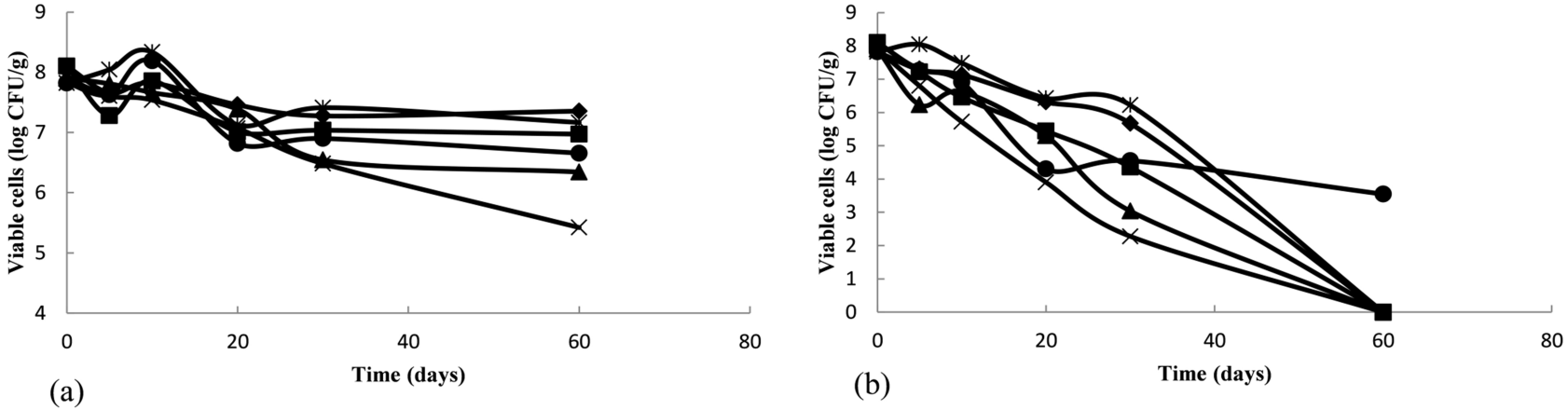

Figure 4.2. Effect of different wall materials on the survival of spray-dried *L. acidophilus* NRRL B-4495 microcapsules stored under 97 and 10% vacuum at (a) refrigerated (4 °C) and (b) room (23 °C) temperature. HM = high maize starch, MD = maltodextrin, GA = gum arabic, Vac = 97% vacuum, Air = 10% vacuum, and LA = *L. acidophilus* NRRL B-4495. GA-Vac ( ), and GA-Air ( ) = spray-dried LA with GA stored under 97 and 10% vacuum, respectively; HM-Vac ( ) and HM-Air ( ) = spray-dried LA with HM stored under 97 and 10% vacuum, respectively; and MD-Vac ( ) and MD-Air ( ) = spray-dried LA with MD stored under 97 and 10% vacuum, respectively.

4.3.3. Viability of spray-dried L. acidophilus NRRL B-4495 powders during storage at refrigerated (4 °C) temperature

Similar to the previous result, GA showed better protective effects on preserving LA during storage at 4 °C (Figure 4.2a) than HM and MD. At 60 days of storage, LAGA-vac powders had higher cell viability than the other powder samples with 7.36 log CFU/g of cells, corresponding to a 0.75 log reduction in viability, while the number of live bacteria for LAGA-air powders was 6.98 log CFU/g. The number of live bacteria for LAMD-vac and LAMD-air powders after 60 days had decreased to 7.18 and 6.66 log CFU/g, respectively. Meanwhile, the number of live bacteria for LPHM-vac powders was 6.35 log CFU/g. The results showed that more than 6 log CFU/g of *L. acidophilus* survived at 4^{o}C after 60 days of storage. Zhao et al. (2008) claim that a minimum concentration of *L. acidophilus* equivalent to 10^{6} CFU/mL or gram of product is needed to have therapeutic benefits in the human body. Also, Kailasapathy and Chin (2000) state that regular consumption of yogurt containing 1.0×10^{6} CFU/g of human origin *L. acidophilus*, which is able to survive the upper regions of the gastrointestinal tract, is essential to achieve therapeutic benefits.

4.3.4. Viability of spray-dried L. acidophilus NRRL B-4495 microcapsules stored at room (23 °C) temperature

The *L. acidophilus* powders stored at room temperature (Figure 4.2b) experienced a steep decline in cell viability. At the 10^{th} day of storage, the number of viable cells in all LA powders was above of 6 log CFU/g. However, at the 20^{th} and 30^{th} day of storage, only LAMD-vac and LAGA-vac were able to keep a cell viability of more than 6 log CFU/g. Unlike LP, there were no viable cells of LA detected at 60 days of storage at room temperature regardless wall material or vacuum conditions, except for the LAMA-air powders which had 3.56 log CFU/g. Overall, cell counts for LA powders stored at room temperature failed to meet the requirement for probiotic products. The results indicated that LA was more susceptible to the tested storage conditions

than LP. Golowczyc et al. (2010), report that *L. plantarum* species had a greater thermotolerance throughout spray drying compared with *L. kefir* and *Saccharomyces lipolytica*. Soukoulis et al. (2014), state that *L. acidophilus* is a thermo-sensitive probiotic strain and their viability is critically affected by the operating spray drying conditions. Also, Fu and Chen (2011) classify *L. plantarum* as a thermophilic bacteria and *L. acidophilus* as a heat sensitive bacteria. According to Soukoulis et al. (2014) some lactobacilli strains have greater thermotolerance throughout spray drying and this characteristic is strictly strain specific. Probably, the effects of spray drying on cell damage could subsequently affect the viability of *L. acidophilus* during the storage conditions.

4.4. Scanning electron microscopy of spray-dried probiotic powders

The spray-dried lactobacilli powders were observed by a scanning electron microscopy (SEM) (Figures 4.3 and 4.4). Spray-dried powders had a surface without mechanical fissures and the presence of concavities. These concavities were the result of the rapid evaporation of the atomized liquid drops during spray drying (Fritzen-Freire et al., 2012). Both LPHM and LAHM powders had a donut shape, possibly because of a particular property of starch when it is subjected to spray drying (O'riordan, Andrews, Buckle, & Conway, 2001). LPMD and LAMD powders consisted of particles with a wrinkled surface or with concavities. Rodríguez-Huezo et al. (2007) reported that the use of moderate inlet drying temperatures in spray dried powders (140 °C) could produce concavities, making the particles stronger against mechanical fracture and solute diffusion. LPGA and LAGA powders had spherical shape and a flat ball effect on their surfaces, which was also found in other studies using gum arabic (Desmond et al., 2002; Lian, Hsiao, & Chou, 2002) as a wall material for the production of spray-dried probiotic powders.

