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Faculty of Graduate Studies **Department of Agricultural Sciences**

Layer-by-layer coating of single cell *Lactobacillus rhamnosus* GG to increases viability under simulated gastrointestinal conditions.

By

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This Thesis was defended successfully on 23/12/2020 and approved by:

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Dedication

Every challenging work, won't be achieved without hard work, systemized strategy and good thinking. These cannot be reached without guidance, support and love.

To my parents, my first teachers, who picked me up and taught me to trust Allah, and believe in hard work and wise steps.

To my sisters and brothers, I am really grateful to all of you. Indeed, thanks for your patience.

To Hammam, who didn't hesitate to encourage me to go on every adventure, especially this one. And tolerate with love all consequences of taking this step.

To a person who always used to stand up with me, and will not read it. For you Asala.

To Aseel, my real 'listener' and supportive friend.

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Last but not least, my precious family and sincere friends who stand beside me and support me with their love and confidence all the time.

الإقرار

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل عنوان:

Layer-by-layer coating of single cell *Lactobacillus rhamnosus* GG to increases viability under simulated gastrointestinal conditions.

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة اليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم من قبل لنيل أي درجة علمية أو بحث علمي أو بحثي لأي مؤسسة علمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالبة:**

Signature: **التوقيع:**

Date: **التاريخ:**

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List of Abbreviations

Abbrev.	
GIT	Gastrointestinal tract
NsDSC	Nigella sativa Defatted Seeds Cake
BSP	Black seeds protein
PC	Protein concentrate
ALG	Alginate
SGF	Simulated gastric fluids
SIF	Simulated intestinal fluids
ALD	Alcoholic liver disease
BCA	Bicinchoninic acid
WR	Working reagent
IBD	Inflammatory bowel disease
EPS	Exopolysaccharides
LbL	Layer-by-layer
IBS	Irritable bowel syndrome
NASH	Non-alcoholic steatohepatitis
NEC	Necrotizing enterocolitis

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Abstract

This study was conducted to evaluate the viability of *Lactobacillus rhamnosus* GG microencapsulated by layer-by-layer (LbL) technique with black seed protein (BSP) extracted from *Nigella sativa* defatted seeds cakes (NsDSC), as a coating material, with alginate, inulin or glucomannan, separately, and the final coating layers number was three. The viable cell counts of the plain and coated *L. rhamnosus* GG were determined under sequential simulated gastric fluid (SGF) for 120 min and simulated intestinal fluid (SIF) for 180 min. Additionally, the viability after exposure to 37, 45 and 55˚C for 30 min was also determined. Generally, the survivability of coated *L. rhamnosus* GG showed significant ($p<0.05$) improvement (<4, 3 and 1.5 logs reduction) comparing to plain cells (~6.7 log cycle reduction) under sequential exposure to SGF and SIF. Moreover, the coated cells were superior over plain cells under treatment with high temperatures. The study has shown that, BSP and inulin have provided the best protection of *L. rhamnosus* GG against either in simulated GI conditions or under heating temperatures. In conclusion, the LbL technique showed a significant protection of probiotic cells, making it a promising method to maintain high viable count of probiotics through host's GI.

Chapter One
Introduction

1.1. Probiotics:

In 2001, the World Health Organization defined probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host". In 2014, the definition was reworded, to be more grammatically correct, as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." (Hill et al., 2014)

The term probiotics includes strains belonging to several genera of bacteria and yeasts, such as *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Enterococcus*, *Escherichia*, *Bacillus*, and *Saccharomyces*. These microorganisms are naturally found in fermented food like yogurt and dairy products, pickles, sauerkraut, kefir, Kombucha...etc. Besides, they are available in the market in form of probiotic foods or as supplements.

Specifically, *lactobacillus rhamnosus* GG was originally isolated from healthy human gut. It was identified as a potential probiotic strain because of its resistance to acid and bile salts, good growth characteristics (Segers et al., 2014) and its high capacity of adhesion to mucosal surfaces, thanks to its superficial exopolysaccharides (EPS) and pili. The adhesion to mucosal surface is facilitated by the adhesion protein LR GG-0186. Moreover, *L. rhamnosus* GG normalize permeability of intestine, also, its ability to express a long galactose-rich EPS which may play a role in

adhesiveness. More positive effects of *L. rhamnosus* GG and its products were reported on the progression of diseases including GI infections and diarrhoea, Irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), liver diseases such as Alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), necrotizing enterocolitis (NEC), respiratory tract infections, cancer and cystic fibrosis (Capurso, 2019).

1.2. Probiotics market:

The interest of probiotics and its introduction to foods is massively increased during the past few years owing to reported evidence about probiotics positive effect on the human health. Probiotics promote health status and play an essential role against colonization of pathogenic microbes in intestine by production of antimicrobial compounds, increase gut integrity by stimulating mucus production, improve enzymes formation, regulate the composition of gastrointestinal (GI) microbiota, and act as immunity modulators. Nowadays, the investigations about the importance of GI microbiota are widely and intensively increased. It was reported that probiotics and GI microbiota composition demonstrate a major role in the progression of many diseases and disorders such as obesity, allergies, diabetes, inflammations, inflammatory bowel diseases (IBD), cancer, infectious diseases, and even neurodegenerative diseases (Kechagia et al., 2013).

Therefore, the food industry had never missed the chance to increase the profits by producing novel probiotics. From the economic point of view, the global probiotics market expected to reach USD 77.09 billion by 2025 (Grand View Research, 2019). Moreover, economic analysts have expected an increase in the demand for functional food especially during the COVID-19 pandemic. This increase is owing to consumer's awareness about the benefits of probiotics and its effect on health and, particularly, immunity boosting, and their tendency towards healthier food in general. On the other hand, analysts have expected a depression in the consumption of meats, poultry, and seafood while the demand for plant and animal protein supplements will increase (Grand View Research, 2019). This may affect the distribution of food items that used to be probioticated, or even under ongoing researches.

To achieve a better usefulness, we need to be sure that the probioticated products have taken into account the differences in needs amongst all population categories, and the consumption of that product in specific market. For example, (1) if we probioticate bread, we should consider celiac disease population and gluten-free dieters, (2) milk products probiotication should take in account lactose intolerant patients, so we think about probiotication of alternatives suitable for this category such as almond or soy milk, (3) take in consideration the coating agents in case of vegan consumers. Also, as mentioned before, the expectations are going toward increasing the demand for plant and animal proteins excluding the meat, poultry and seafood, so the probiotication of food should take into

account these economic expectations during the coming 5 years (Grand View Research, 2019).

To obtain the maximum health benefits claimed about probiotics, the number of viable probiotic cells should not be lower than 10^6-10^7 cfu/ ml or gm ingested according to Food and Drug Administration- World Health Organization (Thomas et al., 2014). Unfortunately, the numbers of probiotics ingested affected by numerous physiological factors, including (1) chemical: low pH, gastrointestinal conditions, food matrix properties, processing and storage conditions, and (2) physical factors that affected the adhesion and colonization of probiotics in intestines like rapid transit time (Anselmo et al., 2016).

To overcome these problems, the scientists thought about several strategies to improve probiotics viability, including nanoparticles, polymer gels and microencapsulation to protect the sensitive probiotic cells against harsh conditions in gastrointestinal tract and during storage, and to improve the adhesion to mucosal lining.

1.3. Layer-by-Layer microencapsulation:

Microencapsulation refers to physicochemical process to entrap an active compound or cell in a material in order to improve its functionality. In 1955, the chemist Barrett K. Green received a patent for the process of microencapsulation (Nguon et al., 2018), at first it was for typing paper then widened to include the pharmaceuticals and the food industries.

In addition, scientists have developed techniques to perform microencapsulation and obtain a proper form of final product with higher viability and stability. These techniques, including extrusion, emulsion, spray drying, spray freezing, spray-freeze drying and layer-by-layer were investigated (Călinoiu et al., 2019).

