BIOPROCESS OPTIMIZATION FOR HIGH CELL MASS PRODUCTION OF *Lactobacillus acidophilus* AS PROBIOTIC

AFIF NAJIHAH BINTI KEPLI

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

School of Chemical and Energy Engineering
Faculty of Engineering
Universiti Teknologi Malaysia

JULY 2021

ACKNOWLEDGEMENT

In the Name of Allah, The Most Gracious, and Merciful. Firstly, I would like to express my deepest appreciation to all those who provided me the possibility to complete the experiments as well as the thesis. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my main thesis supervisor, Professor Dr. Hesham A. El. Enshansy, for encouragement, guidance, critics, and friendship. I am also very thankful to my co-supervisor Dr. Daniel Joe Dailin, Dr. Siti Zulaiha Hanapi (Asistant Director of IBD) and Madam Roslinda Malek (Head of laboratory) for their guidance, advice, and motivation. Without their continued support and interest, this thesis would not have been the same as presented here.

My warms thanks to the bioprocess team for their help, tips, and assistance especially to Mr. Solleh Ramli and Jared for his technical support. My lovely colleagues and friends Farah Diana, Jenifer, Hani, Kurgan Kumar, Azrini, Syimah, and Izzatul nadia for encourage and support. I owe my loving thanks to my husband (Muhammad Asyraf), mother, father, mother in-law, father in-law, and sibling for their unconditional support, both financially and emotionally throughout my study and without their advice, spirit and understanding it would have been impossible to finish this work.

Last but not least. My outmost gratitude goes to Universiti Teknologi Malaysia through UTM Zamalah scholarships and Harita Go Green Sdn. Bhd.

ABSTRACT

Lactobacillus from the lactic acid bacteria group, mainly synonym as a good bacterium known as probiotics that give a beneficial effect on human health. *Lactobacillus acidophilus* is one of the important bacteria that can maintain and restore gastrointestinal microflora, rebuild the digestive system from harmful bacteria, and fight vaginal infection. However, the biomass production of this bacteria is one of the industrial challenges. Therefore, this study was carried out to maximize cell mass production through optimization of medium composition and scaling up of the process to semi-industrial scale. Twelve different media were screened for the potential effect on cell growth. The best medium was composed of (g L^{-1}): glucose, 30; yeast extract, 6; ammonium citrate, 1; citric acid, 0.5; potassium dihydrogen phosphate (KH_2PO_4), 1.5; magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), 0.4; manganese (II) sulphate monohydrate ($MnSO_4.H_2O$), 0.082; sodium acetate, 1; and tween 80, 1. The biomass produced in this medium reached 2.46 g L^{-1}. Further medium optimization using one factor at a time (OFAT) and statistical method response surface methodology (RSM) improved biomass production up to 4.64 g L^{-1} and 5.36 g L^{-1}, respectively. The RSM optimized medium supported biomass production by approximately 15.52 % compared to OFAT optimized medium. Thus, the RSM optimized medium was used further in a 16-L bioreactor operated in batch cultivation mode to increase cell mass production. Cultivations in the bioreactor were carried out under controlled and uncontrolled pH conditions. High cell mass production was achieved in a controlled pH bioreactor (pH 6.5) and reached 6.41 g L^{-1} compared to in an un-controlled pH bioreactor which produced 4.56 g L^{-1} only. The biomass obtained from the controlled pH bioreactor was used for microencapsulation process. The cell viability after encapsulation was 9.45 log CFU/g with 76.95 % of encapsulation efficiency. The encapsulated *L. acidophilus* exhibited good resistance to bile salt concentration with 77 % of cells survived at bile salt concentration of 0.3%. However, resistance to the bile salt was found to be affected by pH value as well. After two hours of treatment, cell viability was 31.84 % at pH 4, whereas, cells were completely inactivated at pH 1. Thus, it can be concluded that statistically optimized medium composed of (g L^{-1}): glucose, 50; yeast extract, 20.91; ammonium citrate, 3.42; citric acid, 0.5; KH_2PO_4, 1.5; $MgSO_4.7H_2O$, 0.4; MnSO4.H_2O, 0.082; sodium acetate, 1; and tween 80, 1 produced the highest biomass production under pH-controlled condition of 6.5. Microencapsulation was also a suitable approach to protect cell viability when further applied to the human gastrointestinal tract.

ABSTRAK

Lactobacillus daripada kumpulan bakteria asid laktik, sangat sinonim sebagai bakteria baik yang dikenali sebagai probiotik yang memberikan kesan baik kepada kesihatan manusia. *Lactobacillus acidophilus* adalah salah satu bakteria penting yang dapat mengekalkan dan memulihkan mikroflora gastrousus, membina semula sistem pencernaan daripada bakteria berbahaya, dan memerangi jangkitan faraj. Walau bagaimanapun, pengeluaran biojisim bakteria ini adalah salah satu cabaran kepada industri. Oleh itu, kajian ini dilakukan untuk memaksimumkan pengeluaran sel melalui pengoptimuman komposisi medium, dan meningkatkan proses ke skala semi-industri. Dua belas media yang berbeza disaring untuk mengesan potensi pada pertumbuhan sel. Medium terbaik mengandungi komposisi berikut (g L^{-1}): glukosa, 30; ekstrak ragi, 6; ammonium sitrat, 1; asid sitrik, 0.5; kalium dihidrogen fosfat (KH_2PO_4), 1.5; magnesium sulfat heptahidrat ($MgSO_4.7H_2O$), 0.4; mangan (II) sulfat monohidrat ($MnSO4.H_2O$), 0.082; natrium asetat, 1; dan tween 80, 1. Biojisim yang dihasilkan dalam medium ini mencapai 2.46 g L^{-1}. Medium dioptimumkan lagi dengan menggunakan kaedah satu faktor pada satu masa (OFAT) dan kaedah statistik permukaan (RSM), masing-masing menghasilkan biojisim 4.64 g L^{-1} dan 5.36 g L^{-1}. Medium yang dioptimumkan secara RSM menyokong pengeluaran biojisim sebanyak 15.52 % berbanding medium dioptimumkan secara OFAT. Oleh itu, untuk meningkatan lagi pengeluaran sel, medium yang dioptimumkan secara RSM digunakan di dalam bioreaktor 16-L yang beroperasi pada mod pengkulturan kelompok. Pengeluaran sel yang lebih tinggi diperhatikan dalam keadaan pH terkawal dan tidak terkawal. Pengeluaran sel yang tinggi dicapai dalam bioreaktor pH terkawal (pH 6.5) dan mencapai 6.41 g L^{-1} berbanding dengan bioreaktor pH tidak terkawal yang menghasilkan 4.56 g L^{-1} sahaja. Biojisim yang diperoleh dari bioreaktor pH terkawal digunakan untuk proses mikroenkapsulasi. Keupayaan sel selepas enkapsulasi adalah 9.45 log CFU/g dengan kecekapan enkapsulasi 76.95 %. *L. acidophilus* yang telah dienkapsulasi menunjukkan ketahanan yang baik terhadap garam hempedu dengan 77 % sel bertahan pada kepekatan garam hempedu 0.3 %. Walau bagaimanapun, ketahanan terhadap garam hempedu juga dipengaruhi oleh nilai pH. Setelah dua jam rawatan, keupayaan sel pada pH 4 ialah 31.84 %, sementara sel tidak aktif sepenuhnya pada pH 1. Oleh itu, dapat disimpulkan bahawa medium yang dioptimumkan secara RSM yang mengandungi (g L^{-1}): glukosa, 50; ekstrak ragi, 20.91; ammonium sitrat, 3.42; asid sitrik, 0.5; KH_2PO_4, 1.5; $MgSO_4.7H_2O$, 0.4; $MnSO_4.H_2O$, 0.082; natrium asetat, 1; dan tween 80, 1 dengan kawalan pH 6.5 adalah medium yang menghasilkan pengeluaran biojisim yang tertinggi. Mikroenkapsulasi juga merupakan pendekatan yang tepat untuk melindungi keupayaan sel apabila digunakan pada saluran gastrousus manusia pada masa akan datang.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
$CaCl_2$	-	Calcium chloride
CCD	-	Central composite design
CDW	-	Cell dry weight
CO2	-	Carbon Dioxide
FDA	-	Food and drug administration
GRAS	-	Generally regarded as safe
HCl	-	Hydrochloric acid
K_2HPO_4	-	dipotassium hydrogen phosphate
$KHPO_4$	-	Potassium phosphate
L. acidophilus	-	*Lactobacillus acidophilus*
LAB	-	Lactic acid bacteria
$MgSO_4.7H_2O$	-	Magnesium sulfate heptahydrate
$MnSO_4.H_2O$	-	Manganese (II) sulfate monohydrate
MRS	-	Man Rogosa Sharpe
NaCl	-	Sodium Chloride
NaOH	-	Sodium hydroxide
OD	-	Optical density
OD_{600}	-	Optical density 600 nm
OFAT	-	One factor at a time
RSM	-	Response surface methodology
Sp	-	Species
%	-	Percentage
λ	-	Wavelength
°C	-	Degree Celsius
g	-	Gram
$g\ L^{-1}$	-	Gram per liter
min	-	Minutes
ml	-	Millilitre
h	-	Hour