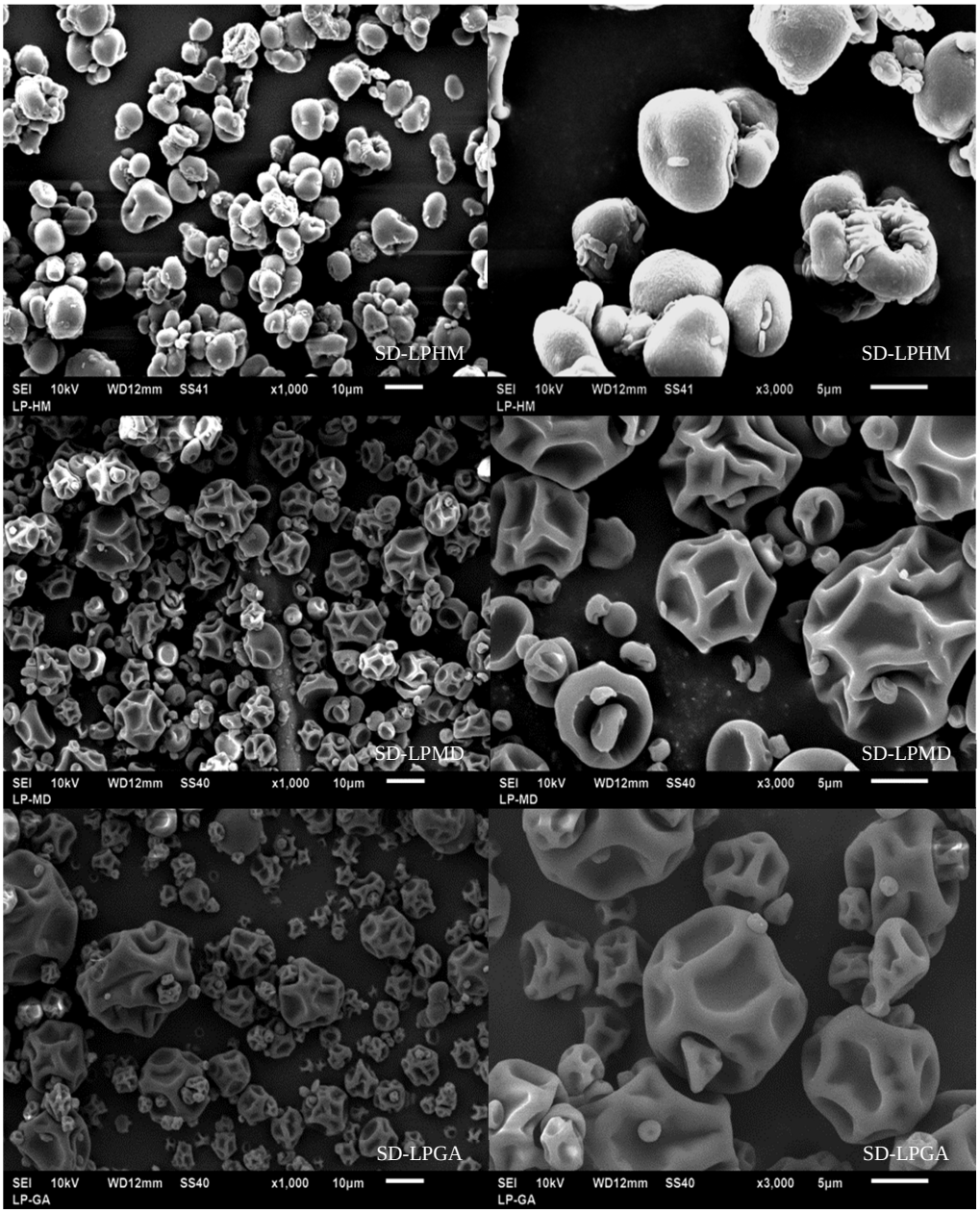

Figure 4.3. Scanning electron micrographs of spray-dried *L. plantarum* NRRL B-4496 powders. HM = high maize starch, MD = maltodextrin, GA = gum arabic, and LP = *L. plantarum* NRRL B-4496. SD-LPHM = spray-dried microcapsules of LP with high maize starch, SD-LPMD = spray-dried microcapsules of LP with maltodextrin, and SD-LPGA = spray-dried microcapsules of LP with gum arabic. Magnification: left side = 1000x, right side = 3000x.

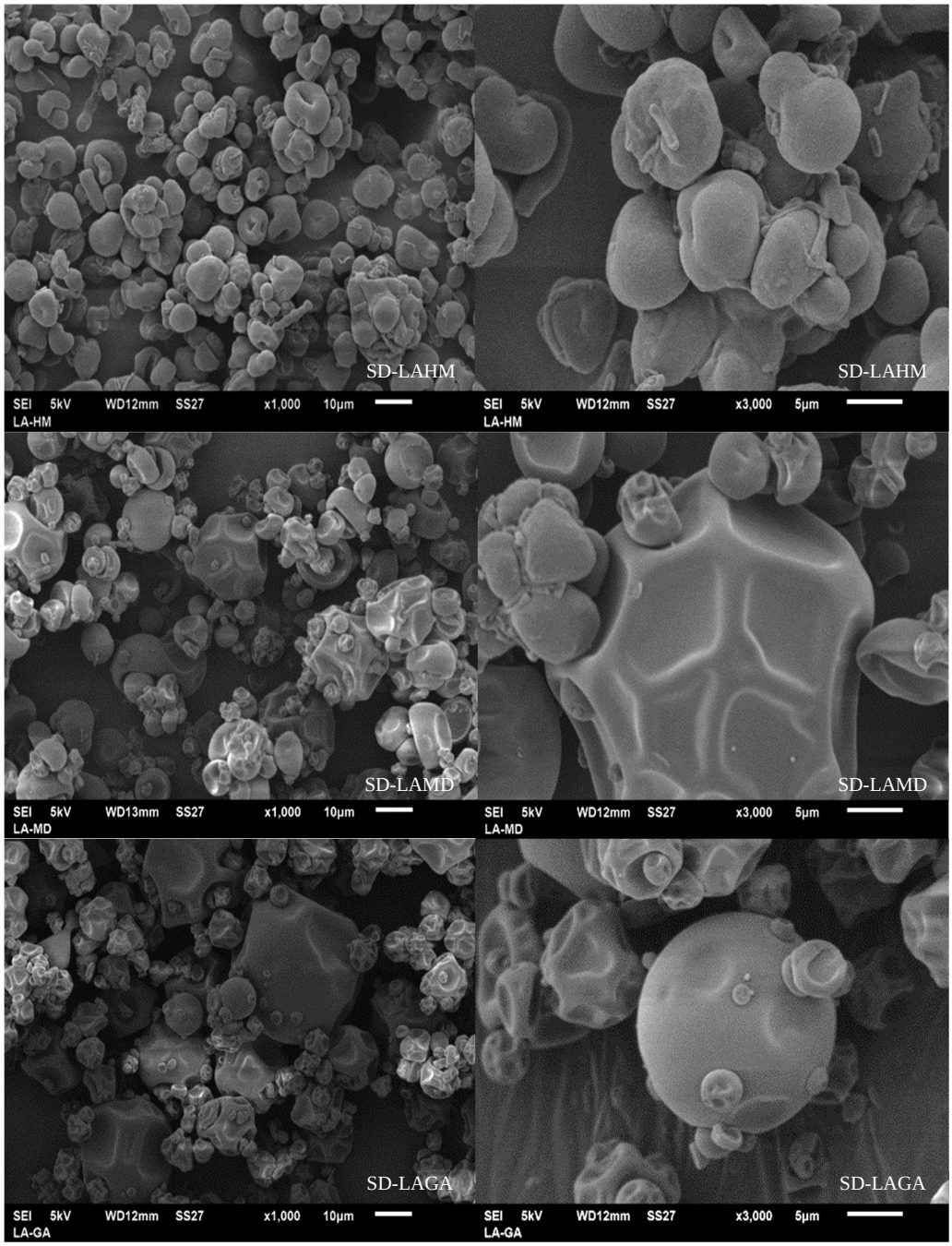

Figure 4.4. Scanning electron micrographs of spray-dried *L. acidophilus* NRRL B-4495 powders. HM = high maize starch, MD = maltodextrin, GA = gum arabic, and LA = *L. acidophilus* NRRL B-4495. SD-LAHM = spray-dried microcapsules of LA with high maize starch, SD-LAMD = spray-dried microcapsules of LA with maltodextrin, and SD-LAGA = spray-dried microcapsules of LA with gum arabic. Magnification: left side = 1000x, right side = 3000x.

CHAPTER 5. SUMMARY AND CONCLUSIONS

The objectives of the present study were to a) investigate the effect of high maize starch (HM), maltodextrin (MD), and gum arabic (GA) on the viability of *L. plantarum* NRRL B-4496 (LP) and *L. acidophilus* NRRL B-4495 (LA) after spray drying and during storage at different conditions and b) to evaluate the spray drying conditions to produce the aforementioned probiotic powders.

Probiotic solutions mixed with wall materials were separately spray dried at an inlet drying air temperature of 140°C to obtain six LP and LA-powders. The actual production rate of powders ranged from 0.091 to 0.105 (kg dry solids/h). The energy used during spray drying was not significantly different for any of the powders. The estimated evaporation rate of water from the six powders during spray drying ranged from 0.758 to 0.830 (kg water/h) and was not affected by wall materials or probiotic strains. The moisture content of LP and LA-powders ranged from 5.63 to 8.98 % and regardless of probiotic strain, the maltodextrin treatment had lower moisture content than gum arabic and high maize starch treatments.