Layer-by-layer as a technique was raised up few decades ago and proposed by the German chemist Gero Decher in 1991 (Matsusaki et al., 2012). At first, the applications of this technique have been limited on increasing the effectiveness of drugs and pharmaceuticals, then it was outspreaded until it linked to food processing by encapsulation of some types of food pigments and single cell probiotics (Ram et al., 2001; Priya et al., 2011; Marais et al., 2014; and Jung et al., 2015).

Layer-by-Layer technique described by electrostatic attractions and ionic interactions between the layers used in coating. This approach based mainly on alternately exposure of the probiotic cell to negative and positive charged substrates. In order to remove the excess of the coating materials, buffer solution is used as a washing solution after each coating step. In the end, the encapsulated probiotics might be dried, depending on the application (Priya et al., 2011).

The main negative-charged molecules used in the coating of probiotics were alginate and pectin polysaccharides. While the studies on the positive-charged layer were covering (1) polysaccharides as chitosan and, (2) proteins as milk proteins or, as in the current study, protein concentrate

extracted from *Nigella sativa* defatted cake (NsDSC). Moreover, the compounds used in microencapsulation must be food grade, for example: biodegradable polysaccharides, and able to tolerate harsh conditions such as acidic pH, bile salts, digestive enzymes and storage conditions, including temperature.

This technique steps ahead of other microencapsulation techniques, it fulfils the maximum saving of coating materials and minimizes the wasting occur in the bead-form microcapsule. It also allows delivering of probiotics cells, growth, and colonisation in GIT with no need to releasing apart from coating material (Anselmo et al., 2016).

Generally, all studies previously published approved the positive effect of microencapsulation on protecting of probiotics bacteria and yeast. Particularly, LbL approach also enhance the viability of probiotics under harsh conditions as detailed in Table (1).

Table 1: Effect of layer-by-layer of probiotics on its viability under harsh conditions

Author (year)	+ve layer	-ve layer	Probiotic	Results		
				Gastrointestinal	Adhesion	Heat treatment
Xiao et al., (2020)	Whey protein isolates (WPI)	Xanthan Gum	*L. bulgaricus* *L. paracasei*	-Mortality of the cells decreased sig. -Survivability further increased when the micro-capsules coated with xanthan gum. -Coated L. paracasie showed higher resistance than L. bulgaricus		-Free cells: viable count decreased 1, 4 and 6 log cycle at 55, 65 and 75°C for 10 min, respectively. -Coated: less than 0.5, 1 and 3 log cfu/ml reduction at same temperatures, respectively.
Wang et al., (2019)	Chitosan	Sodium phytate	*L. pentosus*	-Plain: 7.4 and 6.09 log cfu/ml reduction in SGF for 2h, and bile salts for 3h, respectively. - Coated: 4.34 and 2.33 log cfu/ml reduction under the same conditions, respectively.		At 45°C, 1 and 0.5 log cfu/ml reduction were provided for plain and coated, respectively. At 65°C, no growth of plain cells while 3.2 log cfu/ml of coated cells were viable.

Author (year)	**+ve layer**	**-ve layer**	**Probiotic**	**Results**		
				Gastrointestinal	Adhesion	Heat treatment
Anselmo et al. (2016)	Chitosan	Alginate	*Bacillus coagulans*	(CHI/ALG)1 showed protection against both bile salts and gastric fluids (4 log reduction) (CHI/ALG)2→ 1log reduction	(CHI/ALG)2 BC exhibited nearly 1.5-fold higher adherence to mucosal surface of porcine intestines compared to plain-BC	-
Thomas et al. (2014)	Chitosan	Dextran sulfate	*S. boulardii*	-Almost 2 log and 0.5 log cycles reduction after 2h SGF for plain and coated LP respectively. -Coated cells showed a reduction in viability under bile salts but not as drastic as uncoated cells.		
Priya et al., (2011)	Chitosan	Carboxymethyl cellulose (CMC)	*L. acidophilus*	Survival of 1 and 6.3 log cfu/500mg of plain and coated LA, respectively, after sequential exposure to 2h of SGF, 2h bile salts.		79% and 93% survival of plain and coated cells, respectively, after freeze drying.

1.4. *Nigella sativa:* Global market:

Black seeds oil market (*Nigella sativa*) size was over USD 15 million in 2018 and the industry expects significant increase by 2025 to reach USD 25 Million. This gain is owing to black seed oil health benefits and non-toxic nature and an increased demand on pharmaceuticals containing natural ingredients (Ahuja and Singh, 2019). The wastes by-product from black seed oil extraction will increase subsequently. Generally, NsDSC often discarded, and reusage of these leftovers is very limited, indeed, as far as we know, the reusage is not found at all in Palestine other than for experimental and research purposes. These leftovers contain fiber, proteins, fat residues, carbohydrates and are rich in vitamins and minerals (Kour and Gani, 2020; Thilakarathne et al., 2018).

1.5. Viability of commercial probiotics under simulated GI conditions:

As mentioned earlier, the viability of probiotics cells affected by several harsh conditions including gastric and intestinal juices, and storage conditions in general. Interestingly, Millette et al. (2013) compared the resistance of different commercial probiotics products against SGF for 120 min and SIF for 180 min. They reported low resistance or no survival of most of probiotics products in form of powder and capsules without enteric coats. While probiotics capsules covered with an enteric coating had a higher survival rate than uncoated. However, 1/3 of probiotics of fermented milk showed a good resistance (<1 log reduction), while the others showed no resistance. This demonstrates the importance of coatings and proper

vehicle for the probiotics to reach the colon with the beneficial number to confer health benefits (Millete et al., 2013; Garcia-Geja et al., 2015; and Nami et al., 2020).

The aim of this study was to encapsulate probiotic single cell, *lactobacillus rhamnosus* GG, using protein extracted from NsDSC and different biodegradable polysaccharides (inulin, alginate or glucomannan) applying layer-by-layer approach. In addition, to determine the coatings effect on viability of probiotic cells under gastrointestinal conditions and different heating temperatures.

Chapter Two
Materials and Methods

2.1. Materials and Equipment:

L. rhamnosus GG powder was purchased from Dicofarm (Italy). *Nigella sativa* defatted cake was purchased from Al-Hethnawi company for oil extraction (Jenin, Palestine). Polysaccharides (inulin, alginate and glucomannan) kindly donated by M. Altamimi from An-Najah National University. The enzyme mTrans-Glutaminase (TGase) was kindly donated by M. Sabbah from An-Najah National University. MRS agar, MRS broth and 2.5L AnaeroJar assembly purchased from OXOID company (Basingstoke, UK). Bile salts and bromelain enzyme from pineapple stem brought from SIGMA (Dorset, UK). Peptone water from (DIFCO). Memmert CO2 incubator, refrigerated centrifuge (ALC PK120R), Memmert shaking water bath, thermo mix (ONILAB HM100-Pro), drying oven (Raypa), microcentrifuge (Denever Instrument), pH meter 3310 (JENWAY), and autoclave (Hydra).

2.2. Bacterial strain and growth conditions:

Lactobacillus rhamnosus GG, was (1 pack powder) reconsitiuted in 10 ml of De Man, Rogosa, and Sharpe (MRS) broth to be activated. The cultures were transferred twice to ensure purity then inoculated in 500 ml of MRS broth. The cultures were incubated at 37˚C for 48 h without agitation in a memmert CO_2 incubator.

Bacterial cells were harvested by centrifugation (ALC PK120R) at 4000 rpm for 20 min at 4 ^{o} C. The cell pellets were washed twice with sterile peptone water then twice with 0.15M NaCl. The counting of *L. rhamnosus* GG was performed using standard plate counting method using MRS agar under anaerobic conditions.

2.3. Extraction of protein from Nigella sativa defatted cake (NsDSC):

Protein concentrate was extracted from *Nigella sativa* defatted cake by acid base extraction method as described by Sabbah et al. (2020) with slight modifications. Dried NsDSC was ground and dispersed in DW (1:20, w/v), pH adjusted up to 12.0 with 1M NaOH and stirred at room temperature for 2 hours at constant speed. The suspension was centrifuges (4500 rpm, 20 min) and the supernatant was collected then pH was adjusted to 6.0 with 1M HCl to form precipitate. The precipitate was collected by centrifugation (4000 rpm, 20 min) and uniformly distributed on an aluminium foil sheet for drying at 30 ^{o} C and 20% relative humidity. The obtained protein concentrate (PC) was ground to fine particles.