L	-	Liter
L h^{-1}	-	Liter per hour
Mg	-	Milligram
P	-	Pressure
µ	-	Specific growth rate (h^{-1})
µmax	-	Maximum specific growth rate (h^{-1})
v/v/m	-	Volume per volume per minute
rpm	-	Rotations per minutes
M	-	Molarity

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Research Background

Health awareness nowadays is increased especially in terms of nutritional intake from the food supplement. The human intestine is one of the major organs that can contribute to the serious illness if no protection given from the beginning. Thus, one of the market's demands for gut microflora is probiotic from lactic acid bacteria (LAB). Lactic acid bacteria are commercially important in many branches of industries like the pharmaceutical and food industry as dietary supplements and probiotics product. There are also many products in the market that contain LAB cells (Polak-Berecka et al., 2010). Lactic acid bacteria are fastidious microorganisms and have complex nutrient requirements due to their limited specific minerals, vitamins B, amino acids, purine and pyrimidine bases (Taskila and Ojama, 2013). Therefore, a rich medium is crucial to achieving a higher growth profile. Lactic acid is an organic acid that can be obtained either by the action of fermentative microorganisms or chemical synthesis. Lasprilla et al. (2012) reported that approximately 90% of lactic acid production was produced by bacterial fermentation.

According to FAO/WHO, probiotics are living microorganisms in which if administered in sufficient amounts, can confer a health benefit on the host (Senz et al., 2015). Probiotic bacteria could suppress the growth of pathogens, prevention of diarrhea and constipation diseases, as well as prevention of cancer and mutation activities in the human gut (Yari et al., 2015). Hence, for the host to get beneficial health impact from probiotic foods, the viability and metabolic action of probiotic microorganism integrated into food product ought to be maintained in all step of food digestion processing operation and they must additionally be capable to survive within the gastrointestinal tract (Chang and Liew, 2013).

Lactobacillus group are commonly best known for human digestive health. The *Lactobacillus* genus has a variety of heterogeneous bacteria with more than 100 species and sub-species. Most of them were utilized as probiotics, silage inoculants and as starters in the fermented food industry (Machado et al., 2013). Salvetti (2012) also stated that *Lactobacillus* contains a high number of GRAS (Generally Recognized as Safe) species, which has a functional group consisting of Gram-positive, as well as catalase-negative bacteria which produce lactic acid as a major metabolic end-product of carbohydrate fermentation.

The main important elements in the growth medium of *Lactobacillus* species are carbon, nitrogen, and mineral. Man-Rogosa-Sharpe (MRS) is the standard medium for the growth of *Lactobacillus acidophilus*, which is expensive for industrial applications (Pedram and Ataei, 2014). By decreasing the concentration of nutrients at the minimum level or using low-cost components could possibly reduce the cost of growth media for industrial biomass production. After all, a high concentration of lactic acid components from product cultivation can lead to an inhibition of organism growth and a reduction of the metabolic product yield (Kostov et al., 2011).

Despite that, by selected the major compositions of the medium capable influenced much in the growth of *L. acidophilus* cells especially in large scale processes. The screening method was carried out before getting an optimized medium composition. During screening, each composition with best concentration will be selected in further optimization process. Thus, One Factor at A Time (OFAT) and statistical experiment design using response surface methodology (RSM) expected to be the as efficient approach used in this study to achieve the optimal growth medium composition, by evaluated the effect of each medium component on *L. acidophilus* growth.

Then, in this study optimized medium compositions and cultivation conditions were carried out in 16-L bioreactor under controlled and uncontrolled pH batch fermentation to produce high cell mass production. Followed by that, the high cell mass of *L. acidophilus* was encapsulated with aseptically adding the *L. acidophilus* pure cell culture in feed solution to turn into a powdered form under the microencapsulation process using a spray drying method.

Other than medium optimization study, there are some important factors like acidity and bile salts which can affect the probiotic cells to remain their function in a host to ensure that *L. acidophilus* can be delivered in the gut system with higher viability. Thus, acidity and bile salts test was prepared by added encapsulated cells in artificial juice solutions at the pH range 1.0 to 4.0 and in media containing different concentrations of ox gall for 0 min, 30 min, 60 min, 90 min, and 120 min. The probiotic bacteria tolerance toward acid and bile was determined by the capability of the cells growth in the extreme environment.

1.2 Problem Statement

The demand for probiotic products like *Lactobacillus acidophilus* is increasing as it is well-known that it can confer significant health benefits to the human gut. However, the challenging part faced by industry with this microaerophilic probiotic is to produce high cell mass and economical fermentaion process. Besides that, development of Fermentation process from shake flask to bioreactor involved many challenges due to the complication in evaluating the factors affecting the process during cultivation. For example, in shake flask study there are limiting factors such as unable to control pH during fermentation and production of secondary metabolites that renders the production of cells. In addition to that, the downstream processing which involves preparation of microbial powder via spray drying technique is challenging due to cell loss during drying process.

An optimized drying conditions couple with microencapsulation technique are required to produce high viability of cells. The cells produced also need to be able to withstand the gastrointestinal tract condition so that it can benefits the host when consumed.Thus, the aim of this research is to develop highly efficient production medium, to get optimum drying conditions and to ecaluate the efficacy of the cells to withstand the gastrointestinal tract condition.

1.3 Objectives of Study

This study was conducted to achieve the following objectives:

i. To select and optimize a suitable medium composition to support high cell mass production of *L. acidophilus* in shake flasks level.
ii. To determine the effect of pH on growth kinetic *L. acidophilus* in 16 -L bioreactor.
iii. To evaluate the effectiveness of the *L. acidophilus* encapsulation process using a spray drying method.
iv. To evaluate the efficacy of *L. acidophilus* in the gastrointestinal tract condition.

1.4 Scopes of StudyThe scopes of this study are:

i. Selected the best medium among twelve different media as mentioned in Table 3.1 to support high cell mass production of *L. acidophilus* in shake flask level.
ii. Optimization of medium composition for cell mass production of *L. acidophilus* in shake flasks level using One Factor At the Time (OFAT) method and Respond Surface Methodology (RSM) method on the selected medium.

iii. Batch cultivation study under controlled and uncontrolled pH in the 16-L bioreactor. The pH was controlled at 6.5. The temperature was maintained at 37°C with 200 rpm of agitation and 0.5 v/v/m of aeration. 8 L of working volume with 10% inoculum was used to initiate the cultivation.
iv. Encapsulated of *L. acidophilus* cell using spray drying method obtained from controlled pH batch cultivation. Feed solution was containing 20% m/v of final concentration with 170°C of inlet temperature and 85°C of outlet temperature. 0.48 L/h of feed solution, 40 L/min of drying air flow rate with 6 Bar of air pressure was used to produce microencapsulation cell powder.
v. Evaluated the efficacy test on *L. acidophilus* powder in different concentrations of bile salts in 0.5, 1.0, 2.0, 3.0 and 4.0%. Then, 1.0-4.0 of pH concentrations of gastric juice was used for viability test of acidic condition.