At the end of the storage period, LPHM powders packed under 10% and 97% vacuum and stored at 4^{o}C had significantly higher cell viability than the other powder samples. LA powders had lower numbers of surviving cells than LP powders for the same protective agents and storage conditions. The results showed that cell viability of both LP and LA powders was more stable at refrigerated temperature (4 °C) than those powders stored at ambient temperature (23 °C). Also, both LP and LA powders packed at 97% vacuum had a higher viability than those powders stored at 10% vacuum. The data obtained showed that high maize starch can be used as a protective agent to maintain the viability of *L. plantarum* powder at recommended levels for up to 60 days of storage.

REFERENCES

Aghbashlo, M., Mobli, H., Madadlou, A., & Rafiee, S. (2012). The correlation of wall material composition with flow characteristics and encapsulation behavior of fish oil emulsion. *Food Research International, 49*(1), 379-388.

Agnihotri, N., Mishra, R., Goda, C., & Arora, M. (2012). Microencapsulation–a novel approach in drug delivery: a review. *Indo Global Journal of Pharmaceutical Sciences, 2*(1), 1-20.

Agrawal, R. (2005). Probiotics: an emerging food supplement with health benefits. *Food Biotechnology, 19*(3), 227-246.

Anal, A. K., & Singh, H. (2007). Recent advances in microencapsulation of probiotics for industrial applications and targeted delivery. *Trends in Food Science & Technology, 18*(5), 240-251.

Anandharamakrishnan, C., & Ishwarya, S. P. (2015). Introduction to encapsulation of food ingredients. *Spray Drying Techniques for Food Ingredient Encapsulation*, 37-64.

Ananta, E., Volkert, M., & Knorr, D. (2005). Cellular injuries and storage stability of spray-dried Lactobacillus rhamnosus GG. *International Dairy Journal, 15*(4), 399-409.

Anekella, K. (2012). *Microencapsulation of probiotics (Lactobacillus acidophilus and Lactobacillus rhamnosus) in raspberry powder by spray drying: optimization and storage stability studies*: McGill University (Canada).

Aundhia, C., Raval, J., Patel, M., Shah, N., Chauhan, S., Sailor, G., Javia, A., Mahashwari, R. (2011). Spray Drying in the pharmaceuticaul Industry-A Review. *Journal of Pharmaceutical Research, 2*, 63-65.

Aureli, P., Capurso, L., Castellazzi, A., Clerici, M., Giovannini, M., Morelli, L.,Poli, A., Pregliasco, F.,Salvini, F., Zuccotti, G. (2011). Probiotics and health: an evidence-based review. *Pharmacological Research, 63*(5), 366-376.

Bandyopadhyay, B., & Mandal, N. C. (2014). Probiotics, Prebiotics and Synbiotics-In Health Improvement by Modulating Gut Microbiota: The Concept Revisited. *Int. J. Curr. Microbiol. App. Sci, 3*(3), 410-420.

Barbosa, J., Borges, S., Amorim, M., Pereira, M. J., Oliveira, A., Pintado, M. E., & Teixeira, P. (2015). Comparison of spray drying, freeze drying and convective hot air drying for the production of a probiotic orange powder. *Journal of Functional Foods, 17*, 340-351.

Barbosa, J., Borges, S., & Teixeira, P. (2016). Effect of Different Conditions of Growth and Storage on the Cell Counts of Two Lactic Acid Bacteria after Spray Drying in Orange Juice. *Beverages, 2*(2), 8.

Bouchard, D. S., Rault, L., Berkova, N., Le Loir, Y., & Even, S. (2013). Inhibition of Staphylococcus aureus invasion into bovine mammary epithelial cells by contact with live Lactobacillus casei. *Applied and Environmental Microbiology, 79*(3), 877-885.

Boyle, R. J., Robins-Browne, R. M., & Tang, M. L. (2006). Probiotic use in clinical practice: what are the risks? *The American Journal of Clinical Nutrition, 83*(6), 1256-1264.

Brady, L. J., Gallaher, D. D., & Busta, F. F. (2000). The role of probiotic cultures in the prevention of colon cancer. *The Journal of Nutrition, 130*(2), 410S-414S.

Bucio, A., Hartemink, R., Schrama, J. W., Verreth, J., & Rombouts, F. M. (2005). Survival of Lactobacillus plantarum 44a after spraying and drying in feed and during exposure to gastrointestinal tract fluids in vitro. *The Journal of General and Applied Microbiology, 51*(4), 221-227.

Bukowska, H., Pieczul-Mróz, J., Jastrzebska, M., Chelstowski, K., & Naruszewicz, M. (1998). Decrease in fibrinogen and LDL-cholesterol levels upon supplementation of diet with *Lactobacillus plantarum* in subjects with moderately elevated cholesterol. *Atherosclerosis, 137*(2), 437-438.

Bull, M., Plummer, S., Marchesi, J., & Mahenthiralingam, E. (2013). The life history of *Lactobacillus acidophilus* as a probiotic: a tale of revisionary taxonomy, misidentification and commercial success. *FEMS Microbiology Letters, 349*(2), 77-87.

Burgain, J., Gaiani, C., Linder, M., & Scher, J. (2011). Encapsulation of probiotic living cells: From laboratory scale to industrial applications. *Journal of Food Engineering, 104*(4), 467-483.

Canani, R., Cirillo, P., Terrin, G., Cesarano, L., Spagnuolo, M., De Vincenzo, A., Albano, F., Passariello, A., De Marco, G., Manguso, F. (2007). Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations. *Bmj, 335*(7615), 340

Carneiro, H. C., Tonon, R. V., Grosso, C. R., & Hubinger, M. D. (2013). Encapsulation efficiency and oxidative stability of flaxseed oil microencapsulated by spray drying using different combinations of wall materials. *Journal of Food Engineering, 115*(4), 443-451.

Champagne, C. P., Gardner, N. J., & Roy, D. (2005). Challenges in the addition of probiotic cultures to foods. *Critical Reviews in Food Science and Nutrition, 45*(1), 61-84.

Chandan, R. C., White, C. H., Kilara, A., & Hui, Y. H. (2008). *Manufacturing Yogurt and Fermented Milks*: Wiley.

Chávez, B., & Ledeboer, A. (2007). Drying of probiotics: optimization of formulation and process to enhance storage survival. *Drying Technology, 25*(7-8), 1193-1201.

Chmielewska, A., Ruszczynski, M., & Szajewska, H. (2008). *Lactobacillus reuteri* strain ATCC 55730 for the treatment of acute infectious diarrhoea in children: a meta-analysis of randomized controlled trials. *Pediatria Wspolczesna, 10*(1), 32.

Chuayana Jr, E. L., Ponce, C. V., Rivera, M. R. B., & Cabrera, E. C. (2003). Antimicrobial activity of probiotics from milk products. *Phil J. Microbiol. Infect. Dis, 32*(2), 71-74.

Chun, H., Kim, C.-H., & Cho, Y.-H. (2014). Microencapsulation of Lactobacillus plantarum DKL 109 using External Ionic Gelation Method. *Korean Journal for Food Science of Animal Resources, 34*(5), 692.

Coghetto, C. C., Flores, S. H., Brinques, G. B., & Ayub, M. A. Z. (2016). Viability and alternative uses of a dried powder, microencapsulated *Lactobacillus plantarum* without the use of cold chain or dairy products. *LWT-Food Science and Technology, 71*, 54-59.

Comunian, T. A., Monterrey-Quintero, E. S., Thomazini, M., Balieiro, J. C., Piccone, P., Pittia, P., & Favaro-Trindade, C. S. (2011). Assessment of production efficiency, physicochemical properties and storage stability of spray-dried chlorophyllide, a natural food colourant, using gum Arabic, maltodextrin and soy protein isolate-based carrier systems. *International Journal of Food Science & Technology, 46*(6), 1259-1265.

Crittenden, R., Laitila, A., Forssell, P., Mättö, J., Saarela, M., Mattila-Sandholm, T., & Myllärinen, P. (2001). Adhesion of bifidobacteria to granular starch and its implications in probiotic technologies. *Applied and Environmental Microbiology, 67*(8), 3469-3475.