To obtain a high content of soluble protein, the protein concentrate (PC) was proteolyzed using bromelain, a proteolytic enzyme extracted from pineapple stem. As follows: 20g of PC were suspended with 100 ml DW and pH was adjusted to 7.0, then 2.5g of bromelain were added. The suspension was placed in a shaking water bath at 37 ^{o} C for 4h, then was boiled for 2 min to stop the enzymatic activity. The precipitate was separated by centrifugation (4000, 20 min) and discarded; the supernatant

was freeze- dried. The powder obtained was named black seeds protein (BSP).

2.4. Determination of total protein content using BCA protein assay:

To quantify the total protein content in protein concentrate (PC), BCA protein assay protocol described by He (2019) has been followed. Initially, working reagent solution (WRS) was prepared by mixing 50 parts of BCA (reagent A) with 1 part of BCA (reagent B) (50:1, A:B). Then 100 µl of PC was dissolved in 2.0 ml of WRS in order to get the sample to WRS ratio 1:20. The Eppendorf tube containing the mixture was covered with aluminium foil and incubated at 37°C for 30 min. After that, the tubes were kept at room temperature for 10 min before measuring. Absorbance at 562 nm was measured and compared with BSA standard curve to calculate the protein content and percentage.

2.5. Preparation of coating solutions:

Briefly, 2 g of black seeds protein powder (BSP) was dissolved in 100 ml of distilled water (DW) and stirred overnight for sufficient hydration. Polysaccharide solutions of inulin, alginate and glucomannan, were prepared by dissolving 2mg/ml then sterilising by microfiltration. pH was drop wisely adjusted to 6.5 by adding either 1M NaOH or HCl.

2.6. Layer-by-layer synthesis:

The layer-by-layer encapsulation of *L. rhamnosus* GG was conducted according to a previous study (Wang et al., 2019) with some modifications.

A cell -to -polymer solution ratio of 1:8 was obtained by adding 5 mL concentrated cells into 40 ml of BSP. To induce the gelation, TGase (8 units/g protein), which used for strengthen the BSP layer by catalysing the formation of isopeptide bonds between glutamine and lysine, was added into the mixture (stirring for 30 min). The protein microcapsules were collected by centrifugation (4000 rpm, 15 min). Then, washed twice with peptone water then twice with NaCl (0.15 M) and recollected by centrifugation (4000 rpm, 15 min) after each wash.

Fresh protein microcapsules (2 g) were added into 20 ml alginate solution, mixed and stirred at constant speed for 30 minutes in prewarmed 40°C water bath. Then, microcapsules coated by alginate were collected by centrifugation (4000 rpm, 15 min) and washed twice with sterile peptone water, and NaCl solution (0.15 M) twice.

As shown in Figure (1), the obtained microcapsules were coated by BSP to apply the third layer following the same steps. The same procedures were applied for BSP and replacing alginate with either inulin or glucomannan.

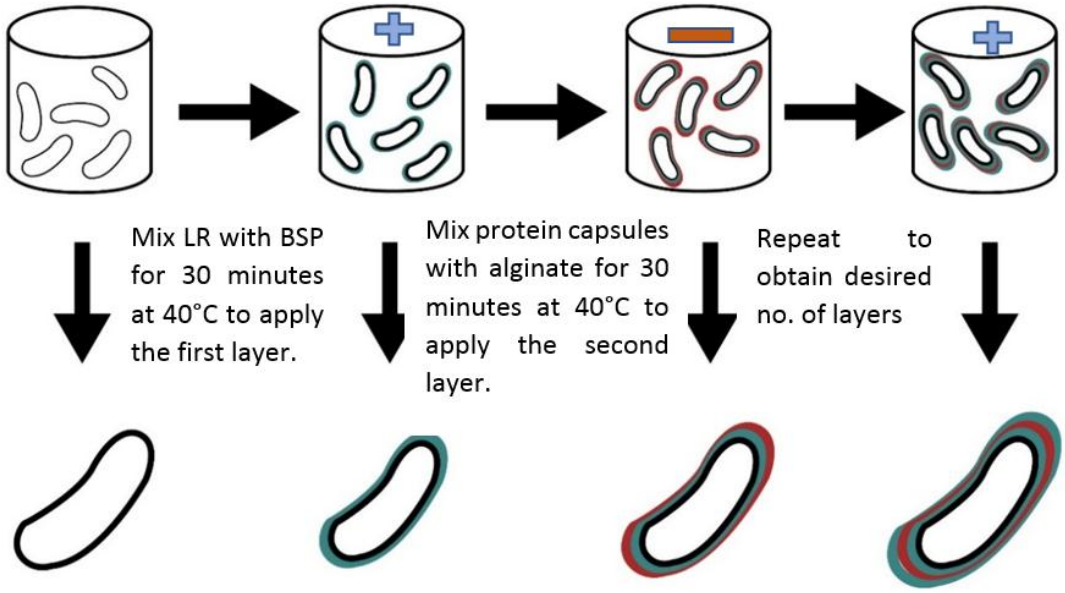

Figure 1: Layer-by-layer microencapsulation**.**

2.7. Response of coated cells to simulated gastric fluid (SGF) and simulated intestinal fluids (SIF):

Survivability of plain and encapsulated *L. rhamnosus* GG were evaluated after sequential exposure to SGF for 2h and 3h bile salts solutions (4%, w/v). One litre of stock simulating gastric fluid solution was prepared, using previously reported method (Wang et al., 2019) with slight modifications. The solution has contained 16.4 ml hydrochloric acid (10%, v/v), sodium chloride (2 g), bromelain (10 g), and 983.6 ml deionized water. The pH was adjusted to 2.8 using 1M HCl and/or NaOH.

Bile salts (4 g) were added into 100 ml deionized water, and pH adjusted to 6.8, using 1M NaOH and/or HCl, to form bile salt solutions (4%, w/v).

For digestion in SGF, plain- *L. rhamnosus* and all microencapsulated cells were mixed (separately) with SGF and placed in a water bath at 37 °C.

Samples were obtained every 30 min up to 120 min. Cells were palleted via centrifugation (4000 rpm, 20 min), resuspended and washed twice in peptone water. The suspension was diluted to an appropriate concentration using the gradient dilution method and then plain- *L. rhamnosus*, and other microencapsulated cells were transferred to MRS agar plates and incubated anaerobically for 48 h at 37 °C.

Sequentially, plain- *L. rhamnosus* and other microencapsulated cells (obtained after gastric simulation) were mixed with 4% bile salt solution and placed in a water bath at 37 °C. Similarly, samples were taken at 30, 60, 120 and 180 min. The cells were collected via centrifugation (4000, 20 min) and washed twice in peptone water as described previously.

The overall survival rate of *L. rhamnosus* GG was calculated after exposure to SGF and SIF by equation of Li et al. (2016):

$$\textit{Survival (\%)} = N^t/N^o * 100\%$$

Where N^t is the cell count at the end of different incubation time, N^o is the viable cell number prior to exposure to SGF and SIF.

2.8. Heat stability:

The effect of layer-by-layer encapsulation on the heat resistance of *L. rhamnosus* was measured using plain- *L. rhamnosus*, (BSP-INU/BSP)- *L. rhamnosus*, (BSP-ALG/BSP)- *L. rhamnosus*, and (BSP-GLU/BSP)- *L. rhamnosus* incubated at 37, 45 or 55 °C for 30 min. The viable number of each sample were calculated using SPC method (MRS agar).

2.9. Experimental Design and Statistical Analysis:

Three samples obtained by coating of *L. rhamnosus* GG with BSP and different types of polysaccharides (inulin, alginate or glucomannan) using LbL technique, were compared to plain *L. rhamnosus*'s behaviour under simulated GI conditions and heat treatments.

The experiment, starting from LbL synthesis to simulation process, was carried out in duplicate. In addition, the sampling for bacterial culturing also was performed in duplicate. All results obtained were analysed using SPSS Statistics 21. Repeated measures, then one-way ANOVA test, with p-value= 0.05 have been conducted.