REFERENCES

Agheyisi, R. (2005). Ga-121 probiotics: ingredients, supplements, foods. Publisher: BCC Research Inc.

Aguirre-Ezkauriatza, E. J., Aguilar-Yáñez, J. M., Ramírez-Medrano, A., & Alvarez, M. M. (2010). Production of probiotic biomass (Lactobacillus casei) in goat milk whey: Comparison of batch, continuous and fed-batch cultures. *Bioresource Technology*, *101*(8), 2837-2844.

Alfaro, L., Hayes, D., Boeneke, C., Xu, Z., Bankston, D., Bechtel, P. J., & Sathivel, S. (2015). Physical properties of a frozen yogurt fortified with a nano-emulsion containing purple rice bran oil. *LWT-Food Science and Technology*, *62*(2), 1184-1191.

Al-Madboly, L. A., Khedr, E. G., & Ali, S. M. (2017). Optimization of reduced glutathione production by a *Lactobacillus plantarum* isolate using Plackett–Burman and Box–Behnken designs. *Frontiers in Microbiology*, *8*, 772.

Altermann, E., Russell, W. M., Azcarate-Peril, M. A., Barrangou, R., Buck, B. L., McAuliffe, O., & Lick, S. (2005). Complete genome sequence of the probiotic lactic acid bacterium *Lactobacillus acidophilus* NCFM. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(11), 3906-3912.

Alvarez, M. M., Aguirre-Ezkauriatza, E. J., Ramírez-Medrano, A., & Rodríguez-Sánchez, Á. (2010). Kinetic analysis and mathematical modeling of growth and lactic acid production of *Lactobacillus casei var. rhamnosus* in milk whey. *Journal of dairy science*, *93*(12), 5552-5560.

Amara, A. A. (2012). Toward healthy genes Ed. *Amro Amara*.

Amara, A. A., & Shibl, A. (2015). Role of Probiotics in health improvement, infection control and disease treatment and management. *Saudi pharmaceutical journal*, *23*(2), 107-114.

Amin, T., Thakur, M., & Jain, S. C. (2013). Microencapsulation-the future of probiotic cultures. *The Journal of Microbiology, Biotechnology and Food Sciences*, *3*(1), 35.

Amrane, A., & Prigent, Y. (1998). Influence of yeast extract concentrationon batch cultures of *Lactobacillus helveticus*: growth and production coupling. *World Journal of Microbiology and Biotechnology, 14*(4), 529-534.

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.

Anekella, K., & Orsat, V. (2013). Optimization of microencapsulation of probiotics in raspberry juice by spray drying. *LWT-Food Science and Technology, 50*(1), 17-24.

Anvari, M., Khayati, G., & Rostami, S. (2014). Optimisation of medium composition for probiotic biomass production using response surface methodology. *Journal of Dairy Research, 81*(1), 59-64.

Aragón-Rojas, S., Ruiz-Pardo, R. Y., Hernández-Sánchez, H., & Quintanilla-Carvajal, M. X. (2018). Optimization of the production and stress resistance of the probiotic *Lactobacillus fermentum* K73 in a submerged bioreactor using a whey-based culture medium. *CyTA-Journal of Food, 16*(1), 1064-1070.

Aslim, B. and Alp, G. (2009). Relationship between the Resistance to Bile Salts and Low Ph with Exopolysaccharide (EPS) Production of *Bifidobacterium* sp. Isolated from Infants Feces and Breast Milk. *Journal of Food Microbiology. 16*, 101-105.

Azcarate-Peril MA, Tallon R, Klaenhammer TR (2009). Temporal gene expression and probiotic attributes of *Lactobacillus acidophilus* during growth in milk. *Journal of Dairy Science*, *92*, 870–886. doi:10.3168/jds.2008-1457.

Banaszkiewicz, T. (2011). Nutritional value of soybean meal. *Soybean and nutrition,* 1-20.

Barbosa, J., & Teixeira, P. (2017). Development of probiotic fruit juice powders by spray-drying: A review. *Food Reviews International, 33*(4), 335-358.

Barrangou, R., Azcarate-Peril, M. A., Duong, T., Conners, S. B., Kelly, R. M., & Klaenhammer, T. R. (2006). Global analysis of carbohydrate utilization by *Lactobacillus acidophilus* using cDNA microarrays. *Proceedings of the National Academy of Sciences of the United States of America, 103*(10), 3816-3821.

Bermúdez-Humarán, L. G., Aubry, C., Motta, J. P., Deraison, C., Steidler, L., Vergnolle, N., & Langella, P. (2013). Engineering lactococci and lactobacilli for human health. *Current opinion in Microbiology*, *16*(3), 278-283.

Botrel, D. A., Rodrigues, I. C. B., de Souza, H. J. B., & de Barros Fernandes, R. V. (2016). Application of inulin in thin-layer drying process of araticum (*Annona crassiflora*) pulp. *LWT-Food Science and Technology, 69*, 32-39.

Brinques, G. B., do Carmo Peralba, M., & Ayub, M. A. Z. (2010). Optimization of probiotic and lactic acid production by *Lactobacillus plantarum* in submerged bioreactor systems. *Journal of industrial microbiology & biotechnology, 37*(2), 205-212.

Brinques, G. B., do Carmo Peralba, M., & Ayub, M. A. Z. (2010). Optimization of probiotic and lactic acid production by *Lactobacillus plantarum* in submerged bioreactor systems. *Journal of industrial microbiology & biotechnology, 37*(2), 205-212.

Bron, P. A., M. Marco, S. M. Hoffer, E. V. Mullekom, W. M. de Vos, and M. Kleerebezem. (2004). Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters in vitro and in situ in the gastrointestinal tract. *Journal of Bacteriology*, *186*, 7829-7835.

Budryn, G., Zaczyńska, D., & Oracz, J. (2016). Effect of addition of green coffee extract and nanoencapsulated chlorogenic acids on aroma of different food products. *LWT, 73*, 197-204.

Bulatović, M. L., Rakin, M. B., Vukašinović-Sekulić, M. S., Mojović, L. V., & Krunić, T. Ž. (2014). Effect of nutrient supplements on growth and viability of *Lactobacillus johnsonii* NRRL B-2178 in whey. *International Dairy Journal*, *34*(1), 109-115.

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.

Burns, P., Patrignani, F., Serrazanetti, D., Vinderola, G. C., Reinheimer, J. A., Lanciotti, R., & Guerzoni, M. E. (2008). Probiotic Crescenza cheese containing *Lactobacillus casei* and *Lactobacillus acidophilus* manufactured with high-pressure homogenized milk. *Journal of Dairy Science*, *91*(2), 500-512.

Bustamante M, Villarroel M, Rubilar M, Shene C (2015) *Lactobacillus acidophilus* La-05 encapsulated by spray drying: effect of mucilage and protein from flaxseed (*Linum usitatissimum* L.) *LWT-Food Science and Technology, 62*, 1162–1168. https://doi.org/10.1016/ j.lwt.2015.02.017

Bustos, A. Y., Saavedra, L., de Valdez, G. F., Raya, R. R., & Taranto, M. P. (2012). Relationship between bile salt hydrolase activity, changes in the internal pH and tolerance to bile acids in lactic acid bacteria. *Biotechnology Letters*, *34*(8), 1511-1518.

Bustos, P., & Bórquez, R. (2013). Influence of osmotic stress and encapsulating materials on the stability of autochthonous *Lactobacillus plantarum* after spray drying. *Drying technology, 31*(1), 57-66.

Butel, M. J. (2014). Probiotics, gut microbiota and health. *Médecine et Maladies Infectieuses*, *44*(1), 1-8.

Cai, S., Zhao, M., Fang, Y., Nishinari, K., Phillips, G. O., & Jiang, F. (2014). Microencapsulation of *Lactobacillus acidophilus* CGMCC1. 2686 via emulsification/internal gelation of alginate using Ca-EDTA and CaCO3 as calcium sources. *Food hydrocolloids*, *39*, 295-300.