D'Aimmo, M. R., Modesto, M., & Biavati, B. (2007). Antibiotic resistance of lactic acid bacteria and *Bifidobacterium spp*. isolated from dairy and pharmaceutical products. *International Journal of Food Microbiology, 115*(1), 35-42.

da Silva, F. C., da Fonseca, C. R., de Alencar, S. M., Thomazini, M., de Carvalho Balieiro, J. C., Pittia, P., & Favaro-Trindade, C. S. (2013). Assessment of production efficiency, physicochemical properties and storage stability of spray-dried propolis, a natural food additive, using gum Arabic and OSA starch-based carrier systems. *Food and Bioproducts Processing, 91*(1), 28-36.

Das, A., Ray, S., Raychaudhuri, U., & Chakraborty, R. (2014). Microencapsulation of probiotic bacteria and its potential application in food technology. *International Journal of Agriculture, Environment and Biotechnology, 7*(1), 47.

Dauqan, E., & Abdullah, A. (2013). Utilization of gum Arabic for industries and human health. *American Journal of Applied Sciences, 10*(10), 1270-1279.

Davis, C. D., & Milner, J. A. (2009). Gastrointestinal microflora, food components and colon cancer prevention. *The Journal of Nutritional Biochemistry, 20*(10), 743-752.

de Araújo Etchepare, M., Raddatz, G., Cichoski, A., Flores, É. , Barin, J., Zepka, L., Jacob-Lopes, E., Grosso, C., de Menezes, C. R. (2016). Effect of resistant starch (Hi-maize) on the survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate. *Journal of Functional Foods, 21*, 321-329.

de Barros Fernandes, R. V., Borges, S. V., & Botrel, D. A. (2014). Gum arabic/starch/maltodextrin/inulin as wall materials on the microencapsulation of rosemary essential oil. *Carbohydrate Polymers, 101*, 524-532.

De Castro-Cislaghi, F. P., Carina Dos Reis, E. S., Fritzen-Freire, C. B., Lorenz, J. G., & Sant'Anna, E. S. (2012). *Bifidobacterium* Bb-12 microencapsulated by spray drying with

whey: Survival under simulated gastrointestinal conditions, tolerance to NaCl, and viability during storage. *Journal of Food Engineering, 113*(2), 186-193.

De Oliveira, M. A., Maia, G. A., De Figueiredo, R. W., De Souza, A. C. R., De Brito, E. S., & De Azeredo, H. M. C. (2009). Addition of cashew tree gum to maltodextrin-based carriers for spray drying of cashew apple juice. *International Journal of Food Science & Technology,, 44*(3), 641-645.

de Vos, P., Faas, M. M., Spasojevic, M., & Sikkema, J. (2010). Encapsulation for preservation of functionality and targeted delivery of bioactive food components. *International Dairy Journal, 20*(4), 292-302.

De Vries, M. C., Vaughan, E. E., Kleerebezem, M., & de Vos, W. M. (2006). *Lactobacillus plantarum*—survival, functional and potential probiotic properties in the human intestinal tract. *International Dairy Journal, 16*(9), 1018-1028.

Deis, R. (1997). Spray-drying-innovative use of an old process. *Food Product Design, 7*(2), 97-113.

Desmond, C., Ross, R., O'callaghan, E., Fitzgerald, G., & Stanton, C. (2002). Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. *Journal of Applied Microbiology, 93*(6), 1003-1011.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids, 17*(1), 25-39.

Ding, W., & Shah, N. (2007). Acid, bile, and heat tolerance of free and microencapsulated probiotic bacteria. *Journal of Food Science, 72*(9), M446-M450.

Domingo, J. J. S. (2017). Review of the role of probiotics in gastrointestinal diseases in adults. *Gastroenterología y Hepatología (English Edition)*.

Erickson, K. L., & Hubbard, N. E. (2000). Probiotic immunomodulation in health and disease. *The Journal of Nutrition, 130*(2), 403S-409S.

Estevinho, B. N., Rocha, F., Santos, L., & Alves, A. (2013). Microencapsulation with chitosan by spray drying for industry applications–A review. *Trends in Food Science & Technology, 31*(2), 138-155.

Fahimdanesh, M., Mohammadi, N., Ahari, H., Zanjani, M. A., Hargalani, F. Z., & Behrouznasab, K. (2013). Effect of microencapsulation plus resistant starch on survival of *Lactobacillus casei* and *Bifidobacterium bifidum* in mayonnaise sauce. *African Journal of Microbiology Research, 6*(40), 6853-6858.

FAO/WHO. (2001). Report of a joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. *Córdoba, Argentina.* October, 1-4.

Farnworth, E. R. (2008). *Handbook of Fermented Functional Foods, Second Edition*: CRC Press.

Figueroa-González, I., Quijano, G., Ramírez, G., & Cruz-Guerrero, A. (2011). Probiotics and prebiotics—perspectives and challenges. *Journal of the Science of Food and Agriculture, 91*(8), 1341-1348.

Foligné, B., Daniel, C., & Pot, B. (2013). Probiotics from research to market: the possibilities, risks and challenges. *Current Opinion in Microbiology, 16*(3), 284-292.

Freire, A. C., Fertig, C. C., Podczeck, F., Veiga, F., & Sousa, J. (2009). Starch-based coatings for colon-specific drug delivery. Part I: The influence of heat treatment on the physico-chemical properties of high amylose maize starches. *European Journal of Pharmaceutics and Biopharmaceutics, 72*(3), 574-586.

Fritzen-Freire, C. B., Prudêncio, E. S., Amboni, R. D., Pinto, S. S., Negrão-Murakami, A. N., & Murakami, F. S. (2012). Microencapsulation of bifidobacteria by spray drying in the presence of prebiotics. *Food Research International, 45*(1), 306-312.

Fu, N., & Chen, X. D. (2011). Towards a maximal cell survival in convective thermal drying processes. *Food Research International, 44*(5), 1127-1149.

Fuentes-Zaragoza, E., Riquelme-Navarrete, M., Sánchez-Zapata, E., & Pérez-Álvarez, J. (2010). Resistant starch as functional ingredient: A review. *Food Research International, 43*(4), 931-942.

Fuller, R. (1989). Probiotics in man and animals. *Journal of Applied Bacteriology, 66*(5), 365-378.

Gardiner, G., O'sullivan, E., Kelly, J., Auty, M., Fitzgerald, G., Collins, J., Ross, R., Stanton, C. (2000). Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. *Applied and Environmental Microbiology, 66*(6), 2605-2612.

Garg, S., Sharma, P., Jayaprakashan, S., & Subramanian, R. (2009). Spray evaporation of liquid foods. *LWT-Food Science and Technology, 42*(1), 119-124.

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., & Saurel, R. (2007). Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International, 40*(9), 1107-1121.

Gill, H. (1998). Stimulation of the immune system by lactic cultures. *International Dairy Journal, 8*(5-6), 535-544.

Gilliland, S., & Speck, M. (1977). Antagonistic action of *Lactobacillus acidophilus* toward intestinal and foodborne pathogens in associative cultures 1. *Journal of Food Protection, 40*(12), 820-823.

Goderska, K., & Czarnecki, Z. (2008). Influence of microencapsulation and spray drying on the viability of Lactobacillus and Bifidobacterium strains. *Polish Journal of Microbiology, 57*(2), 135-140.

Golowczyc, M., Silva, J., Abraham, A., De Antoni, G., & Teixeira, P. (2010). Preservation of probiotic strains isolated from kefir by spray drying. *Letters in Applied Microbiology, 50*(1), 7-12.

Goula, A. M., & Adamopoulos, K. G. (2005). Spray drying of tomato pulp in dehumidified air: II. The effect on powder properties. *Journal of Food Engineering, 66*(1), 35-42.

Gupta, A. (2015). *Characterization of Potentially Active New Probiotics Strains Isolated from Different Sources and to Study their Prospects as Neutraceuticals Agents.* Doctoral dissertation.

Hamsupo, K., Sukyai, P., Loiseau, G., Nitisinprasert, S., Montet, D., & Wanchaitanawong, P. (2005). Prediction on the stability of spray-dried Lactobacillus reuteri KUB-AC5 by Arrhenius equation for long-term storage. *Journal of Microbiology and Biotechnology, 15*(6), 1178-1182.

Harish, K., & Varghese, T. (2006a). Probiotics in humans–evidence based review. *Calicut Medical Journal, 4*(4), e3.

Harish, K., & Varghese, T. (2006b). Probiotics in humans–evidence based review. *Calicut Medical Journal 4*(4), e3.

