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Impact Of Gums On The Growth, Viability, And Enzyme Activity Of Lactobacillus Species

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Impact of Gums on the Growth, Viability, and Enzyme Activity of *Lactobacillus* Species

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Biographical Sketch

Bernice D. Karlton-Senaye was born in 1975 in Accra, Ghana. She attended primary school in Togo, where she studied French as a second language, and secondary school in Ho. Bernice received her B.S degree in Home Science at the University of Ghana, Legon in 2002. Then, she worked as an Assistant Research Scientist at Food Research Institute of the Council for Scientific Research of Ghana (FRI-CSIR) for 6 years. In 2006, Bernice earned a scholarship from the United State Initiative for Long term Capacity Product (UILTCB) of USAID (United State Agency for International Development) through Michigan State University to pursue a M.S degree in Food Science at Purdue University. Whiles at Purdue she received an award that gave her fee waiver to participate in the 27th Annual Workshop of Rapid Methods and Automation in Kansas State University in 2007. After finishing her M.S degree, Bernice returned to Ghana to work as Research Scientist at FRI-CSIR. Bernice was involved in several Nutrition, Sensory Evaluation, Quality Assurance and microbiological projects. She was also a team leader of the FRI branch of the National Salt Iodization project in Ghana which achieved a great success. Bernice was among young women researchers selected by AWARD (African Women Association for Research and Development) to be trained in grant proposal writing prior to her departure to the state to pursue a doctoral program. In 2010, Bernice was awarded graduate Research/Teaching Assistanceship to pursue a PhD. in Energy and Environmental System Program at North Carolina A&T State University. Bernice has been involved in several activities beside research since her enrolment in 2010.

As Graduate Teaching Assistant, Bernice taught Food Safety (FCS 310) course, Physical Science Laboratory (CHEM110) and General Chemistry lab (CHEM 652). Bernice was responsible for organizing training workshops under K-12 Food Safety Program for High/Middle

School. She also assisted in Research Apprenticeship Program (RAP) for high school students. Bernice was selected to participate in the development of teaching module for high/middle school teachers under NASA Instruct Project. She also developed a teaching module and a website on "Food and Climate Change" under NASA Instruct Project.

Bernice is a member of a number of professional and honor society such as Institute of Food Technologists (IFT), American Society of Microbiologists (ASM) and American Chemists Society (ACS) and Phi Kappa Phi Society. Bernice is a recipient of several awards including Recognition Award by Phi Kappa Phi for Outstanding Academic Records and HonorSociety at NCAT. She also received Dr. Wadaran L. Kennedy 4.0 Scholars Award from the Graduate School and The International Student and Scholars Office (ISSO) at A &T for maintaining a 4.0 GPA. She also received Donald McNair scholar award for second position for graduate poster presentation.

Bernice's research areas are research in viability and enzyme activity of probiotics/ prebiotics in dairy products and other fermented foods, research in microbiological, characterization and identification of vegetables and fermented traditional foods, research into Food Safety and antimicrobial activities against pathogens, Nutrition, Sensory Evaluation, Product Development and Quality Assurance and HACCP. Bernice is the first author and corresponding author in six articles two of which are in-press.

After completing her Ph.D. degree, Bernice intends to work in the academia, teaching and conducting research to investigate effects of novel gums as functional ingredients in dairy food to enhance, growth, viability, enzyme activity and protein expression. She is ready to explore other research areas in which her carrier and professional development will lead her.

Dedication

To almighty God who has been so gracious to me every day of my life. To my mother Juliet, who brought me up to be hardworking and God-fearing. To my dear husband, Kallis, whom out of love for me, sacrificed everything for my pursuit for higher education. To my lovely kids, Joshua, Jesse, and Jessica who give me a reason to forge ahead and never give up. To my siblings, Julie, Eric, Patience, and Anthony who supported me with their prayers.

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My deepest gratitude goes to my mother Juliet for her support, my husband, Kallis for his sacrifice and reliable care and support for me and our kids, and for my children Joshua, Jesse and Jessica for giving me unconditional love and joy.