Chapter Three
Results

3.1 Total protein content:

The percentage of total protein content in the original protein concentrate after enzymatic hydrolysis of NsDSC was 73.4%.

3.2 Survival of plain and coated *L. rhamnosus* GG under simulated gastric fluids and bile salts:

The main purpose of studying the survivability of plain and coated *L. rhamnosus* in SGF and SIF is to simulate the effect of low pH during transition through GI tract. The effect of coating of the *L. rhamnosus* on protection against SGF was tested by studying the differences in viability between plain and coated *L. rhamnosus* GG over varying time periods (30, 60, 120 min). Since SGF treatment is carried out with fresh plain cells, and BSP-based coated cells with inulin, alginate and glucomannan, the initial cell counts of plain and coated cells were 9.5 ±0.5 log cfu/ml and 8.8 ±0.6 log cfu/ml, respectively, with survival rate of 50%, 15% and 0.1%. As shown in Figure (2), The viability of plain *L. rhamnosus* was decreased to 9.2 log cfu/ml, 8.06 log cfu/ml and 7.04 log cfu/ml, over 30, 60 and 120 min of exposure to SGF, respectively. While coating of *L. rhamnosus* GG with BSP and glucomannan showed better results, the viability reduced from initial count 8.2 log cfu/ml to 8.14 log cfu/ml, 8.04 log cfu/ml and 7.2 log cfu/ml with survival rate of 70%, 20% and 10%, respectively over the same time periods. On the other hand, using alginate and inulin as coating

materials with BSP confer a higher protection against low pH. Where BSP/alginate coated cells viability reduced to 9.15 log cfu/ml, 9.06 log cfu/ml and 8.28 log cfu/ml, and the survival rates were 75%, 30% and 14% after 30, 60 and 120 min respectively. Also, higher readings were obtained, 9.16 log cfu/ml, 9.1 log cfu/ml and 9.06 log cfu/ml with % survival of 80%, 50% and 30% over same time period respectively, when *L. rhamnosus* GG cells coated with inulin in polysaccharides layer. Survival rates for plain and coated *L. rhamnosus* GG under SIF are presented in Figure (3).

Since the major constituent of SIF is bile salts, the survivability of plain and coated *L. rhamnosus* GG in bile salts were assessed over varying time period (30, 60, 120, 180 min). As shown in Figure (4), in the presence of bile salts, the viability of plain cells was reduced to 6.28 log cfu/ml, 6.06 log cfu/ml, 4.04 log cfu/ml and 3.2 log cfu/ml after 30, 60, 120 and 180 min respectively, with survival rates of 0.07%, 0.015%, 1×10^{-4} % and 5×10^{-5} %. Over the same periods, the viability of coated cells with BSP and glucomannan was reduced from 7.2 log cfu/ml to 5.2 log cfu/ml, 5.04 log cfu/ml, 4.32 log cfu/ml and 4.04 log cfu/ml respectively, with survival rates of 0.1%, 0.02%, 0.016% and 0.002%. However, the resistance of cells coated with BSP/ ALG or inulin showed higher results under the same conditions. While the BSP and ALG- coated *L. rhamnosus*'s viability reduced to 8.2 log cfu/ml, 8.06 log cfu/ml, 7.2 log cfu/ml and 6.3 log cfu/ml after 30, 60, 120 and 180 min respectively, with survival rates of 10%, 3%, 1% and 0.15%. The viability of cells coated with BSP and inulin decreased from 9.06 log cfu/ml to 9.04 log cfu/ml, 8.36 log cfu/ml, 8.18 log

cfu/ml and 7.2 log cfu/ml, and the survival rates were 20%, 18%, 9% and 1% respectively over the same time period. Survival rates for plain and coated *L. rhamnosus* GG under SIF are presented in Figure (5).

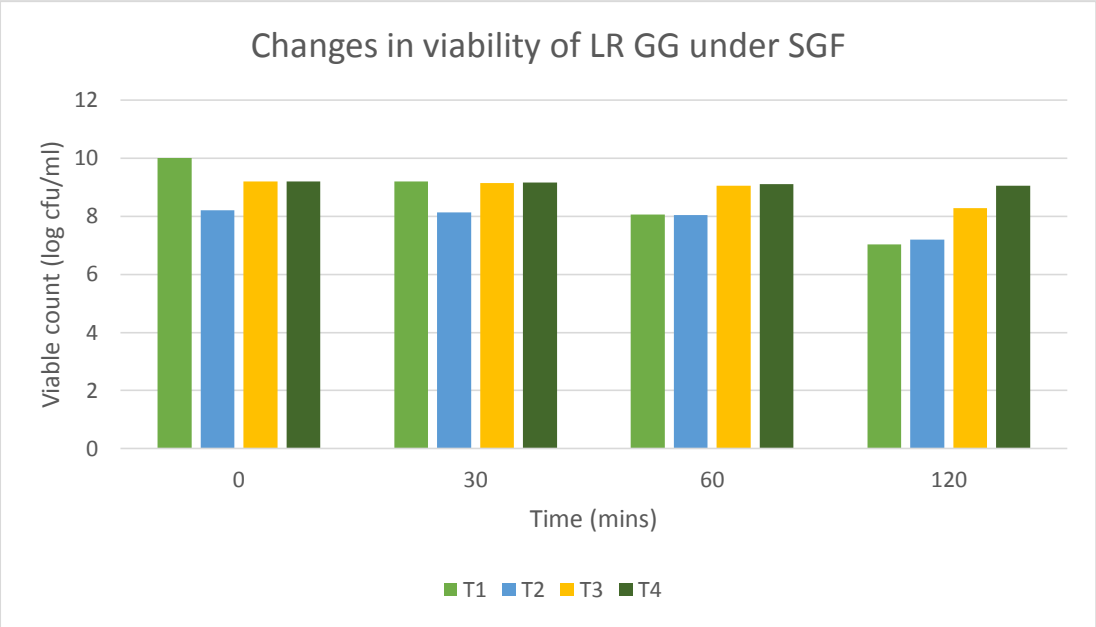

Figure 2: Viable counts changes of plain and coated *L. rhamnosus* GG under SGF, while T1: plain *L. rhamnosus*, T2: BSP/Glucomannan, T3: BSP/Alginate, T4: BSP/Inulin.

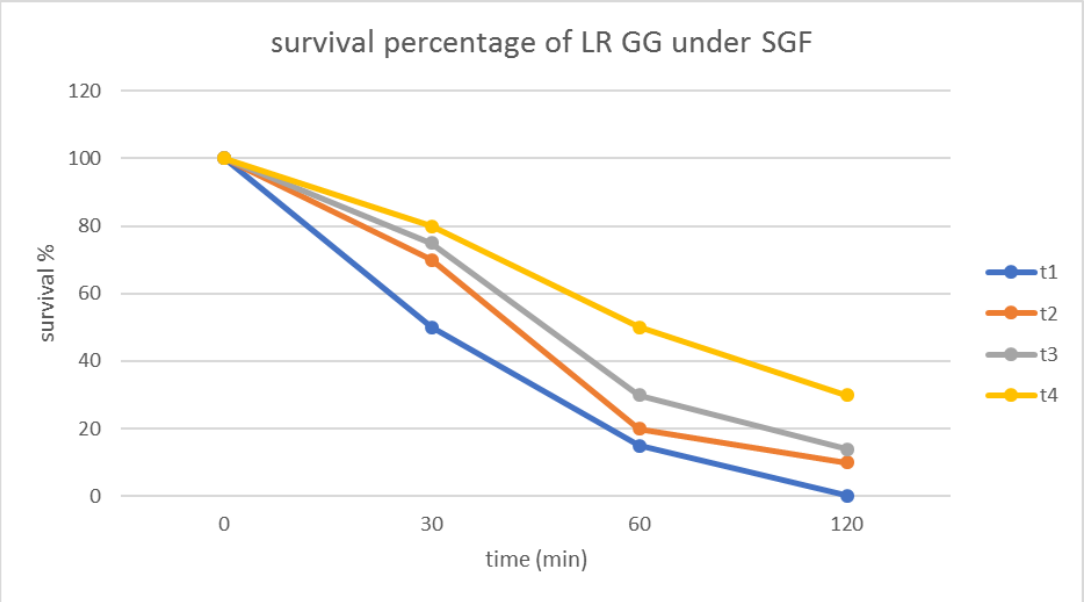

Figure 3: Survival of plain and coated *L. rhamnosus* GG under SGF, while T1: plain *L. rhamnosus*, T2: BSP/Glucomannan, T3: BSP/alginate, T4: BSP/inulin.