Hemaiswarya, S., Raja, R., Ravikumar, R., & Carvalho, I. S. (2013). Mechanism of action of probiotics. *Brazilian Archives of Biology and Technology, 56*(1), 113-119.

Hertzler, S. R., & Clancy, S. M. (2003). Kefir improves lactose digestion and tolerance in adults with lactose maldigestion. *Journal of the American Dietetic Association, 103*(5), 582-587.

Hirayama, K., & Rafter, J. (1999). The role of lactic acid bacteria in colon cancer prevention: mechanistic considerations *Lactic Acid Bacteria: Genetics, Metabolism and Applications* (pp. 391-394): Springer.

Homayouni, A., Azizi, A., Ehsani, M., Yarmand, M., & Razavi, S. (2008). Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. *Food Chemistry, 111*(1), 50-55.

Huang, S., Vignolles, M.-L., Chen, X. D., Le Loir, Y., Jan, G., Schuck, P., & Jeantet, R. (2017). Spray drying of probiotics and other food-grade bacteria: A review. *Trends in Food Science & Technology*.

Iaconelli, C., Lemetais, G., Kechaou, N., Chain, F., Bermúdez-Humarán, L., Langella, P., Gervais, P., Beney, L. (2015). Drying process strongly affects probiotics viability and functionalities. *Journal of Biotechnology, 214*, 17-26.

Indira, K., Jayalakshmi, S., Gopalakrishnan, A., & Srinivasan, M. (2011). Biopreservative potential of marine lactobacillus spp. *African Journal of Microbiology Research, 5*(16), 2287-2296.

Iyer, C., Kosters, A., Sethi, G., Kunnumakkara, A. B., Aggarwal, B. B., & Versalovic, J. (2008). Probiotic *Lactobacillus reuteri* promotes TNF-induced apoptosis in human myeloid leukemia-derived cells by modulation of NF-κB and MAPK signalling. *Cellular Microbiology, 10*(7), 1442-1452.

Jayalalitha, V. (2013). Microencapsulation of Probiotics to Prepare Functional Dairy Products.

Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., & Thun, M. J. (2008). Cancer statistics, 2008. *CA: A Cancer Journal for Clinicians, 58*(2), 71-96.

Jose, N. M. (2015). *Isolation, Screening and Characterization of Probiotic Isolates from Commercial Dairy Food and Bovine Rumen*. Lincoln University.

Kailasapathy, K. (2002). Microencapsulation of probiotic bacteria: technology and potential applications. *Current Issues in Intestinal Microbiology, 3*(2), 39-48.

Kailasapathy, K., & Chin, J. (2000). Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium spp*. *Immunology and Cell Biology, 78*(1), 80-88.

Kajiyama, T., & Park, K. J. (2011). Influence of feed parameters on spray drying energy consumption. *Journal of Food Process Engineering, 34*(2), 414-426.

Kandansamy, K., & Somasundaram, P. D. (2012). Microencapsulation of colors by spray drying-a review. *International Journal of Food Engineering, 8*(2).

Kechagia, M., Basoulis, D., Konstantopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., & Fakiri, E. M. (2013). Health benefits of probiotics: a review. *ISRN nutrition, 2013*.

Klarin, B., Wullt, M., Palmquist, I., Molin, G., Larsson, A., & Jeppsson, B. (2008). *Lactobacillus plantarum* 299v reduces colonisation of *Clostridium difficile* in critically ill patients treated with antibiotics. *Acta Anaesthesiologica Scandinavica, 52*(8), 1096-1102.

Kopp-Hoolihan, L. (2001). Prophylactic and therapeutic uses of probiotics: a review. *Journal of the American Dietetic Association, 101*(2), 229-241.

Kumar, S. R. (2011). *Studies on Probiotics and Antimicrobial Properties of Lactic Acid Bacteria Isolated from Marine Fish, Shrimp and South Indian Fermented Foods.* Department of Biotechnology, Pondicherry University.

Kurozawa, L. E., Park, K. J., & Hubinger, M. D. (2009). Effect of maltodextrin and gum arabic on water sorption and glass transition temperature of spray dried chicken meat hydrolysate protein. *Journal of Food Engineering, 91*(2), 287-296.

Landis, S. H., Murray, T., Bolden, S., & Wingo, P. A. (1998). Cancer statistics, 1998. *CA: A Cancer Journal for Clinicians, 48*(1), 6-29.

Lapsiri, W., Bhandari, B., & Wanchaitanawong, P. (2012). Viability of *Lactobacillus plantarum* TISTR 2075 in different protectants during spray drying and storage. *Drying Technology, 30*(13), 1407-1412.

Lebeer, S., Vanderleyden, J., & De Keersmaecker, S. C. (2008). Genes and molecules of lactobacilli supporting probiotic action. *Microbiology and Molecular Biology Reviews, 72*(4), 728-764.

LeBlanc, J., Matar, C., Valdez, J., LeBlanc, J., & Perdigon, G. (2002). Immunomodulating effects of peptidic fractions issued from milk fermented with *Lactobacillus helveticus*. *Journal of Dairy Science, 85*(11), 2733-2742.

Leroy, F., & De Vuyst, L. (2004). Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science & Technology, 15*(2), 67-78.

Lian, W.-C., Hsiao, H.-C., & Chou, C.-C. (2002). Survival of bifidobacteria after spray-drying. *International journal of food microbiology, 74*(1), 79-86.

Lin, W.-H., Hwang, C.-F., Chen, L.-W., & Tsen, H.-Y. (2006). Viable counts, characteristic evaluation for commercial lactic acid bacteria products. *Food Microbiology, 23*(1), 74-81.

Longdet, I. Y., Kutshik, R. J., & Nwoyeocha, I. G. (2011). The Probiotic efficacy of lactobacillus of *Lactobacillus casei* from human breast milk against Shigellosis in Albino rats.

Marco, M. L., Pavan, S., & Kleerebezem, M. (2006). Towards understanding molecular modes of probiotic action. *Current Opinion in Biotechnology, 17*(2), 204-210.

Marteau, P., Seksik, P., & Jian, R. (2002). Probiotics and health: new facts and ideas. *Current Opinion in Biotechnology, 13*(5), 486-489.

McIntosh, G. H. (1996). Probiotics and colon cancer prevention. *Asia Pacific Journal of Clinical Nutrition, 5*, 48-52.

Meng, X., Stanton, C., Fitzgerald, G., Daly, C., & Ross, R. (2008). Anhydrobiotics: The challenges of drying probiotic cultures. *Food Chemistry, 106*(4), 1406-1416.

Mestry, A., Mujumdar, A., & Thorat, B. (2011). Optimization of spray drying of an innovative functional food: fermented mixed juice of carrot and watermelon. *Drying Technology, 29*(10), 1121-1131.

Michail, S. (2005). The mechanism of action of probiotics. *Practical Gastroenterology, 2*, 29-47.

Miller, D. A., & Gil, M. (2012). Spray-drying technology *Formulating Poorly Water Soluble Drugs* (pp. 363-442): Springer.

Mombelli, B., & Gismondo, M. R. (2000). The use of probiotics in medical practice. *International Journal of Antimicrobial Agents, 16*(4), 531-536.

More Swati, K., & Wagh, M. (2014). Review on spray drying technology. *International Journal of Pharmaceutical, Chemical and Biological Sciences, 4*, 219-225.

Mosilhey, S. H. (2003). Influence of different capsule materials on the physiological properties of microencapsulated *Lactobacillus acidophilus*. *Abstract Dissertation. Institut für Lebensmitteltechn. Bonn*.

Mujumdar, A. S. (1995). *Handbook of Industrial Drying, Second Edition, Revised and Expanded*: Taylor & Francis.

Murugesan, R., & Orsat, V. (2012). Spray drying for the production of nutraceutical ingredients—a review. *Food and Bioprocess Technology, 5*(1), 3-14.

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science, 1*, 1806-1815.

Ng, S., Hart, A., Kamm, M., Stagg, A., & Knight, S. (2009). Mechanisms of action of probiotics: recent advances. *Inflammatory Bowel Diseases, 15*(2), 300-310.

O'Hara, A. M., & Shanahan, F. (2007). Mechanisms of action of probiotics in intestinal diseases. *The Scientific World Journal, 7*, 31-46.