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Abstract

This study investigated the impact of ten selected gums (carrageenan, carrageenan-maltodextrin, guar, locust bean, pectin, pectin- dextrose, pectin-carrageenan, guar-locust bean-carrageenan, inulin, and xanthan) on the growth of eight *Lactobacillus* strains in laboratory media. The bacterial population for eight *Lactobacillus* strains was determined in modified basal media at different concentration (0%, 0.25% and 0.5% w/v) of gums stored up to 12 hours at 37 °C. Findings show significant differences ($p < 0.05$) between the treated and control sample. Guar was found to be the best gum to enhance growth of *L. rhamnosus* GG B101, *L. rhamnosus* GG B101, *L. delbrueckii* subsp. *bulgaricus* SD35, *L. delbrueckii* subsp. *bulgaricus* SD33, *L. acidophilus* SD 16, *L. acidophilus* EF7, *L. reuteri* DSM20016, *L. reuteri* CF2-2F during 12 hours of storage at 37 C in laboratory media. The population of *L. rhamnosus* GGB101 in the samples containing gums were significantly ($p < 0.05$) higher than the control without gums. Samples containing 0.5% gums showed slightly higher bacterial population than those with 0.25% of gum. A minimum of 12 hour of incubation period was shown to be adequate to reach a bacterial population of 6 log CFU/mL in the presence of gums. Addition of guar (0.5%) led to the highest (1.31 log CFU/mL) increase in population of the treated *L. rhamnosus* GGB101 over the control. There was no marked ($p < 0.05$) change in pH and titratable acidity.

Four different gums (carrageenan, carrageenan-maltodextrin, pectin-carrageenan, guar-locust bean-carrageenan) were selected to determine the effect on the growth, viability and beta-galactosidase activity of four selected *Lactobacillus* strains (*L. rhamnosus* GG B101, *L. rhamnosus* GG B101, *L. reuteri* DSM20016, *L. reuteri* CF2-2F). Effect of the four selected gums on thegrowth *L. rhamnosus* GG B101, *L. rhamnosus* GG B101, *L. reuteri* DSM20016 in milk were compared to those in modified basal media. Results showed that xanthan induced

highest growth of *L. rhamnosus* GG B103, *L. rhamnosus* GG B101 in milk whereas carrageenan-maltodextrin led to highest growth of *L. reuteri* DSM 20016 in the media. Marked differences ($p < 0.05$) in viability were observed between milk samples containing gums and the control. The presence of carrageenan-maltodextrin led to the highest viable counts (8.76 ± 0.03 log CFU/mL) of *L. rhamnosus* GGB103 during storage. The average population of *L. rhamnosus* GGB101 and *Lactobacillus rhamnosus* GGB103 was 8 log CFU/mL, whereas that of *Lactobacillus reuteri* DSM20016 and *Lactobacillus reuteri* SD2112 decreased slightly (0.15-0.64 log CFU/mL) to 7 log CFU/mL during the storage period. Viable counts were retained at 7-8 log CFU with no significant difference in the control versus treated strains except in the presence of carrageenan-maltodex. Guar-locust bean-carrageenan led to significant ($p < 0.05$) highest levels of β-gal activity of *L. rhamnosus* GGB103 (1287 ± 4.24 Miller units/ mL) compared to the control (78 ± 2.83 Miller units/ mL). *L. rhamnosus GG B101and L. rhamnosus GG B103* were the best strains to attain enhanced levels of β-galactosidase activity. This shows that the inclusion of carrageenan-maltodextrin and guar-locust bean-carrageenan in milk could improve growth, viability and achieve an enhanced level of *β*-galactosidase activity in *L. rhamnosus* GG B101 and *L. rhamnosus* GG B103. The information gleaned from this study could be used in the food industry to improve the quality of probiotic functional foods and thereby contribute to the alleviation of lactose malabsorption.

CHAPTER 1

General Introduction

The increased recognition of probiotics as health promoting food supplement has heightened interest in identifying substances that can selectively stimulate and promote their growth and viability. Probiotics are "live microorganisms that, when administered in adequate amounts, confer health benefits to the host" (FAO/WHO, 2002). Many researchers have isolated intestinal bacteria including *Lactobacilli, Streptococci, Enterococci, Lactococci, Bifidobacteria* but also *Bacillus* spp. and fungi such as *Saccharomyces*, spp. and *Aspergillus* spp. as probiotics (Gibson, Probert, Loo, Rastall, & Roberfroid, 2004; Karlton-Senaye & Ibrahim, 2013) and established their health benefits. These include reinforcement of gut mucosal immunity, decreased risk associated with mutagenicity and carcinogenicity, alleviation of lactose intolerance, acceleration of intestinal mobility, hypocholesterolemic effect, reduced duration of diarrheas, prevention of inflammatory bowel disease, prevention of colon cancer, inhibition of Helicobacter pylori and intestinal pathogens, and treatment and prevention of allergy (Passos & Ribeiro, 2009). The claimed health benefits of probiotics microorganisms could be lost since the efficacy of the use of probiotics is related to the number of live active culture cells consumed at a minimum level of 10^6 CFU/ml and stability (Donkor, Nilmini, Stolic, Vasiljevic, & Shah, 2007).