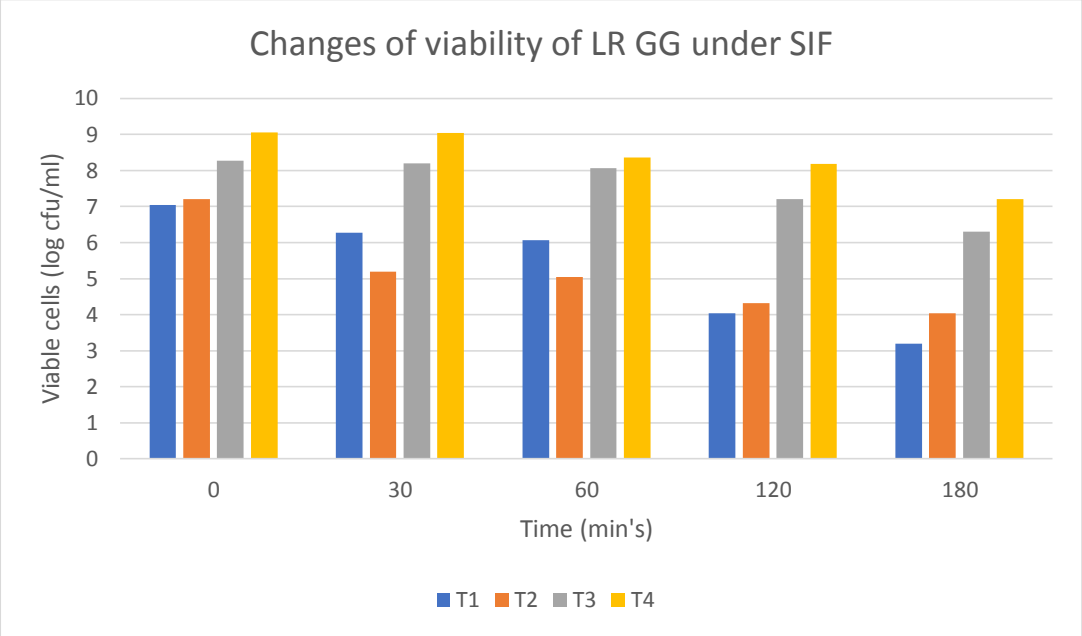

Figure 4: Viable count changes of plain and coated *L. rhamnosus* GG under SIF, while T1: plain *L. rhamnosus*, T2: BSP/Glucomannan, T3: BSP/Alginate, T4: BSP/Inulin.

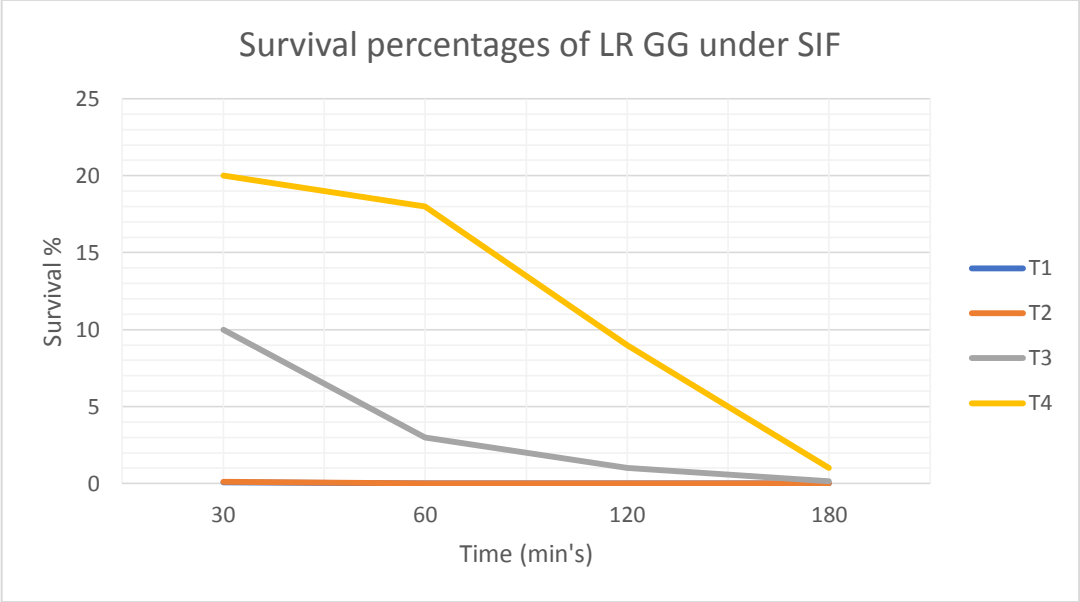

Figure 5: Survival of plain and coated *L. rhamnosus* GG under SIF, while T1: plain *L. rhamnosus*, T2: BSP/Glucomannan, T3: BSP/Alginate, T4: BSP/Inulin.

3.3 Heat stability:

As mentioned previously, the initial count of 1 ml of plain *L. rhamnosus* GG was 9.5 ±0.5 log cfu/ml. As shown in Figure (6), the cell count of coated cells with BSP and different types of polysaccharides (glucomannan, alginate and inulin) were 8.0 log cfu/ml, 9.2 log cfu/ml and 9.2 log cfu/ml, respectively. After exposure of all samples to different heating temperatures 37, 45 and 55°C for 30 min, the viability of both coated and plain *L. rhamnosus* was decreased differently.

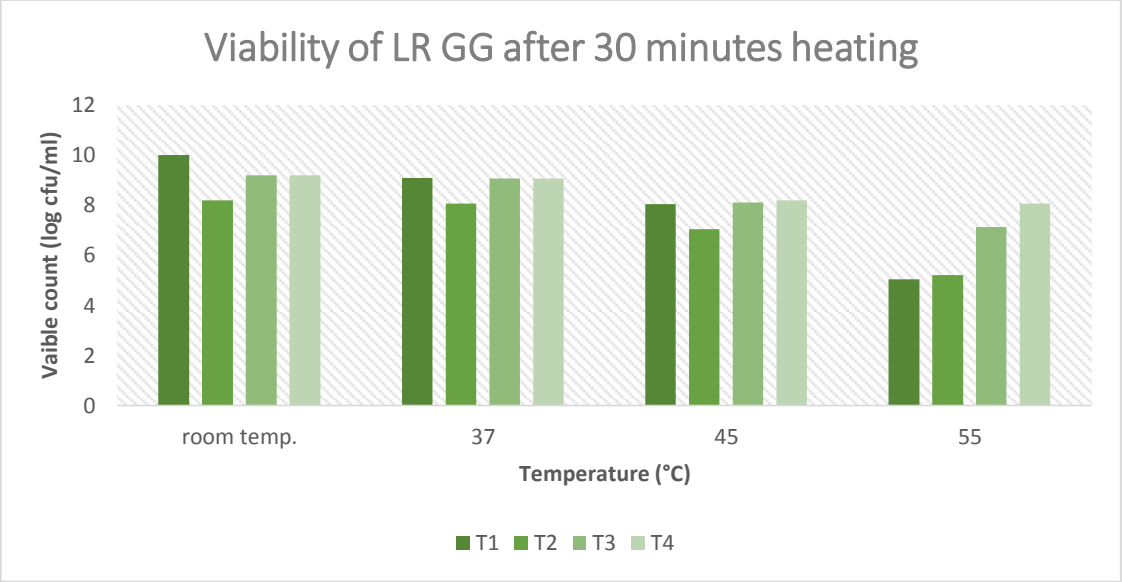

Figure 6: Changes in viability of plain and coated *L. rhamnosus* GG when exposed to different heating temperature for 30 min, while T1: plain *L. rhamnosus*, T2: BSP/Glucomannan, T3: BSP/Alginate, T4: BSP/Inulin.