O'riordan, K., Andrews, D., Buckle, K., & Conway, P. (2001). Evaluation of microencapsulation of a Bifidobacterium strain with starch as an approach to prolonging viability during storage. *Journal of Applied Microbiology, 91*(6), 1059-1066.

Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions–a review. *International Journal of Medical Microbiology, 300*(1), 57-62.

Otles, S., & Cagindi, O. e. (2003). Kefir: a probiotic dairy-composition, nutritional and therapeutic aspects. *Pakistan Journal of Nutrition, 2*(2), 54-59.

Paéz, R., Lavari, L., Vinderola, G., Audero, G., Cuatrin, A., Zaritzky, N., & Reinheimer, J. (2012). Effect of heat treatment and spray drying on lactobacilli viability and resistance to simulated gastrointestinal digestion. *Food Research International, 48*(2), 748-754.

Parker, R. B. (1974). Probiotics, the other half of the antibiotics story. *Animal Nutrition and Health, 29*, 4-8.

Parra Huertas, R. A. (2010). Food microencapsulation: a review. *Revista Facultad Nacional de Agronomía, Medellín, 63*(2), 5669-5684.

Parvez, S., Malik, K. A., Ah Kang, S., & Kim, H. Y. (2006). Probiotics and their fermented food products are beneficial for health. *Journal of Applied Microbiology, 100*(6), 1171-1185.

Patel, R., Patel, M., & Suthar, A. (2009). Spray drying technology: an overview. *Indian Journal of Science and Technology, 2*(10), 44-47.

Peighambardoust, S., Tafti, A. G., & Hesari, J. (2011). Application of spray drying for preservation of lactic acid starter cultures: a review. *Trends in Food Science & Technology, 22*(5), 215-224.

Perdana, J., Bereschenko, L., Fox, M. B., Kuperus, J. H., Kleerebezem, M., Boom, R. M., & Schutyser, M. A. (2013). Dehydration and thermal inactivation of *Lactobacillus plantarum* WCFS1: Comparing single droplet drying to spray and freeze drying. *Food Research International, 54*(2), 1351-1359.

Picot, A., & Lacroix, C. (2004). Encapsulation of bifidobacteria in whey protein-based microcapsules and survival in simulated gastrointestinal conditions and in yoghurt. *International Dairy Journal, 14*(6), 505-515.

Pourashouri, P., Shabanpour, B., Razavi, S. H., Jafari, S. M., Shabani, A., & Aubourg, S. P. (2014). Impact of wall materials on physicochemical properties of microencapsulated fish oil by spray drying. *Food and Bioprocess Technology, 7*(8), 2354-2365.

Pu, J., Bankston, J. D., & Sathivel, S. (2011). Developing microencapsulated flaxseed oil containing shrimp (*Litopenaeus setiferus*) astaxanthin using a pilot scale spray dryer. *Biosystems engineering, 108*(2), 121-132.

Raigond, P., Ezekiel, R., & Raigond, B. (2015). Resistant starch in food: a review. *Journal of the Science of Food and Agriculture, 95*(10), 1968-1978.

Rathore, S., Desai, P. M., Liew, C. V., Chan, L. W., & Heng, P. W. S. (2013). Microencapsulation of microbial cells. *Journal of Food Engineering, 116*(2), 369-381.

Reddy, K. B. P. K., Madhu, A. N., & Prapulla, S. G. (2009). Comparative survival and evaluation of functional probiotic properties of spray-dried lactic acid bacteria. *International Journal of Dairy Technology, 62*(2), 240-248.

Reid, G., Jass, J., Sebulsky, M. T., & McCormick, J. K. (2003). Potential uses of probiotics in clinical practice. *Clinical Microbiology Reviews, 16*(4), 658-672.

Ringø, E., & Gatesoupe, F.-J. (1998). Lactic acid bacteria in fish: a review. *Aquaculture, 160*(3), 177-203.

Riveros, B., Ferrer, J., & Borquez, R. (2009). Spray drying of a vaginal probiotic strain of *Lactobacillus acidophilus*. *Drying Technology, 27*(1), 123-132.

Rodríguez-Huezo, M., Durán-Lugo, R., Prado-Barragán, L., Cruz-Sosa, F., Lobato-Calleros, C., Alvarez-Ramírez, J., & Vernon-Carter, E. (2007). Pre-selection of protective colloids for enhanced viability of *Bifidobacterium bifidum* following spray-drying and storage, and evaluation of aguamiel as thermoprotective prebiotic. *Food Research International, 40*(10), 1299-1306.

Rolfe, R. D. (2000). The role of probiotic cultures in the control of gastrointestinal health. *The Journal of Nutrition, 130*(2), 396S-402S.

Rousseaux, C., Thuru, X., Gelot, A., Barnich, N., Neut, C., Dubuquoy, L., Dubuquoy, C., Merour, E., Geboes, K., Chamaillard, M. (2007). *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. *Nature Medicine, 13*(1), 35-37.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J.-M., & Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. *LWT-Food Science and Technology, 50*(1), 1-16.

Saavedra-Leos, Z., Leyva-Porras, C., Araujo-Díaz, S. B., Toxqui-Terán, A., & Borrás-Enríquez, A. J. (2015). Technological application of maltodextrins according to the degree of polymerization. *Molecules, 20*(12), 21067-21081.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch–a review. *Comprehensive Reviews in Food Science and Food Safety, 5*(1), 1-17.

Salminen, S. (2001). Human studies on probiotics: aspects of scientific documentation. *Näringsforskning, 45*(1), 8-12.

Salminen, S., Bouley, C., Boutron, M.-C., Cummings, J., Franck, A., Gibson, G., Isolauri, E., Moreau, M., Roberfroid, M., Rowland, I. (1998). Functional food science and gastrointestinal physiology and function. *British Journal of Nutrition, 80*(S1), S147-S171.

Salminen, S., Ouwehand, A., Benno, Y., & Lee, Y. (1999). Probiotics: how should they be defined? *Trends in Food Science & Technology, 10*(3), 107-110.

Sanders, M. E. (2000). Considerations for use of probiotic bacteria to modulate human health. *The Journal of Nutrition, 130*(2), 384S-390S.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2007). Alternative drying processes for the industrial preservation of lactic acid starter cultures. *Biotechnology Progress, 23*(2), 302-315.

Santivarangkna, C., Kulozik, U., & Foerst, P. (2008). Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. *Journal of Applied Microbiology, 105*(1), 1-13.

Sarowska, J., Choroszy-Król, I., Regulska-Ilow, B., Frej-Madrzak, M., & Jama-Kmiecik, A. (2013). The therapeutic effect of probiotic bacteria on gastrointestinal diseases. *Advances in Clinical and Experimental Medicine, 22*(5), 759-766.

Saxelin, M., Tynkkynen, S., Mattila-Sandholm, T., & de Vos, W. M. (2005). Probiotic and other functional microbes: from markets to mechanisms. *Current Opinion in Biotechnology, 16*(2), 204-211.

Schultz, M., Linde, H.-J., Lehn, N., Zimmermann, K., Grossmann, J., Falk, W., & Schölmerich, J. (2003). Immunomodulatory consequences of oral administration of *Lactobacillus rhamnosus* strain GG in healthy volunteers. *Journal of Dairy Research, 70*(02), 165-173.

Selle, K., Klaenhammer, T., & Russell, W. (2014). LACTOBACILLUS| *Lactobacillus acidophilus.*

Semyonov, D., Ramon, O., & Shimoni, E. (2011). Using ultrasonic vacuum spray dryer to produce highly viable dry probiotics. *LWT-Food Science and Technology, 44*(9), 1844-1852.

Serna-Cock, L., & Vallejo-Castillo, V. (2013). Probiotic encapsulation. *African Journal of Microbiology Research, 7*(40), 4743-4753.

Shah, N. (2000). Probiotic bacteria: selective enumeration and survival in dairy foods. *Journal of Dairy Science, 83*(4), 894-907.

Sherman, P. M., Ossa, J. C., & Johnson-Henry, K. (2009). Unraveling mechanisms of action of probiotics. *Nutrition in Clinical Practice, 24*(1), 10-14.

Shokri, Z., Fazeli, M. R., Ardjmand, M., Mousavi, S. M., & Gilani, K. (2015). Factors affecting viability of *Bifidobacterium bifidum* during spray drying. *DARU Journal of Pharmaceutical Sciences, 23*(1), 7.