Additionally, probiotic bacteria are able to colonize the gastrointestinal tract at level of 10^7 -10^9 CFU/ml (Hernandez-Hernandez et al., 2012). Various technology for enhancing and maintaining the growth and viability of probiotics including selection of acid and bile resistant strains, use of oxygen impermeable containers, two-step fermentation, stress adaptation, incorporation of micro-nutrient, sonication of bacteria and micro-encapsulation (Sarkar, 2010) have been adopted. Nonetheless, difficulties of viability of probiotics still persist. To be

effective, probiotics must remain viable and stable during processing, storage and survive intestinal stressors such as gastric acidity, bile acid secretion and pancreatic enzymes (Bruno, Lankaputhra, & Shah, 2002; Lacroix & Yildirim, 2007). However, work done on the viability of *Lactobacillus* and *Bifidobacteria* species has showed low counts or absence of these probiotics in commercial yogurt (Adhikari, Mustapha, & Grün, 2003; Ibrahim & Carr., 2006). Temmerman, Scheirlinck, Huy, and Swings (2003) reported that only 6 out of 55 commercial products contained all claimed bacteria, 11 contained no viable counts (Temmerman et al., 2003). According to Szajewska et al., (2004) 40% (3 out of 5) of the products assessed revealed poor microbial quality of the products (Szajewska et al., 2004). Mortazavian et al. reported 98.7 % and 98.9 % lost in viability of *L. lactis* and *L. acidophilus* in yogurt during 20 days of refrigerated storage (Mortazavian et al., 2007).

The use of certain polysaccharides as prebiotics to promote growth and viability of lactic acid bacteria (LAB) in dairy products (Ranadheera, Baines, & Adams, 2010) has been noted with inulin and fructooligosaccharide (FOS) being the most recognized (Akalin, Fenderya, & Akbulut, 2004; Donkor et al., 2007; Gibson et al., 2004; Oliveira, Perego, Converti, & Oliveira, 2009b). A prebiotic is defined as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson et al., 2004). Techniques that combine both probiotics and prebiotics have been called synbiotics. Synbiosis is a combination of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting

bacteria, and thus improving host welfare (Gibson & Roberfroid, 1995; Iacono, Raso, Canani, Calignano, & Meli, 2011).

Moreover, various gums have been extensively used in dairy products as stabilizers improving viscosity and texture by preventing "wheying off" (Schmidt, 1994). Like other polysaccharides, gums contribute fiber and enhance sensory qualities of foods (Riedo, Scalarone, & Chiantore, 2010). They are used in other industries including pharmaceutical, cosmetic, paint, inks, paper, color and adhesive industries (Riedo et al., 2010). Chemically, gums are complex polysaccharides made up of cellulose, starches, sugars, oxidation products of these materials, acids, salts of carbon, hydrogen and oxygen (Mantell, 1949). Gums are categorized as plant, algal and plant exudates gums, names that are indicative of their sources. Gums are capable of imbibing and holding large amounts of water (Grindrod & Nickerson, 1968) through gel formation which may enhance stability and activity of probiotics. Most gums are non-digestible and non-degradable. These properties promote gums as good source of nutrients for bacterial growth. Limited studies have been done using gums to enhance viability. Encapsulation of *L. casei* and *B. lactis* in calcium alginate beads raised the survival rate of these probiotics by 30% during 180 days storage at −20 °C. In one recent study done by Ghasempour, Alizadeh, and Bari (2012) to optimize probiotic yogurt production using a novel zedo gum to enhance viability was not established. However, microbial counts of all yogurt samples were maintained at beneficial minimum level of 10^6-10^7 CFU/ml (Ghasempour, Alizadeh, and Bari et al., 2012). Preliminary studies have shown that selected gums enhanced growth of *Lactobacillus reuteri* (Karlton-Senaye & Ibrahim, 2013). Therefore, the aim of this study is to determine the impact of gums (carrageenan, carrageenan-maltodextrin, guar, locust bean, pectin, pectin- dextrose, pectin-

carrageenan, guar-locust bean-carrageenan, inulin, and xanthan) on growth, viability and enzyme activity of *Lactobacillus* species in milk.