Champagne, C.P. & Kailasapathy, K. (2008). Encapsulation of probiotics. In *Delivery and Controlled Release of Bioactives in Foods and Nutraceuticals* (pp. 344-369). Woodhead, Cambridge, UK.

Chang, C. P., & Liew, S. L. (2013). Growth medium optimization for biomass production of a probiotic bacterium, *Lactobacillus rhamnosus* ATCC 7469. *Journal of Food Biochemistry*, *37*(5), 536-543.

Chen, H., Niu, J., Qin, T., Ma, Q., & Wang, L. (2015). Optimization of the medium for Lactobacillus acidophilus by Plackett-Burman and steepest ascent experiment. *ACTA Scientiarum Polonorum Technologia Alimentaria, 14*(3), 227-232.

Coghetto, C. C., Vasconcelos, C. B., Brinques, G. B., & Ayub, M. A. Z. (2016). *Lactobacillus plantarum* BL011 cultivation in industrial isolated soybean protein acid residue. *Brazilian Journal of Microbiology*, *47*(4), 941-948

Comunian, T. A., Thomazini, M., Alves, A. J. G., de Matos Junior, F. E., de Carvalho Balieiro, J. C., & Favaro-Trindade, C. S. (2013). Microencapsulation of ascorbic acid by complex coacervation: Protection and controlled release. *Food Research International, 52*(1), 373-379.

Cook, M. T., Tzortzis, G., Charalampopoulos, D., & Khutoryanskiy, V. V. (2012). Microencapsulation of probiotics for gastrointestinal delivery. *Journal of controlled release, 162*(1), 56-67.

Corcoran, B. M., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2004). Comparative survival of probiotic lactobacilli spray-dried in the presence of prebiotic substances. *Journal of Applied Microbiology, 96*(5), 1024-1039.

Corona-Hernandez, R. I., Álvarez-Parrilla, E., Lizardi-Mendoza, J., Islas-Rubio, A. R., de la Rosa, L. A., & Wall-Medrano, A. (2013). Structural stability and viability of microencapsulated probiotic bacteria: a review. *Comprehensive Reviews in Food Science and Food Safety, 12*(6), 614-628.

Corrêa-Filho, L. C., Moldão-Martins, M., & Alves, V. D. (2019). Advances in the application of microcapsules as carriers of functional compounds for food products. *Applied Sciences, 9*(3), 571.

Costa, S. S., Machado, B. A. S., Martin, A. R., Bagnara, F., Ragadalli, S. A., & Alves, A. R. C. (2015). Drying by spray drying in the food industry: micro-encapsulation, process parameters and main carriers used. *African Journal of Food Science, 9*(9), 462-470.

Crittenden, R., Weerakkody, R., Sanguansri, L., & Augustin, M. (2006). Synbiotic microcapsules that enhance microbial viability during nonrefrigerated storage and gastrointestinal transit. *Applied and environmental microbiology*, *72*(3), 2280-2282.

Cui, J. H., Goh, J. S., Kim, P. H., Choi, S.H., Lee, B. J. (2000) Survival and stability of bifidobacteria loaded in alginate poly-l-lysinemicroparticles. *International Journal of Pharmaceutics, 210*, 51–59. doi:10.1016/S0378-5173(00)00560-3.

de Araújo Etchepare, M., Raddatz, G. C., de Moraes Flores, É. M., Zepka, L. Q., Jacob-Lopes, E., Barin, J. S., Grosso, C. R. F. & de Menezes, C. R. (2016). Effect of resistant starch and chitosan on survival of *Lactobacillus acidophilus* microencapsulated with sodium alginate. *LWT-Food Science and Technology*, *65*, 511-517.

de Barros Fernandes, R. V., Botrel, D. A., Silva, E. K., Borges, S. V., de Oliveira, C. R., Yoshida, M. I., & de Paula, R. C. M. (2016). Cashew gum and inulin: New alternative for ginger essential oil microencapsulation. *Carbohydrate polymers, 153*, 133-142.

de Moraes Filho, M. L., Busanello, M., Prudencio, S. H., & Garcia, S. (2018). Soymilk with okara flour fermented by *Lactobacillus acidophilus*: Simplex-centroid mixture design applied in the elaboration of probiotic creamy sauce and storage stability. *LWT*, *93*, 339-345.

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.

Delcour, J., Ferain, T., Deghorain, M., Palumbo, E., & Hols, P. (1999). The biosynthesis and functionality of the cell-wall of lactic acid bacteria. In *Lactic acid bacteria: genetics, metabolism and applications* (pp. 159-184). Springer, Dordrecht.

Desai, K. G. H., & Jin Park, H. (2005). Recent developments in microencapsulation of food ingredients. *Drying technology, 23*(7), 1361-1394.

Desmond, C., Ross, R. P., 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.

Dias, D. R., Botrel, D. A., Fernandes, R. V. D. B., & Borges, S. V. (2017). Encapsulation as a tool for bioprocessing of functional foods. *Current Opinion in Food Science, 13*, 31-37.

Dima, C., Pătraşcu, L., Cantaragiu, A., Alexe, P., & Dima, Ş. (2016). The kinetics of the swelling process and the release mechanisms of *Coriandrum sativum* L. essential oil from chitosan/alginate/inulin microcapsules. *Food chemistry, 195*, 39-48.

Dixit, G., Samarth, D., Tale, V., & Bhadekar, R. (2013). Comparative studies on potential probiotic characteristics of *Lactobacillus acidophilus* strains. *EurAsian Journal of BioSciences*, *7*.

Donnet-Hughes, A., Rochat, F., Serrant, P., Aeschlimann, J.M., Schiffrin, E.J., 1999. Modulation of nonspecific mechanisms of defence by lactic acid bacteria: effective dose. *Journal of Dairy Science, 82*, 863-869.

Elmarzugi, N., El Enshasy, H., Abd Malek, R., Othman, Z., Sarmidi, M. R., & Abdel Aziz, R. (2010). Optimization of cell mass production of the probiotic strain *Lactococcus lactis* in batch and fed-bach culture in pilot scale levels. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Technology, 2*, 873-879.

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.

FAO/WHO. (2002). *Guidelines for the evaluation of probiotics in food.* Report of a joint FAO/WHO working group on drafting guidelines for the evaluation of probiotics in food.

Favaro-Trindade, C. S., & Grosso, C. R. F. (2002). Microencapsulation of *L. acidophilus* (La-05) and *B. lactis* (Bb-12) and evaluation of their survival at the pH values of the stomach and in bile. *Journal of microencapsulation, 19*(4), 485-494.

Fernández, M., Hudson, J. A., Korpela, R., & de los Reyes-Gavilán, C. G. (2015). Impact on human health of microorganisms present in fermented dairy products: an overview. *BioMed. Research International*, 2015. doi: http://dx.doi.org/10.1155/2015/412714.

Fernandez, M.F., Boris, S. and Barbes, C. (2003). Probiotic properties of human *lactobacilli* strains to be used in the gastrointestinal tract. *Journal of Applied Microbiology*, 94: 449-455.

Ferreira, S. C., Bruns, R. E., Ferreira, H. S., Matos, G. D., David, J. M., Brandao, G. C., & Dos Santos, W. N. L. (2007). Box-Behnken design: an alternative for the optimization of analytical methods. *Analytica chimica acta, 597*(2), 179-186.

Food and Agriculture Organization of the Unite Nations (2001). *Health and nutritional properties of probiotics in food including poder milk with live lactic acid bacteria.* World Health Organization.

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.

Fu, N., Huang, S., Xiao, J., & Chen, X. D. (2018). Producing powders containing active dry probiotics with the aid of spray drying. In *Advances in food and nutrition research* (Vol. 85, pp. 211-262). Academic Press.

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

Fung, W. Y., Woo, Y. P., & Liong, M. T. (2008). Optimization of growth of *Lactobacillus acidophilus* FTCC 0291 and evaluation of growth characteristics in soy whey medium: a response surface methodology approach. *Journal of Agricultural and Food Chemistry*, *56*(17), 7910-7918.