Simakachorn, N., Pichaipat, V., Rithipornpaisarn, P., Kongkaew, C., Tongpradit, P., & Varavithya, W. (2000). Clinical evaluation of the addition of lyophilized, heat-killed *Lactobacillus acidophilus* LB to oral rehydration therapy in the treatment of acute diarrhea in children. *Journal of Pediatric Gastroenterology and Nutrition, 30*(1), 68-72.

Singh, R. P., & Heldman, D. R. (2001). *Introduction to Food Engineering*. San Diego, CA: Academic Press.

Singh, S., & Dixit, D. (2014). A review on spray drying: emerging technology in food industry. *International Journal of Applied Engineering and Technology, 4*(1), 1-8.

Solval, K. M. (2011). *Spray Drying Technology for the Production and Processing of Microencapsulated Omega-3 Fish Oil with Egg Powder*. Louisiana State University.

Solval, K. M., Sundararajan, S., Alfaro, L., & Sathivel, S. (2012). Development of cantaloupe (*Cucumis melo*) juice powders using spray drying technology. *LWT-Food Science and Technology, 46*(1), 287-293.

Soukoulis, C., Behboudi-Jobbehdar, S., Yonekura, L., Parmenter, C., & Fisk, I. (2014). Impact of milk protein type on the viability and storage stability of microencapsulated *Lactobacillus acidophilus* NCIMB 701748 using spray drying. *Food and Bioprocess Technology, 7*(5), 1255-1268.

Stiles, M. E., & Holzapfel, W. H. (1997). Lactic acid bacteria of foods and their current taxonomy. *International Journal of Food Microbiology, 36*(1), 1-29.

Su, L.-C., Lin, C. W., & Chen, M. J. (2007). Development of an Oriental-style dairy product coagulated by microcapsules containing probiotics and filtrates from fermented rice. *International Journal of Dairy Technology, 60*(1), 49-54.

Teixeira, P., Castro, H., & Kirby, R. (1996). Evidence of membrane lipid oxidation of spray-dried *Lactobacillus bulgaricus* during storage. *Letters in Applied Microbiology, 22*(1), 34-38.

Tonon, R. V., Brabet, C., Pallet, D., Brat, P., & Hubinger, M. D. (2009). Physicochemical and morphological characterisation of açai (*Euterpe oleraceae Mart.*) powder produced with different carrier agents. *International Journal of Food Science & Technology, 44*(10), 1950-1958.

Topping, D. L., Fukushima, M., & Bird, A. R. (2003). Resistant starch as a prebiotic and synbiotic: state of the art. *Proceedings of the Nutrition Society, 62*(01), 171-176.

Tripathi, M., & Giri, S. (2014). Probiotic functional foods: Survival of probiotics during processing and storage. *Journal of Functional Foods, 9*, 225-241.

Tuohy, K. M., Probert, H. M., Smejkal, C. W., & Gibson, G. R. (2003). Using probiotics and prebiotics to improve gut health. *Drug Discovery Today, 8*(15), 692-700.

Uccello, M., Malaguarnera, G., Basile, F., D'agata, V., Malaguarnera, M., Bertino, G., Vacante, M., Drago, F., Biondi, A. (2012). Potential role of probiotics on colorectal cancer prevention. *BMC Surgery, 12*(1), S35.

Umer, H., Nigam, H., Tamboli, A. M., & Nainar, M. S. M. (2011). Microencapsulation: Process, techniques and applications. *International Journal of Research in Pharmaceutical and Biomedical Sciences, 2*(2), 474-481.

Vasiljevic, T., & Shah, N. P. (2008). Probiotics—from Metchnikoff to bioactives. *International Dairy Journal, 18*(7), 714-728.

Vega-Mercado, H., Góngora-Nieto, M. M., & Barbosa-Cánovas, G. V. (2001). Advances in dehydration of foods. *Journal of Food Engineering, 49*(4), 271-289.

Viernstein, H., Raffalt, J., & Polheim, D. (2005). Stabilisation of probiotic microorganisms *Applications of Cell Immobilisation Biotechnology* (pp. 439-453): Springer.

Vinderola, C., Bailo, N., & Reinheimer, J. (2000). Survival of probiotic microflora in Argentinian yoghurts during refrigerated storage. *Food Research International, 33*(2), 97-102.

Vinderola, C., Mocchiutti, P., & Reinheimer, J. (2002). Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. *Journal of Dairy Science, 85*(4), 721-729.

Vonk, R. J., Reckman, G. A., Harmsen, H. J., & Priebe, M. G. (2012). *Probiotics and Lactose Intolerance*: INTECH Open Access Publisher.

Wisniewski, R. (2015). *Spray Drying Technology Review*.

Wollowski, I., Rechkemmer, G., & Pool-Zobel, B. L. (2001). Protective role of probiotics and prebiotics in colon cancer. *The American Journal of Clinical Nutrition, 73*(2), 451s-455s.

Ying, D., Sun, J., Sanguansri, L., Weerakkody, R., & Augustin, M. A. (2012). Enhanced survival of spray-dried microencapsulated *Lactobacillus rhamnosus* GG in the presence of glucose. *Journal of Food Engineering, 109*(3), 597-602.

Yonekura, L., Sun, H., Soukoulis, C., & Fisk, I. (2014). Microencapsulation of *Lactobacillus acidophilus* NCIMB 701748 in matrices containing soluble fibre by spray drying: Technological characterization, storage stability and survival after in vitro digestion. *Journal of Functional Foods, 6*, 205-214.

Zhao, R., Sun, J., Torley, P., Wang, D., & Niu, S. (2008). Measurement of particle diameter of *Lactobacillus acidophilus* microcapsule by spray drying and analysis on its microstructure. *World Journal of Microbiology and Biotechnology, 24*(8), 1349-1354.

Ziemer, C. J., & Gibson, G. R. (1998). An overview of probiotics, prebiotics and synbiotics in the functional food concept: perspectives and future strategies. *International Dairy Journal, 8*(5-6), 473-479.

APPENDIX A. EFFECT OF DIFFERENT WALL MATERIALS ON THE SURVIVAL OF SPRAY-DRIED L. PLANTARUM NRRL B-4496.

Table A1. Viability of *L. plantarum* NRRL B-4496 stored under 97 or 10% vacuum at room (23 °C) temperature.

Time	HM-air	HM-vac	MD-air	MD-vac	GA-air	GA-vac
0	8.31 ± 0.00^{Aa}	8.31 ± 0.00^{Aa}	8.09 ± 0.00^{Aa}	8.09 ± 0.00^{Aa}	8.80 ± 0.00^{Aa}	8.80 ± 0.00^{Aa}
5	8.42 ± 0.01^{Aa}	8.01 ± 0.02^{Bb}	7.53 ± 0.05^{Bb}	7.77 ± 0.01^{Ab}	7.87 ± 0.04^{Ab}	7.87 ± 0.02^{Ab}
10	7.73 ± 0.04^{Ab}	7.78 ± 0.05^{Ac}	7.08 ± 0.08^{Ac}	7.12 ± 0.03^{Ac}	7.81 ± 0.02^{Ab}	7.85 ± 0.06^{Abc}
20	7.56 ± 0.02^{Ac}	7.16 ± 0.08^{Bd}	7.14 ± 0.00^{Ac}	7.12 ± 0.02^{Ac}	7.69 ± 0.03^{Abc}	7.71 ± 0.01^{Abc}
30	7.21 ± 0.02^{Ad}	6.93 ± 0.00^{Be}	6.64 ± 0.04^{Bd}	6.98 ± 0.07^{Ac}	7.54 ± 0.09^{Ac}	7.68 ± 0.07^{Ac}
60	6.11 ± 0.04^{Ae}	5.96 ± 0.00^{Bf}	4.16 ± 0.07^{Be}	6.02 ± 0.04^{Ad}	6.33 ± 0.00^{Bd}	6.97 ± 0.04^{Ad}

^{AB}Means with the same letter in a row are not significantly different ($P \leq 0.05$). ^{abcde}Means with the same letter in a column are not significantly different ($P \leq 0.05$). HM = high maize starch, MD = maltodextrin, GA = gum arabic, Vac = 97% vacuum, Air = 10% vacuum, and LP = *L. plantarum* NRRL B-4496. GA-Vac and GA-Air = spray-dried LP with GA stored under 97 and 10% vacuum, respectively; HM-Vac and HM-Air = spray-dried LP with HM stored under 97 and 10% vacuum, respectively; and MD-Vac and MD-Air = spray-dried LP with MD stored under 97 and 10% vacuum, respectively.