Generally, LbL coating of cells showed higher survivals compared with plain cells. Particularly, survival (%) of plain *L. rhamnosus* was 25%, 1% and 0.001% at 37, 45 and 55°C respectively. Under the same temperatures, the cells coated with BSP and glucomannan showed 30% survival, 2% and 0.1% at 37, 45 and 55°C, respectively. Moreover, presence of alginate or inulin revealed a higher tolerance against heating temperatures. For BSP and alginate coated *L. rhamnosus*, the survivability reduced to 30%, 5% and 0.6%, while results for BSP and inulin coated *L. rhamnosus* GG were 40%, 10% and 3% at 37, 45 and 55°C respectively.

3.4 Statistical Analysis Results:

The statistical analysis results for sequential exposure to SGF and SIF are represented in Figure (7), which clearly show that there was a huge reduction in viability of plain *L. rhamnosus* when exposed sequentially to

SGF and bile salts. The viability of plain cells was reduced almost by 6 logs cfu/ml along the process, as previously described. While variations in viability reduction were shown depending on the polysaccharide incorporated. There were significant differences ($p<0.05$) between treatments under sequential SGF and SIF comparing to plain cells, in addition to significant differences ($p< 0.05$) were observed between coating treatments.

Moreover, the significant differences obtained by statistical analysis of viable count of plain and coated cells when exposed to different heating temperatures are represented in Table (2). The differences were existing between coating treatments and plain cells, in addition to differences between the coating treatments.

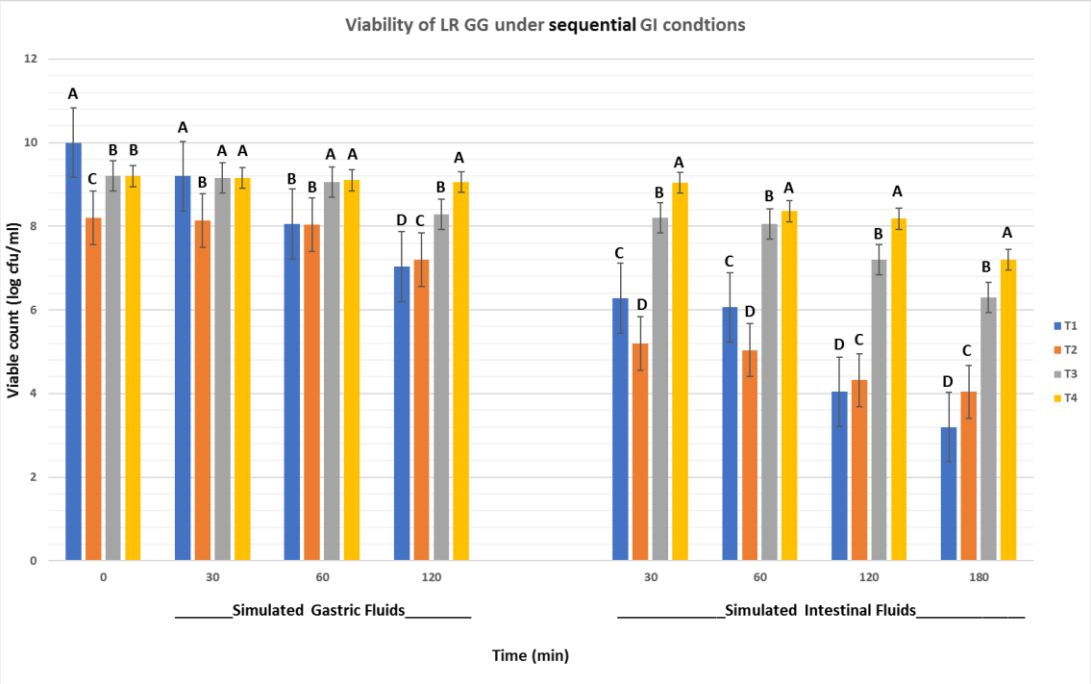

Figure 7: Viability of plain and coated *L. rhamnosus* GG under simulated gastric and intestinal fluids over time periods, while T1: plain *L. rhamnosus*, T2: BSP/Glucomannan, T3: BSP/Alginate, T4: BSP/Inulin. * Columns with the same letter in the same time period are not significantly different ($p >0.05$).

Table 2: Viable count of *L. rhamnosus* GG (mean log cfu/ml) when exposed to different heating temperatures for 30 minutes.

Parameter	Temperature	Plain	Glucomannan	Alginate	Inulin
Viable count under Heat treatment (log cfu/ml)	37°C	9.095[a]	8.06[b]	9.095[a]	9.085[a]
	45°C	8.04[c]	7.035[d]	8.1[b]	8.205[a]
	55°C	5.035[d]	5.3[c]	7.12[b]	8.06[a]

*Values with the same letter in the same row are not significantly different ($p > 0.05$)

Discussion

The effect of LbL coating on viability of single cell probiotics, and its protective properties against harsh conditions are still under studying and not hugely investigated. There were just few works regarding this method in the last ten years. No single work, as far as we know, has investigated the use of the food industry by-products as LbL coating materials for probiotics.

Generally, all studies previously published approved the positive effect of microencapsulation on protecting of probiotics bacteria and yeast. Particularly, LbL approach also enhance the viability of probiotics under harsh conditions. The newest study, as far as we know, achieved by Xioa and colleagues and published at the beginnings of 2020. They investigated the effect of microencapsulation of *L. bulgaricus* and *L. paracasei* with whey protein isolate and xanthan gum using LbL technique. The results revealed increasing of survivability and significant decreasing in mortality under simulated GI fluids of coated cells comparing to plain cells. In addition to higher tolerance to heating temperatures, while 6 logs reductions have been obtained when plain cells exposed to 75°C, only 3 logs reduction have been recorded for coated cells at same temperature. The same findings were supported previously by others (Priya et al., 2011; Cook et al., 2013; Thomas et al., 2014, Anselmo et al., 2016 and Wang et al., 2019).

In LbL microencapsulation, the role of ionic interaction is an essential to get better results. Black seeds protein is a positively-charged polymer, which can easily form a polyelectrolyte complexes with negative-charged polymers including alginate and inulin. Microencapsulation, in general, with proteins has a positive effect on protection of probiotics under gastric conditions, pH 2 and 37°C (Picot and Lacroix, 2004; Gbassi et al., 2009; Doherty et al., 2011; Gerez et al., 2012; Maciel et al., 2014; Chen et al., 2017; Maleki et al., 2020; De Araújo Etchepare et al., 2020). Specifically, reduction of 2.8 logs - 3.9 logs of microencapsulated *Lactobacillus acidophilus* La-14, depending on numbers of coating layers, under gastric conditions have been obtained. While 7-logs reduction was observed in case of free cells (De Araújo Etchepare et al., 2020). These results are consistent with that of Gbassi et al. (2009) who found that coating of alginate beads with whey protein significantly improve the survivability of probiotics under acidic pH. Maleki et al. (2020) worked on mixing different encapsulating agents, whey protein isolate, inulin and crystalline nanocellulose, in different concentrations to investigate the effect on protecting *Lactobacillus rhamnosus* ATCC 7469. They adduced that higher WPI concentration mixture conferred a higher protection against simulated gastric conditions.

Alginate is the most widespread negative charged polysaccharides in microencapsulation researches and applications. The importance of alginate linked to gelation, viscosity, and stabilizing properties, that alginate attributes to the product in which it is used.

On the other hand, the hydrophilicity of alginate may be partially disadvantageous, using of high molecular-weighed alginate lead to undesirable properties related to viscosity in some products. LbL technique has approved its ability to overcome this problem by using the minimum quantity of polysaccharides rather than use it as a core material.

Probiotic cells that coated with only alginate layer exhibit a higher releasing rate at acidic pH, this explained by higher porosity of alginate that hasn't been treated or coated with other coating material (Chàvarri et al., 2010; De Araújo Etchepare et al., 2020). Dehkordi et al. (2020) reported that the survivability *L. acidophilus* was 40% higher when the alginates bead coated with whey protein isolate. In the current study, the alginate incorporation showed a better protection against harsh conditions and heating than glucomannan did.