García, A. H. (2011). Anhydrobiosis in bacteria: from physiology to applications. *Journal of biosciences, 36*(5), 939-950.

Gareau, M. G., Sherman, P. M., & Walker, W. A. (2010). Probiotics and the gut microbiota in intestinal health and disease. *Nature Reviews Gastroenterology and Hepatology*, *7*(9), 503-514.

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.

Gibbs, F., Selim Kermasha, Inteaz Alli, Catherine N. Mulligan, B. (1999). Encapsulation in the food industry: a review. *International journal of food sciences and nutrition, 50*(3), 213-224.

Gibson, G. R., & Roberfroid, M. B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *The Journal of nutrition, 125*(6), 1401-1412.

Goderska, K., Nowak, J., & Czarnecki, Z. (2008). Comparision of growth of *Lactobacillus acidophilus* and *Bifidobacterium Bifidum* species in media suplemented with selected saccharides including prebiotics. *Acta Scientiarum Polonorum Technologia Alimentaria, 7*(2), 5-20.

Goldstein, E. J., Tyrrell, K. L., & Citron, D. M. (2015). Lactobacillus species: taxonomic complexity and controversial susceptibilities. *Clinical Infectious Diseases*, *60*(suppl 2), S98-S107.

Goncalves, A., Estevinho, B. N., & Rocha, F. (2016). Microencapsulation of vitamin A: A review. *Trends in Food Science & Technology, 51*, 76-87.

Gopal, P. K. (2011). Lactobacillus spp.: *Lactobacillus acidophilus*. Fonterra Research Centre, Palmerston Noth, New Zealand. pp 91- 95.

Goyal, A., Sharma, V., Sihag, M. K., Tomar, S. K., Arora, S., Sabikhi, L., & Singh, A. K. (2015). Development and physico-chemical characterization of microencapsulated flaxseed oil powder: A functional ingredient for omega-3 fortification. *Powder Technology, 286*, 527-537.

Hammes WP, Hertel C (2009) Genus I. *Lactobacillus Beijerink*, 1901. In: De Vos P, Garrity GM, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman WB (eds) Bergey's Manual of Systematic Bacteriology, vol 3, 2nd edn. Springer, Berlin, pp 465–510.

Heriban, V., Sitkey, V., Šturdík, E., & Matuš, P. (1993). Nutrition and broth alterations in the lactic acid fermentation. *Acta biotechnologica*, *13*(3), 283-288.

Hernandez-Hernandez, O., Muthaiyan, A., Moreno, F. J., Montilla, A., Sanz, M. L., & Ricke, S. C. (2012). Effect of prebiotic carbohydrates on the growth and tolerance of Lactobacillus. *Food Microbiology*, *3*0(2), 355-361.

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, 63*, 1-17.

Hwang, C. F., Chang, J. H., Houng, J. Y., Tsai, C. C., Lin, C. K., & Tsen, H. Y. (2012). Optimization of medium composition for improving biomass production of *Lactobacillus plantarum* Pi06 using the Taguchi array design and the Box-Behnken method. *Biotechnology and Bioprocess Engineering*, *17*(4), 827-834.

Jafarei, P., & Ebrahimi, M. T. (2011). *Lactobacillus acidophilus* cell structure and application. *African Journal of Microbiology Research, 5*(24), 4033-4042.

Kamilla, L., Lisa, O. A. G., & Chew, A. L. (2008). Optimization of nitrogen source in addition of carbon source to improvise the growth of *Lactobacillus acidophilus*. In *4th Lıfe Sciences Postgraduate Conference at Universiti Sains Malaysia.* Retrieved from https://www.researchgate.net/publication/ 333746372

Kang, Y. R., Lee, Y. K., Kim, Y. J., & Chang, Y. H. (2019). Characterization and storage stability of chlorophylls microencapsulated in different combination of gum Arabic and maltodextrin. *Food chemistry, 272*, 337-346.

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.*

Khaleghi, M., Kermanshahi, R. K., Yaghoobi, M. M., Zarkesh-Esfahani, S. H., & Baghizadeh, A. (2010). Assessment of bile salt effects on s-layer production, slp gene expression and some physicochemical properties of *Lactobacillus acidophilus* ATCC 4356. *Journal of Microbiology and Biotechnology, 20*(4), 749-756.

Khalid, K. (2011). An overview of lactic acid bacteria. *International Journal of Biosciences*, *1*(3), 1-13.

Kim, P. I., Jung, M. Y., Chang, Y. H., Kim, S., Kim, S. J., & Park, Y. H. (2007). Probiotic properties of Lactobacillus and Bifidobacterium strains isolated from porcine gastrointestinal tract. *Applied microbiology and biotechnology, 74*(5), 1103-1111.

Klotz, S., Kuenz, A., & Prüße, U. (2017). Nutritional requirements and the impact of yeast extract on the d-lactic acid production by Sporo *Lactobacillus inulinus.Green Chemistry, 19*(19), 4633-4641.

Kostov, G., Angelov, M., Denkova, Z., Dobrev, I., & Goranov, B. (2011). Lactic acid production with *Lactobacillus casei* ssp. rhamnosus NBIMCC 1013: Modeling and optimization of the nutrient medium. *Engineering in Lıfe Sciences*, *11*(5), 517-527.

Kumar, M., Ghosh, M., and Ganguli, Abhijit. (2012). Mitogenic Response and Probiotic Characteristics of Lactic Acid Bacteria Isolated from Indigenously Pickled Vegetables and Fermented Beverages. *World Journal of Microbial Biotechnology, 28*(2), 703-311.

Lasprilla, A. J., Martinez, G. A., Lunelli, B. H., Jardini, A. L., & Maciel Filho, R. (2012). Poly-lactic acid synthesis for application in biomedical devices - A review. *Biotechnology Advances*, *30*(1), 321-328.

Leclercq, S., Harlander, K. R., & Reineccius, G. A. (2009). Formation and characterization of microcapsules by complex coacervation with liquid or solid aroma cores. *Flavour and Fragrance Journal, 24*(1), 17-24.

Lee, N. K., Park, Y. L., Choe, G. J., Chang, H. I., & Paik, H. D. (2010). Medium optimization for the production of probiotic *Lactobacillus acidophilus* A12 using response surface methodology. *Korean Journal for Food Science of Animal Resources, 30*(3), 359-364.

Lehtoranta, L. (2012). Probiotics and virus infections: The effects of *Lactobacillus rhamnosus* GG on respiratory and gastrointestinal virus infections. Academic Dissertation, University of Helsinki, Helsinki.

Li, M., Liao, X., Zhang, D., Du, G., & Chen, J. (2011). Yeast extract promotes cell growth and induces production of polyvinyl alcohol-degrading enzymes. *Enzyme research, 2011.*

Liguori, R., Soccol, C. R., Vandenberghe, L. P. D. S., Woiciechowski, A. L., Ionata, E., Marcolongo, L., & Faraco, V. (2015). Selection of the strain *Lactobacillus acidophilus* ATCC 43121 and its application to brewers' spent grain conversion into lactic acid. *BioMed research international, 2015.*

Liu, Q. N., Liu, R. S., Wang, Y. H., Mi, Z. Y., Li, D. S., Zhong, J. J., and Tang, Y. J. (2009). Fed-batch fermentation of *Tuber melanosporum* for the hyperproduction of mycelia and bioactive *Tuber* polysaccharides. *Bioresource Technology, 100*, 3644-3649.

Lopes, S. M., Krausová, G., Rada, V., Gonçalves, J. E., Gonçalves, R. A., & de Oliveira, A. J. (2015). Isolation and characterization of inulin with a high degree of polymerization from roots of *Stevia rebaudiana (Bert.) Bertoni*. *Carbohydrate research, 411*, 15-21.