Table A2. Viability of *L. plantarum* NRRL B-4496 stored under 97 or 10% vacuum at refrigerated (4 °C) temperature.

Time	HM-air	HM-vac	MD-air	MD-vac	GA-air	GA-vac
0	8.31 ± 0.00^{Aa}	8.31 ± 0.00^{Aa}	8.09 ± 0.00^{Aa}	8.09 ± 0.00^{Aa}	8.80 ± 0.00^{Aa}	8.80 ± 0.00^{Aa}
5	8.23 ± 0.00^{Ab}	8.07 ± 0.00^{Bc}	7.58 ± 0.01^{Bb}	8.07 ± 0.05^{Aa}	7.96 ± 0.03^{Bbc}	8.16 ± 0.02^{Ab}
10	8.24 ± 0.00^{Ab}	8.08 ± 0.00^{Bc}	7.62 ± 0.00^{Bb}	8.03 ± 0.02^{Aa}	7.77 ± 0.02^{Ad}	7.74 ± 0.04^{Ad}
20	8.29 ± 0.00^{Aa}	8.20 ± 0.01^{Bb}	7.64 ± 0.09^{Ab}	7.73 ± 0.03^{Ab}	8.02 ± 0.02^{Ab}	8.02 ± 0.07^{Abc}
30	8.29 ± 0.00^{Aa}	8.29 ± 0.02^{Aa}	7.55 ± 0.02^{Bb}	7.83 ± 0.07^{Ab}	8.12 ± 0.05^{Ab}	8.11 ± 0.02^{Abc}
60	8.08 ± 0.01^{Ac}	8.17 ± 0.04^{Ab}	7.34 ± 0.02^{Bc}	7.85 ± 0.00^{Ab}	7.84 ± 0.07^{Acd}	7.94 ± 0.06^{Ac}

^{AB}Means with the same letter in a row are not significantly different ($P \leq 0.05$). ^{abcde}Means with the same letter in a column are not significantly different ($P \leq 0.05$). HM = high maize starch, MD = maltodextrin, GA = gum arabic, Vac = 97% vacuum, Air = 10% vacuum, and LP = *L. plantarum* NRRL B-4496. GA-Vac and GA-Air = spray-dried LP with GA stored under 97 and 10% vacuum, respectively; HM-Vac and HM-Air = spray-dried LP with HM stored under 97 and 10% vacuum, respectively; and MD-Vac and MD-Air = spray-dried LP with MD stored under 97 and 10% vacuum, respectively.

APPENDIX B. EFFECT OF DIFFERENT WALL MATERIALS ON THE SURVIVAL OF SPRAY-DRIED L. ACIDOPHILUS NRRL B-4495.

Table B1. Viability of *L. acidophilus* NRRL B-4496 stored under 97 or 10% vacuum at room (23 °C) temperature.

Time	HM-air	HM-vac	MD-air	MD-vac	GA-air	GA-vac
0	7.90 ± 0.00^{Aa}	7.90 ± 0.00^{Aa}	7.82 ± 0.00^{Aa}	7.82 ± 0.00^{Ab}	8.10 ± 0.00^{Aa}	8.10 ± 0.00^{Aa}
5	6.79 ± 0.04^{Ab}	6.23 ± 0.07^{Bc}	7.26 ± 0.02^{Bb}	8.04 ± 0.05^{Aa}	7.22 ± 0.01^{Ab}	7.30 ± 0.04^{Ab}
10	5.72 ± 0.05^{Bc}	6.61 ± 0.06^{Ab}	6.91 ± 0.03^{Bc}	7.48 ± 0.00^{Ac}	6.45 ± 0.06^{Bc}	7.13 ± 0.02^{Ac}
20	3.89 ± 0.15^{Bd}	5.30 ± 0.04^{Ad}	4.30 ± 0.00^{Be}	6.42 ± 0.09^{Ad}	5.44 ± 0.06^{Bd}	6.31 ± 0.07^{Ad}
30	2.28 ± 0.00^{Be}	3.04 ± 0.05^{Ae}	4.55 ± 0.05^{Bd}	6.22 ± 0.04^{Ae}	4.35 ± 0.07^{Be}	5.67 ± 0.04^{Ae}
60	0.00±0.00	0.00±0.00	3.54 ± 0.13^{Af}	0.00±0.00	0.00±0.00	0.00±0.00

^{AB}Means with the same letter in a row are not significantly different ($P \leq 0.05$). ^{abcde}Means with the same letter in a column are not significantly different ($P \leq 0.05$). HM = high maize starch, MD = maltodextrin, GA = gum arabic, Vac = 97% vacuum, Air = 10% vacuum, and LA = *L. acidophilus* NRRL B-4495. GA-Vac and GA-Air = spray-dried LA with GA stored under 97 and 10% vacuum, respectively; HM-Vac and HM-Air = spray-dried LA with HM stored under 97 and 10% vacuum, respectively; and MD-Vac and MD-Air = spray-dried LA with MD stored under 97 and 10% vacuum, respectively.

Table B2. Viability of *L. acidophilus* NRRL B-4496 stored under 97 or 10% vacuum at refrigerated (4 °C) temperature.

Time	HM-air	HM-vac	MD-air	MD-vac	GA-air	GA-vac
0	7.90 ± 0.00^{Aa}	7.90 ± 0.00^{Aa}	7.82 ± 0.00^{Ab}	7.82 ± 0.00^{Ab}	8.10 ± 0.00^{Aa}	8.10 ± 0.00^{Aa}
5	7.59 ± 0.14^{Aab}	7.81 ± 0.04^{Aab}	7.62 ± 0.02^{Bc}	8.04 ± 0.04^{Aab}	7.28 ± 0.02^{Bc}	7.65 ± 0.03^{Ac}
10	7.53 ± 0.06^{Ab}	7.66 ± 0.02^{Ab}	8.19 ± 0.01^{Aa}	8.33 ± 0.06^{Aa}	7.85 ± 0.04^{Ab}	7.82 ± 0.02^{Ab}
20	7.07 ± 0.07^{Bc}	7.38 ± 0.04^{Ac}	6.82 ± 0.08^{Ad}	7.13 ± 0.07^{Ac}	7.01 ± 0.02^{Bd}	7.46 ± 0.08^{Ad}
30	6.48 ± 0.04^{Ad}	6.54 ± 0.00^{Ad}	6.90 ± 0.01^{Bd}	7.41 ± 0.02^{Ac}	7.03 ± 0.06^{Bd}	7.27 ± 0.00^{Ae}
60	5.42 ± 0.04^{Be}	6.34 ± 0.07^{Ae}	6.65 ± 0.03^{Ae}	7.16 ± 0.17^{Ac}	6.97 ± 0.11^{Bd}	7.36 ± 0.03^{Ade}

^{AB}Means with the same letter in a row are not significantly different ($P \leq 0.05$). ^{abcde}Means with the same letter in a column are not significantly different ($P \leq 0.05$). HM = high maize starch, MD = maltodextrin, GA = gum arabic, Vac = 97% vacuum, Air = 10% vacuum, and LA = *L. acidophilus* NRRL B-4495. GA-Vac and GA-Air = spray-dried LA with GA stored under 97 and 10% vacuum, respectively; HM-Vac and HM-Air = spray-dried LA with HM stored under 97 and 10% vacuum, respectively; and MD-Vac and MD-Air = spray-dried LA with MD stored under 97 and 10% vacuum, respectively.

VITA

Vondel Vandeker Reyes Ortega was born in Langue, Valle, Honduras. He obtained his Bachelor of Science in Food Technology from Universidad Nacional de Agricultura (UNA), Honduras. In fall of 2014, he completed an internship in the School of Nutrition and Food Sciences at Louisiana State University under the guidance of Dr. Subramaniam Sathivel. He started his master's studies under Dr. Sathivel's supervision in January 2015. In April 2016, he was awarded the IFT Gulf Coast Section Scholarship from Tom Quinn & Associates. In June 2017, he received the IFT Institute of Thermal Processing MS Scholarship. Vondel is a candidate to receive his degree in August 2017.