Indeed, incorporation of alginate as a coating material of *L. rhamnosus* GG showed a no significant reduction comparing to plain *L. rhamnosus* after 30 min of SGF. However, significant difference ($p<0.05$) has appeared after 60 min and continued to the end of SIF process. However, it has weaker effect compared with inulin which was acting as alginate until 120 min of SGF, then the significant difference ($p<0.05$) has appeared with alginate coated *L. rhamnosus* until the end of GI simulation process.

Inulin is negatively-charged non-digestible polysaccharides, which considered as prebiotic, that selectively stimulate the growth and activity of probiotics in the colon. Addition of prebiotics significantly provided a

better protection with only 3.1 and 2.9 logs reduction for *L. acidophilus* 5 and *L. casei* 01, respectively, after incubation in simulated gastric juice at pH 1.55 (Krasaekoopt and Watcharapoka, 2014). In the same context, a significant improvement was reported in the viability of *L. casei* and *B. bifidum* when the capsules were containing prebiotics such as inulin (Zanjani et al., 2014).

According to Gandomi et al. (2016) incorporation of chitosan-alginate beads with inulin showed significantly ($P < 0.05$) an additional positive effect in protection of *Lactobacillus rhamnosus* GG against stringent conditions. Furthermore, cooperation of inulin with WPI has provided a positive effect on viability of *Lactobacillus rhamnosus* ATCC 7469 (Maleki et al., 2020). In the current research, incorporation of inulin as a coating layer beside BSP enhanced significantly the survivability of *L. rhamnosus* GG under acidic pH and bile salts as described earlier.

Glucomannan is a natural, water-soluble polysaccharide which is considered an important component for the food industry, biopharmaceuticals, chemical industries and other fields associated with human health. However, as shown in the results, the effectiveness of glucomannan on viability under low pH and bile salts was the lowest, owing to its non-ionic nature. These findings were partially in agreement with Naulkaekul et al. (2013) who observed disability of glucomannan to protect alginate bead or improve cells survivability. While in present study, it has affected positively the survivability but not as well as other coating

polysaccharides. The viability of glucomannan coated *L. rhamnosus* was, surprisingly, lower than plain *L. rhamnosus* during first 60 min under SIF. Then, a significant difference ($p<0.05$) was noted even after 180 min of simulation process.

When the plain and coated *L. rhamnosus* were exposed to different heating temperatures, the effect of temperature has significantly ($P<0.05$) decreased viability as shown previously in Table (2). This may relate to the crucial differences in mechanism of protection of each polysaccharide, where glucomannan has non-ionic nature leading to poor protection, alginate reduce the porosity of surface and inulin provide prebiotic activity, enhance the recovery of injured cells and reduce the porosity.

Conclusion and Future Perspectives

In summary, encapsulation of probiotic strain *L. rhamnosus* GG was successfully performed using layer-by-layer technique, with black seeds protein extracted from *Nigella sativa* deffated seeds cakes and different types of polysaccharides (glucomannan, alginate and inulin). In comparison with plain *L. rhamnosus*, coating materials have shown significant improvement against sequential exposure to SGF. Also, when exposed to high temperatures that may represent storing conditions.

1. These findings support that layer-by-layer is a promising technique to maintain high viable count of probiotics through hosts' GI.

2. Indeed, the polysaccharides used as coating materials affect differently how the probiotics act under GI conditions. While glucomannan showed the weakest protection, inulin confers the highest protection, significantly.

In future studies, the effect of LbL coating of probiotics on adhesion to mucosal lining of colon will be included in researches. In addition, more investigations needed on microencapsulation of probiotics cells with LbL approach using different coating materials, preferably, materials extracted from industrial wastes to reduce the burden on the environment.

Moreover, application of coated cells by LbL technology in food processing, including highly consumed items such as bread, meat products, fruits and vegetable-based products, and special needs products like vegan-milk, gluten-free and lactose-free products, with different probiotics strains and coatings will be the focus of research.

References

- Ahuja K., and Singh S. (2019). **Black Seed Oil Market Size, By Form Type (Capsule, Oil), By Application (Personal Care & Cosmetics [Skin care, Hair care, Fragrances], Pharmaceuticals, Nutraceutical [Food supplement/Dietary supplement, Functional Food], Flavoring & Dressing, Culinary), Regional Outlook, Price Trend, Competitive Market Share & Forecast,** 2019 – 2025. Global Market Insight.

- Călinoiu, L. F., Ştefănescu, B. E., Pop, I. D., Muntean, L., & Vodnar, D. C. (2019). **Chitosan coating applications in probiotic microencapsulation.** Coatings, 9(3), 194.

- Capurso, L. (2019). ***Thirty years of Lactobacillus rhamnosus GG: a review.*** **Journal of clinical gastroenterology**, 53, S1-S41.

- Chávarri, M., Marañón, I., Ares, R., Ibáñez, F. C., Marzo, F., & del Carmen Villarán, M. (2010). ***Microencapsulation of a probiotic and prebiotic in alginate-chitosan capsules improves survival in simulated gastro-intestinal conditions***. **International journal of food microbiology**, 142(1-2), 185-189.

- Chen, H. Y., Li, X. Y., Liu, B. J., & Meng, X. H. (2017). ***Microencapsulation of Lactobacillus bulgaricus and survival assays under simulated gastrointestinal conditions***. **Journal of Functional Foods**, 29, 248-255.

- Cook, M. T., Tzortzis, G., Khutoryanskiy, V. V., & Charalampopoulos, D. (2013). ***Layer-by-layer coating of alginate matrices with chitosan–alginate for the improved survival and targeted delivery of probiotic bacteria after oral administration***. **Journal of Materials Chemistry B**, 1(1), 52-60.

- De Araújo Etchepare, M., Nunes, G. L., Nicoloso, B. R., Barin, J. S., Flores, E. M. M., de Oliveira Mello, R., & de Menezes, C. R. (2020). **Improvement of the viability of encapsulated probiotics using whey proteins**. LWT, 117, 108601.

- Decher, G. (1997). **Fuzzy nanoassemblies: toward layered polymeric multicomposites.** science, 277(5330), 1232-1237.

- Dehkordi, S. S., Alemzadeh, I., Vaziri, A. S., & Vossoughi, A. (2020). **Optimization of alginate-whey protein isolate microcapsules for survivability and release behavior of probiotic bacteria**. Applied Biochemistry and Biotechnology, 190(1), 182-196.

- Doherty, S. B., Gee, V. L., Ross, R. P., Stanton, C., Fitzgerald, G. F., & Brodkorb, A. (2011). **Development and characterisation of whey protein micro-beads as potential matrices for probiotic protection**. Food Hydrocolloids, 25(6), 1604-1617.

- Gandomi, H., Abbaszadeh, S., Misaghi, A., Bokaie, S., & Noori, N. (2016). **Effect of chitosan-alginate encapsulation with inulin on survival of Lactobacillus rhamnosus GG during apple juice storage and under simulated gastrointestinal conditions**. LWT-Food Science and Technology, 69, 365-371.

- Garcia-Ceja, A., Mani-López, E., Palou, E., & López-Malo, A. (2015). **Viability during refrigerated storage in selected food products and during simulated gastrointestinal conditions of individual and combined lactobacilli encapsulated in alginate or alginate-chitosan**. LWT-Food Science and Technology, 63(1), 482-489.

- Gbassi, G. K., Vandamme, T., Ennahar, S., & Marchioni, E. (2009). ***Microencapsulation of Lactobacillus plantarum spp in an alginate matrix coated with whey proteins***. **International journal of food microbiology**, 129(1), 103-105.

- Gerez, C. L., Font de Valdez, G., Gigante, M. L., & Grosso, C. R. F. (2012). **Whey protein coating bead improves the survival of the probiotic Lactobacillus rhamnosus CRL 1505 to low pH**. Letters in applied microbiology, 54(6), 552-556.