Machado, A., Almeida, C., Carvalho, A., Boyen, F., Haesebrouck, F., Rodrigues, L., & Azevedo, N. F. (2013). Fluorescence in situ hybridization method using a peptide nucleic acid probe for identification of *Lactobacillus spp*. in milk samples. *International Journal of Food Microbiology, 162*(1), 64-70.

Maciel, G. M., Chaves, K. S., Grosso, C. R. F., & Gigante, M. L. (2014). Microencapsulation of Lactobacillus acidophilus La-5 by spray-drying using sweet whey and skim milk as encapsulating materials. *Journal of dairy science*, *97*(4), 1991-1998.

Malek, A. R., Hamdan, S., El Enshasy, H., Othman, N. Z., Sarmidi, M. R., and Aziz, R. (2010). High cell mass production and characterization of *Lactobacillus salivarius*, a new probiotic strain from human breast milk. *Journal of Pharmacy and Biological Sciences, 8*, 49-59.

Mandenius, C.F., and Brudin, A. (2008). Bioprocess Optimization Using Design-of Experiment Methodology. *Biotechnology Progress, 24*(6), 1191-1203.

Martins, I. M., Barreiro, M. F., Coelho, M., & Rodrigues, A. E. (2014). Microencapsulation of essential oils with biodegradable polymeric carriers for cosmetic applications. Chemical Engineering Journal, *245*, 191-200.

Masood, M. I., Qadir, M. I., Shirazi, J. H., & Khan, I. U. (2011). Beneficial effects of lactic acid bacteria on human beings. *Critical Reviews in Microbiology, 37*(1), 91-98.

Meena, G. S., Kumar, N., Majumdar, G. C., Banerjee, R., Meena, P. K., & Yadav, V. (2014). Growth characteristics modeling of *Lactobacillus acidophilus* using RSM and ANN. *Brazilian Archives of Biology and Technology, 57*(1), 15-22.

Moraes Filho, M. L., Busanello, M., & Garcia, S. (2016). Optimization of the fermentation parameters for the growth of *Lactobacillus* in soymilk with okara flour. *LWT-Food Science and Technology*, *74*, 456-464.

Mortazavian, A., Razavi, S. H., Ehsani, M. R., & Sohrabvandi, S. (2007). Principles and methods of microencapsulation of probiotic microorganisms. *Iranian Journal of Biotechnology, 5*(1), 1-18.

Muthukumarasamy, P., Allan-Wojtas, P., & Holley, R. A. (2006). Stability of *Lactobacillus reuteri* in different types of microcapsules. *Journal of food science, 71*(1), M20-M24.

Mutukumira, A. N., Ang, J., & Lee, S. J. (2015). Microencapsulation of probiotic bacteria. In *Beneficial Microorganisms in Food and Nutraceuticals* (pp. 63-80). Springer, Cham.

Myers, R. H., & Montgomery, D. C. (1995) *Response Surface Methodology. Process and Product Optimization Using Designed Experiments.* John Wiley & Sons, Inc., NY, USA.

Narayan, S. S., Jalgaonkar, S., Shahani, S., & Kulkarni, V. N. (2010). Probiotics: current trends in the treatment of diarrhoea. *Hong Kong Medical Journal.*, *16*(3), 213-218.

Nawong, S., Oonsivilai, R., Boonkerd, N., & Hansen, L. T. (2016). Entrapment in food-grade transglutaminase cross-linked gelatin–maltodextrin microspheres protects *Lactobacillus* spp. during exposure to simulated gastro-intestinal juices. *Food research international, 85*, 191-199.

Nazzaro, F., Fratianni, F., Nicolaus, B., Poli, A., & Orlando, P. (2012). The prebiotic source influences the growth, biochemical features and survival under simulated gastrointestinal conditions of the probiotic *Lactobacillus acidophilus*. *Anaerobe, 18*(3), 280-285.

Nunes, G. L., de Araújo Etchepare, M., Cichoski, A. J., Zepka, L. Q., Lopes, E. J., Barin, J. S., & de Menezes, C. R. (2018). Inulin, hi-maize, and trehalose as thermal protectants for increasing viability of *Lactobacillus acidophilus* encapsulated by spray drying. *LWT, 89*, 128-133.

Ohland, C. L., & MacNaughton, W. K. (2010). Probiotic bacteria and intestinal epithelial barrier function. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *298*(6), G807-G819.

Okuro, P. K., Thomazini, M., Balieiro, J. C., Liberal, R. D., & Fávaro-Trindade, C. S. (2013). Co-encapsulation of Lactobacillus acidophilus with inulin or polydextrose in solid lipid microparticles provides protection and improves stability. *Food Research International*, *53*(1), 96-103.

Ooi, L. G., & Liong, M. T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: a review of *in vivo* and *in vitro* findings. *International Journal of Molecular Sciences*, *11*(6), 2499-2522.

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.

Pai, D. A., Vangala, V. R., Ng, J. W., Ng, W. K., & Tan, R. B. (2015). Resistant maltodextrin as a shell material for encapsulation of naringin: Production and physicochemical characterization. *Journal of Food Engineering, 161*, 68-74.

Panda, B. P., Ali, M., and Javed, S. (2007). Fermentation process optimization. *Research Journal of Microbiology, 2*, 201-208.

Pedram, N., & Ataei, S. A. (2014). Optimization of a Modified GS Medium for a Probiotic Strain (*L. acidophilus* ATCC4356). *Applied Food Biotechnology, 1*(1), 25-29.

Peighambardoust, S. H., 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.

Pelczar, M.J., Chan, E.C.S. & Krieg, N.R. (2010). Microbiology: An Application-Based Approach. New Delhi: Tata Mc Graw Hill

Petrović, T., Nedović, V., Dimitrijević-Branković, S., Bugarski, B., & Lacroix, C. (2007). Protection of probiotic microorganisms by microencapsulation. *Chemical Industry and Chemical Engineering Quarterly*, *13*(3), 169-174.

Phan, P. (2011). Kinetic Investigation in Lactic Acid Production by *L. Acidophilus. Acidophilus* (March 31, 2011).

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.

Pinto, S. S., Fritzen-Freire, C. B., Muñoz, I. B., Barreto, P. L., Prudêncio, E. S., & Amboni, R. D. (2012). Effects of the addition of microencapsulated Bifidobacterium BB-12 on the properties of frozen yogurt. *Journal of Food Engineering, 111*(4), 563-569.

Polak-Berecka, M., Waśko, A. D. A. M., Kordowska-Wiater, M., Podleśny, M. A. R. C. I. N., Targoński, Z. D. Z. I. S. L. A. W., & Kubik-Komar, A. G. N. I. E. S. Z. K. A. (2010). Optimization of medium composition for enhancing growth of *Lactobacillus rhamnosus* PEN using response surface methodology. *Polish Journal of Microbiology, 59*(2), 113-118.

Poomkokrak, J., Niamnuy, C., Choicharoen, K., & Devahastin, S. (2015). Encapsulation of soybean extract using spray drying. *Journal of Food Science and Agricultural Technology (JFAT), 1*, 105-110.

Poornima, K. & Sinthya, R. (2017). Application of Various Encapsulation Techniques in Food Industries. *International Journal of Latest Engineering Research and Applications (IJLERA), 2* (10), 37-41.

Popa, D., & Ustunol, Z. (2011). Influence of sucrose, high fructose corn syrup and honey from different floral sources on growth and acid production by lactic acid bacteria and bifidobacteria. *International journal of dairy technology, 64*(2), 247-253.

Power, S. E., O'Toole, P. W., Stanton, C., Ross, R. P., & Fitzgerald, G. F. (2014). Intestinal microbiota, diet and health. *British Journal of Nutrition*, *111*(03), 387-402.

Pudziuvelyte, L., Marksa, M., Jakstas, V., Ivanauskas, L., Kopustinskiene, D. M., & Bernatoniene, J. (2019). Microencapsulation of Elsholtzia ciliata Herb Ethanolic Extract by Spray-Drying: Impact of Resistant-Maltodextrin Complemented with Sodium Caseinate, Skim Milk, and Beta-Cyclodextrin on the Quality of Spray-Dried Powders. *Molecules, 24*(8), 1461.