- He, F. (2011). **BCA (bicinchoninic acid) protein assay**. Bio-protocol, 101, e44.

- Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B., ... & Calder, P. C. Jung, J., Cavender, G., Simonsen, J., & Zhao, Y. (2015). ***Investigation of the mechanisms of using metal complexation and cellulose nanofiber/sodium alginate layer-by-layer coating for retaining anthocyanin pigments in thermally processed blueberries in aqueous media***. **Journal of agricultural and food chemistry**, 63(11), 3031-3038.

- 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.

- Kour, J., & Gani, A. (2020). **Nigella sativa Seed Cake: Nutraceutical Significance and Applications in the Food and Cosmetic Industry. In Black cumin (Nigella sativa) seeds: Chemistry, Technology, Functionality, and Applications (pp. 223-229)**. Springer, Cham.

- Krasaekoopt, W., & Watcharapoka, S. (2014). **Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice.** LWT-Food Science and Technology, 57(2), 761-766.

- Li, C., Wang, C. L., Sun, Y., Li, A. L., Liu, F., & Meng, X. C. (2016). ***Microencapsulation of Lactobacillus rhamnosus GG by Transglutaminase Cross‐Linked Soy Protein Isolate to Improve Survival in Simulated Gastrointestinal Conditions and Yoghurt***. **Journal of food science**, 81(7), M1726-M1734.

- 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.

- Maleki, O., Khaledabad, M. A., Amiri, S., Asl, A. K., & Makouie, S. (2020). **Microencapsulation of Lactobacillus rhamnosus ATCC 7469 in whey protein isolate-crystalline nanocellulose-inulin composite enhanced gastrointestinal survivability**. LWT, 109224.

- Marais, A., Utsel, S., Gustafsson, E., & Wågberg, L. (2014). **Towards a super-strainable paper using the layer-by-layer technique**. Carbohydrate polymers, 100, 218-224.

- Matsusaki, M., Ajiro, H., Kida, T., Serizawa, T., & Akashi, M. (2012). **Layer-by-layer assembly through weak interactions and their biomedical applications**. Advanced materials, 24(4), 454-474.

- Millette, M., Nguyen, A., Amine, K. M., & Lacroix, M. (2013). ***Gastrointestinal survival of bacteria in commercial probiotic products***. **International Journal of Probiotics & Prebiotics**, 8(4), 149.

- Nami, Y., Lornezhad, G., Kiani, A., Abdullah, N., & Haghshenas, B. (2020). **Alginate-Persian Gum-Prebiotics microencapsulation impacts on the survival rate of Lactococcus lactis ABRIINW-N19 in orange juice**. LWT, 124, 109190.

- Nguon, O., Lagugné-Labarthet, F., Brandys, F. A., Li, J., & Gillies, E. R. (2018). **Microencapsulation by in situ polymerization of amino resins**. Polymer Reviews, 58(2), 326-375.

- Nualkaekul, S., Cook, M. T., Khutoryanskiy, V. V., & Charalampopoulos, D. (2013). **Influence of encapsulation and coating materials on the survival of Lactobacillus plantarum and Bifidobacterium longum in fruit juices**. Food Research International, 53(1), 304-311.

- 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.

- Priya, A. J., Vijayalakshmi, S. P., & Raichur, A. M. (2011). ***Enhanced survival of probiotic Lactobacillus acidophilus by encapsulation with nanostructured polyelectrolyte layers through layer-by-layer approach*. Journal of agricultural and food chemistry**, 59(21), 11838-11845.

- Ram, M. K., Bertoncello, P., Ding, H., Paddeu, S., & Nicolini, C. (2001). **Cholesterol biosensors prepared by layer-by-layer technique**. Biosensors and Bioelectronics, 16(9-12), 849-856.

- Ramos, P. E., Cerqueira, M. A., Teixeira, J. A., & Vicente, A. A. (2018). **Physiological protection of probiotic microcapsules by coatings**. Critical reviews in food science and nutrition, 58(11), 1864-1877.

- Sabbah, M., Altamimi, M., Di Pierro, P., Schiraldi, C., Cammarota, M., & Porta, R. (2020). ***Black edible films from protein-containing defatted cake of Nigella sativa seeds*. International Journal of Molecular Sciences**, 21(3), 832.

- Segers, M. E., & Lebeer, S. (2014, August). **Towards a better understanding of Lactobacillus rhamnosus GG-host interactions. In Microbial cell factories (Vol. 13, No. 1, pp. 1-16).** BioMed Central.

- Thilakarathne, R. C. N., Madushanka, G. D. M. P., & Navaratne, S. B. (2018). **Comparison of physico-chemical properties of Indian and Ethiopian origin Black cumin (Nigella sativa) seed cake**.

- Thomas, M. B., Vaidyanathan, M., Radhakrishnan, K., & Raichur, A. M. (2014). ***Enhanced viability of probiotic Saccharomyces boulardii encapsulated by layer-by-layer approach in pH responsive chitosan–dextran sulfate polyelectrolytes.* Journal of Food Engineering**, 136, 1-8.

- Wang, M., Yang, J., Li, M., Wang, Y., Wu, H., Xiong, L., & Sun, Q. (2019). **Enhanced viability of layer-by-layer encapsulated Lactobacillus pentosus using chitosan and sodium phytate**. Food chemistry, 285, 260-265.

- Zanjani, M. A. K., Tarzi, B. G., Sharifan, A., & Mohammadi, N. (2014). ***Microencapsulation of probiotics by calcium alginate-gelatinized starch with chitosan coating and evaluation of survival in simulated human gastro-intestinal condition***. **Iranian journal of pharmaceutical research: IJPR**, 13(3), 843

التغليف بالطبقات لخلايا بكتيريا *اللاكتوباسيلس رامنوساس* ج ج المنفردة للزيادة من الحياتية لها في ظروف المحاكاة المعوية.

إعداد

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إشراف

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الملخص

تهدف هذه الدراسة إلى زيادة حياتية خلايا *اللاكتوباسيلس رامنوساس* ج ج تحت ظروف صعبة تتضمن ظروف المحاكاة لسوائل الجهاز الهضمي، بالإضافة لحياتيها عند تعريضها لمعاملات حرارية مختلفة، عن طريق تغليف خلايا *اللاكتوباسيلس رامنوساس* ج ج بتقنية تعدد الطبقات للخلية المنفردة باستخدام بروتين حبة البركة –كمادة تغليف– والمستخلص من مخلفات حبة البركة –من نوع *نايجيلا ساتيفا*– منزوعة الدهون، إضافة لبعض أنواع السكريات المتعددة مثل: الإينولين، الألجينات أو الجلوكومنان كل على حدة، العدد النهائي من الطبقات كان ثلاث. تعداد الخلايا الحية الخاصة *باللاكتوباسيلس رامنوساس* المغلفة وغير المغلفة تم تحديدها عن طريق تعريض الخلايا لعملية متتابعة من المحاكاة لسوائل المعدة لمدة 120 دقيقة، وسوائل الأمعاء لمدة 180 دقيقة. إضافة إلى ذلك، تم حساب الحياتية للخلايا بعد تعريضها لدرجات حرارة مختلفة 37، 45 و55 درجة مئوية لمدة 30 دقيقة. النتائج أظهرت تأثير حقيقي (احتمالية<0.05) للتغليف على حياتية البكتيريا (<4، 3 و1.5 لوغاريتم انخفاض) مقارنة مع ما يقارب 6.7 لوغاريتم انخفاض للخلايا غير المغلفة. بشكل أدق، أظهرت النتائج أن تغليف الخلايا باستخدام بروتين حبة البركة والإينولين منحت الخلايا حماية أفضل ضد ظروف المحاكاة المعوية والمعاملات الحرارية. بشكل عام وكما أظهرت نتائج الدراسة، التغليف باستخدام تقنية تعدد الطبقات للخلية المنفردة يحافظ على الخلايا ويمنحها الحماية لتحمل الظروف القاسية مما يجعلها طريقة واعدة في زيادة حياتية البروبيوتك للوصول إلى وجهتها النهائية –أي القولون– بالعدد المناسب للحصول على الفوائد الصحية المرجوة.