Rafter, J.J., (2002). Scientific basis of biomarkers and benefits of functional foods for reduction of disease risk: cancer. *British Journal of Nutrition, 88*, S219-S224.

Raja, B. R., & Arunachalam, K. D. (2011). Market potential for probiotic nutritional supplements in India. *African Journal of Business Management*, *5*(14), 5418.

Rajam, R., Karthik, P., Parthasarathi, S., Joseph, G. S., & Anandharamakrishnan, C. (2012). Effect of whey protein–alginate wall systems on survival of microencapsulated *Lactobacillus plantarum* in simulated gastrointestinal conditions. *Journal of Functional Foods, 4*(4), 891-898.

Rasche, U., Eppendorf, A. G., & Center, B. (2019). *Bioreactors and Fermentors-Powerful Tools for Resolving Cultivation Bottlenecks*. Technical report, Eppendorf.

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.

Rezvani, F., Ardestani, F., & Najafpour, G. (2017). Growth kinetic models of five species of Lactobacilli and lactose consumption in batch submerged culture. *Brazilian Journal of Microbiology*, *48*(2), 251-258.

Roberfroid, M. B. (1998). Prebiotics and synbiotics: concepts and nutritional properties. *British Journal of Nutrition, 80*(S2), S197-S202.

Rokka, S., & Rantamäki, P. (2010). Protecting probiotic bacteria by microencapsulation: challenges for industrial applications. *European Food Research and Technology, 231*(1), 1-12.

Saccaro, D. M., Hirota, C. Y., Tamime, A. Y., & de Oliveira, N. (2011). Evaluation of different selective media for enumeration of probiotic micro-organisms in combination with yogurt starter cultures in fermented milk. *African Journal of Microbiology Research*, *5*(23), 3901-3906.

Sahadeva, R. P. K., Leong, S. F., Chua, K. H., Tan, C. H., Chan, H. Y., Tong, E. V., & Chan, H. K. (2011). Survival of commercial probiotic strains to pH and bile. *International Food Research Journal, 18*(4).

Şahan, T., Ceylan, H., Şahiner, N., & Aktaş, N. (2010). Optimization of removal conditions of copper ions from aqueous solutions by Trametes versicolor. *Bioresource Technology*, *101*(12), 4520-4526.

Salvetti, E., Torriani, S., & Felis, G. E. (2012). The genus Lactobacillus: a taxonomic update. *Probiotics and Antimicrobial Proteins*, *4*(4), 217-226.

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.

Sarmidi, M. R., El Enshasy, H.A. (2012). Biotechnology for Wellness Industry: Concepts and Biofactories. *International Journal of Biotechnology for Wellness Industries*, *1,* 3-28.

Schär-Zammaretti, P., Dillmann, M. L., D'Amico, N., Affolter, M., & Ubbink, J. (2005). Influence of fermentation medium composition on physicochemical surface properties of *Lactobacillus acidophilus*. *Applied and Environmental Microbiology*, *71*(12), 8165-8173.

Schiraldi, C., Adduci, V., Valli, V., Maresca, C., Giuliano, M., Lamberti, M., & De Rosa, M. (2003). High cell density cultivation of probiotics and lactic acid production. *Biotechnology and bioengineering*, *82*(2), 213-222.

Sengupta, R., Altermann, E., Anderson, R. C., McNabb, W. C., Moughan, P. J., & Roy, N. C. (2013). The role of cell surface architecture of lactobacilli in host-microbe interactions in the gastrointestinal tract. *Mediators of Inflammation, 2013*.

Senz, M., van Lengerich, B., Bader, J. and Stahl, U., (2015). Control of cell morphology of probiotic *Lactobacillus acidophilus* for enhanced cell stability during industrial processing. *International Journal of Food Microbiology, 192*, 34-42.

Shakiba, N., & Zandstra, P. W. (2017). Engineering cell fitness: lessons for regenerative medicine. *Current Opinion in Biotechnology*, *47*, 7-15.

Sharma, K., Sharma, N., & Sharma, R. (2018). Study on optimization of exopolysaccharides from a potentiel *Lactobacillus casei* KL14 KX774469. *International Journal of Current Microbiology and Applied Sciences*, *7*(2), 3410-3418.

Shen, Q., & Quek, S. Y. (2014). Microencapsulation of astaxanthin with blends of milk protein and fiber by spray drying. *Journal of Food Engineering, 123*, 165-171.

Shinde, T., Sun-Waterhouse, D., & Brooks, J. (2014). Co-extrusion encapsulation of probiotic lactobacillus acidophilus alone or together with apple skin polyphenols: an aqueous and value-added delivery system using alginate. *Food and bioprocess technology*, *7*(6), 1581-1596.

Silva, J., Carvalho, A. S., Ferreira, R., Vitorino, R., Amado, F., Domingues, P., & Gibbs, P. A. (2005). Effect of the pH of growth on the survival of *Lactobacillus delbrueckii* subsp. bulgaricus to stress conditions during spray-drying. *Journal of Applied Microbiology, 98*(3), 775-782.

Silva, J., Carvalho, A. S., Pereira, H., Teixeira, P., & Gibbs, P. A. (2004). Induction of stress tolerance in *Lactobacillus delbrueckii* ssp. bulgaricus by the addition of sucrose to the growth medium. *Journal of Dairy Research, 71*(1), 121-125.

Silva, J., Carvalho, A. S., Teixeira, P., & Gibbs, P. A. (2002). Bacteriocin production by spray-dried lactic acid bacteria. *Letters in Applied Microbiology*, *34*(2), 77-81.

Sohail, A., Turner, M. S., Coombes, A., Bostrom, T., & Bhandari, B. (2011). Survivability of probiotics encapsulated in alginate gel microbeads using a novel impinging aerosols method. *International Journal of Food Microbiology*, *145*(1), 162-168.

Soliman, A. H. S., Sharoba, A. M., Bahlol, H. E. M., Soliman A. S., & Radi O. M. M. (2015). Evaluation of *Lactobacillus acidophilus, Lactobacillus casei* and *Lactobacillus plantarum* for probiotic characteristics. *Middle East Journal of Applied Scieces, 5*(1), 94-101.

Srinivas, D., Mital, B. K., & Garg, S. K. (1990). Utilization of sugars by *Lactobacillus acidophilus* strains. *International Journal of Food Microbiology*, *10*(1), 51-57.

Srinu, B., Rao, T. M., Reddy, P. M., & Reddy, K. K. (2013). Evaluation of different lactic acid bacterial strains for probiotic characteristics. *Veterinary World*, *6*(10), 785.

Stanbury, P. F., Whitaker, A., & Hall, S. J. (2013). *Principles of fermentation technology*. Elsevier. 13-16.

Sun, Z. J., Dan, T., Zhang, W and Zhang, H. (2014). Phylogensis and Evolution of Lactic Acid Bacteria. *Lactic Acid Bacteria.* Springer Berlin Heidelberg.

Sun-Waterhouse, D., Wadhwa, S. S., & Waterhouse, G. I. (2013). Spray-drying microencapsulation of polyphenol bioactives: a comparative study using different natural fibre polymers as encapsulants. *Food and Bioprocess Technology, 6*(9), 2376-2388.

Talwalkar, A. & Kailasapathy, K. (2004). The role of oxygen in the viability of probiotic bacteria with reference to *L. acidophilus* and *Bifidobacterium spp. Current Issues in Intestinal Microbiology, 5*(1), 1-8.

Talwalkar, A., Kailasapathy, K., Peiris, P. and Arumugaswamy, R. (2001). Application of RBGR-a simple way for screening of oxygen tolerance in probiotic bacteria. *International Journal of Food Microbiology, 71*, 245-248.

Tambekar, D. H., & Bhutada, S. A. (2010) Studies on antimicrobial activity and characteristics of bacteriocins produced by *Lactobacillus* strains isolated from milk of domestic animals. *International of Food Microbiology, 8*(2).

Tamime, A. Y., Skriver, A., & Nilsson, L. E. (2006). Starter cultures. *Fermented milks*, 11-52.

Tan, S., Kha, T., Parks, S., Stathopoulos, C., & Roach, P. (2015). Optimising the encapsulation of an aqueous bitter melon extract by spray-drying. *Foods, 4*(3), 400-419.

Tang, Y. J., Zhang, W., and Zhong, J. J. (2009). Performance analyses of a pH-shift and DOT-shift integrated fed-batch fermentation process for the production of ganoderic acid and *Ganoderma* polysaccharides by medicinal mushroom *Ganoderma lucidum*. *Bioresource Technology*, 100, 1852-1859.

Taskila, S., & Ojamo, H. (2013). *The current status and future expectations in industrial production of lactic acid by lactic acid bacteria*. INTECH Open Access Publisher.

Tham, C. S. C., Peh, K. K., Bhat, R., & Liong, M. T. (2012). Probiotic properties of bifidobacteria and lactobacilli isolated from local dairy products. *Annals of Microbiology*, *62*(3), 1079-1087.

Thursby, E., & Juge, N. (2017). Introduction to the human gut microbiota. *Biochemical Journal, 474*(11), 1823-1836.

Todhanakasem, T. (2017). Use of rice hull hydrolyzate in the cultivation of *lactobacillus acidophilus*. *Asia-Pacific Journal of Science and Technology, 17*(5), 778-786.

Tomás, M. S. J., Bru, E., Wiese, B., & Nader-Macías, M. E. F. (2010). Optimization of low-cost culture media for the production of biomass and bacteriocin by a Urogenital Lactobacillus salivarius strain. *Probiotics and Antimicrobial Proteins, 2*(1), 2-11.

Trent, N. (2017). Global Probiotics Sales and Consumption 2017 Market Research Report. Retrieved from http://www.crossroadstoday.com/story/34531010/global-probiotics-sales-and-consumption-2017-market-research-report.

Van De Guchte, M., Serror, P., Chervaux, C., Smokvina, T., Ehrlich, S. D., & Maguin, E. (2002). Stress responses in lactic acid bacteria. *Antonie Van Leeuwenhoek, 82*(1-4), 187-216.

Vaughan E. E, Mollet B., Devos W. M. (1999). Functionality of probiotics and intestinal lactobacilli: light in the intestinal tract tunnel. *Current Opinion in Biotechnology*, *10,* 505–510. doi:10.1016/S0958-1669(99)00018-X.

Venkateswarulu, M. T. (2017). Optimization Of Medium Components And Process Parameters For Enhanced Production Of Lactase By A Bacterium Isolated From Dairy Effluent. Doctor of Philosophy Thesis, Vignan's University, Andhra Pradesh.

Vijayakumar, J., Aravindan, R., & Viruthagiri, T. (2008). Recent trends in the production, purification and application of lactic acid. *Chemical and biochemical engineering quarterly, 22*(2), 245-264.

Vollmer, W., Blanot, D., & De Pedro, M. A. (2008). Peptidoglycan structure and architecture. *FEMS microbiology reviews, 32*(2), 149-167.

Waśko, A., Kordowska-Wiater, M., Podleśny, M., Polak-Berecka, M., Targoński, Z., & Kubik-Komar, A. (2010). The plackett-burman design in optimization of media components for biomass production of *Lactobacillus rhamnosus* OXY. *Acta Biologica Hungarica*, *61*(3), 344-355.

Wisniewski, R. (2015, July). Spray drying technology review. In *45th International Conference on Environmental Systems*.

Wood, L. (2015). Probiotic Products Market report 2015-2016 - A Global Market Overview of Probiotic Foods & Beverages, Probiotic Supplements and Probiotic Animal Feed. Retrived from https://globenewswire.com/news-release/2015/12/02/792213/0/en/Probiotic-Products-Market-report-2015-2016-A-Global-Market-Overview-of-Probiotic-Foods-Beverages-Probiotic-Supplements-and-Probiotic-Animal-Feed.html.

Xiaobo, Z., Linyu, H., Yongcheng, L., & Zhongtao, L. (2006). Medium optimization of carbon and nitrogen sources for the production of eucalyptene A and xyloketal A from *Xylaria* sp. 2508 using response surface methodology. *Process Biochemistry*, *41*(2), 293-298.

Yamamoto, Y., Gaudu, P., & Gruss, A. (2011). Oxidative stress and oxygen metabolism in lactic acid bacteria. *Lactic Acid Bacteria and Bifidobacteria: Current Progress in Advanced Research*, 91-102.

Yari, M., Fooladi, J., & Motlagh, M. A. K. (2015). Microencapsulation and Fermentation of *Lactobacillus acidophilus* LA-5 and Bifidobacterium BB-12. *Applied Food Biotechnology, 2*(4), 27-32.

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.

Zacharof, M. P., & Lovitt, R. W. (2013). Partially chemically defined liquid medium development for intensive propagation of industrial fermentation lactobacilli strains. *Annals of microbiology, 63*(4), 1235-1245.

Zacharof, M. P., & Lovitt, R. W. (2014). Economic liquid growth medium development for high-rate production of cellular biomass and lactic acid of *Lactococcus lactis*. *Industrial, medical and environmental applications of microorganisms, 419*.

Zayed, G., & Winter, J. (1996). Batch and continuous production of lactic acid from salt whey using free and immobilized cultures of lactobacilli. *Applied microbiology and biotechnology*, *44*(3-4), 362-366.

Zhang, B., Shu, G., Bao, C., Cao, J., & Tan, Y. (2017). Optimization of Culture Medium for *Lactobacillus bulgaricus* using Box-Behnken Design. *Acta Universitatis Cibiniensis. Series E: Food Technology*, *21*(1), 3-10.

Zhang, Y. J., Li, S., Gan, R. Y., Zhou, T., Xu, D. P., & Li, H. B. (2015). Impacts of gut bacteria on human health and diseases. *International Journal of Molecular Sciences*, 16(4), 7493-7519.

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.

Zhu, H., Cao, C., Zhang, S., Zhang, Y., and Zou, W. (2011). pH-control modes in a 5-L stirred-tank bioreactor for cell biomass and exopolysaccharide production by *Tremella fuciformis* spore. *Bioresource Technology*. 102: 9175-9178.

Zion Market Research. (2018). Global Probiotic Market is Set for Rapid Growth and is Expected to Reach Value Around USD 65.87 Billion by 2024. Retrieved from https://www.zionmarketresearch.com/news/probiotics-market.

Zuidam, N.J. & Shimoni, E. (2009). Overview of microencapsulates for use in food products or processes and methods to take them. In *Encapsulation Technologies for Active Food Ingredients and Food Processing* (3–29). Springer, New York, USA.

LIST OF PUBLICATIONS

Journal with Impact Factor

1. **Kepli, A. N**., Dailin, D. J., Malek, R. A., Elsayed, E. A., Leng, O. M., & El-Enshasy, H. A. (2019). Medium Optimization using Response Surface Methodology for High Cell Mass Production of *Lactobacillus acidophilus*. *Journal of Scientific & Industrial Research*, Vol.78(09). http://nopr.niscair.res.in/handle/123456789/50485. **(Q4, IF: 0.735)**

Non-index Journal

1. **Kepli, A. N.,** El Enshasy, H. A., El Marzugi, N. A., Elsayed, E. A., Ling, O. M., Malek, R. A., & Ramli, S. (2018). Medical and cosmetic applications of fungal nanotechnology: production, characterization, and bioactivity. In *Fungal nanobionics: Principles and applications*, 21-59. https://doi.org/10.1007/978-981-10-8666-3_2.

Non-indexed Conference Proceedings

1. **Kepli, A. N** & Enshasy, H. A. (2018). Effect of High Cell Mass Production of *Lactobacillus acidophilus* By Different Carbon and Nitrogen sources Using One factor-At-A-time (OFAT) Method. In *7th International Graduate Conference of Engineering. Science and Humanities (IGCESH)* (pp 595-597). https://sps.utm.my/igcesh2018/files/2018/08/IGCESH-Proceedings-2018.pdf.