1.1 Objectives

The overall goal of this project is to identify gums that can be used in the food industry as prebiotics to enhance viability of food-grade probiotics, express *β*-galactosidase, and to improve the quality of dairy products and non-dairy products. Information obtained from this study has a great potential in human health especially alleviating the symptoms of lactose intolerance. Specific objectives are:

1. To determine the effects of selected gums on the growth of *Lactobacillus* spp. in the laboratory media.
2. To study the effect of selected gums on the growth, viability and *β*-galactosidase activity of *Lactobacillus* spp. in milk during refrigerated storage (4 °C).

1.2 Organization of Dissertation

Chapter 2 of this dissertation presents a literature review that covers mainly the molecular structure chemical composition, and functionality of gums. Chapter 3 covers prebiotics and their activities, including their sources, uses in the dairy industry, and their health benefits. Chapter 4 delves into probiotics, source of probiotics including *Lactobacillu*s, application of probiotics in the food industry, health benefits and mechanisms probiotics. Chapter 5 discusses enzymatic activity including alpha and *β*-galactosidase activity. Chapter 6 examines the effects of selected gums on the growth of *Lactobacillus* spp. in the laboratory media. Chapter 7 describes the effect of selected gums on the growth; viability of probiotics and enzymatic activities in milk during refrigerated storage (4ºC). Chapter 8 summarizes the main conclusions of this dissertation and presents the recommendations for future experiments.

CHAPTER 2

Literature Review[1]

2.1 Gums

Gums are complex polysaccharides extracted from sources such as endosperm of plant seeds, plant exudates, sea weeds, bacteria, and animal sources (MacDermot, Mehta, Pastores, & Pintos-Morell, 2012; Zárate & Pérez Chaia, 2012). Gums are polymers with hydrophobic ability due to the presence of a hydroxyl bond. The composition and structure of gums enable gums to imbibe large amount of water forming a gel, which makes gums useful in the food industry. They are used as stabilizers improving viscosity and texture by preventing "wheying off" (De Castro et al., 2003). Gums are also added to food to enhance texture and sensory qualities foods (Heydari, Mortazavian, Ehsani, Mohammadifar, & Ezzatpanah , 2011). Gums have also found usefulness in other industries, namely pharmaceutical, cosmetic, paint, inks, paper, color, and adhesive industries (Heydari et al., 2011).

A number of polysaccharide used as prebiotics to promote growth and viability of lactic acid bacteria (LAB) in dairy products (Ranadheera et al., 2010) already exist with inulin and fructooligosaccharide (FOS) being the most recognized (Akalin et a., 2004; Donkor et al., 2007; Gibson et al., 2004; Oliveira, Perego, Converti, & Oliveira, 2009b). A prebiotic is defined as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson et al., 2004). These health benefits include reinforcement of gut mucosal immunity, decreased risk associated with mutagenicity and carcinogenicity, alleviation of lactose intolerance, acceleration of intestinal mobility, hypocholesterolemic effect, reduced duration of

[1] Parts of this chapter were adopted from: Karlton-Senaye, B. D., & Ibrahim, S. A. (2013). Impact of gums on the growth of probiotics. Agro FOOD Industry: Functional food and Nutraceuticals 24:4.

diarrhea, prevention of inflammatory bowel disease, prevention of colon cancer, inhibition of *Helicobacter pylori* and intestinal pathogens, and treatment and prevention of allergy (Maganha et al., 2013). Though gums are known to improve texture of food, limited studies have been conducted using gums to enhance growth and viability.

2.2 Sources of Gums

Gums are classified according to their source of extraction, chemical structure, and physical characteristics. Gums are extractable from land plants (e.g., locust bean, guar, pectin, tara) or marine plants (e.g., carrageenan, alginate, furcelluran), from microorganisms (e.g., xanthan, gellan, pullulan) or animal source (e.g., chitosan). Due to the presence of the hydroxyl group and their hydrophilic nature, gums are able to impact viscosity or gelling properties to their media (Whistler & Hymowitz, 1979).

2.3 Molecular Structure, Chemical Composition and Functionality of Gums

Gums are chemically closely related with carbohydrates, but are comprised of cellulose, starches, sugars, oxidation products of these materials, acids, salts of carbon, hydrogen, and oxygen. Gums also contain calcium, magnesium, potassium and sometimes nitrogen (Mantell, 1949). Gums can be obtained commercially by tapping from certain trees and shrubs, extracting from marine plants, by milling or extracting from some seeds, by thermal treatment of starches from kernels or root crops, by chemical processing of cellulose from tree trunks and the cotton plant, as well as by separating animal by-products and by purification processes (Mantell, 1949). Table 1 shows the chemical composition of different gums (Millar, 2003).

Table 1

Major Monosaccharide Components of Algal, Plant Exudates and Higher Plants (Adopted from Williams & Phillips, 2000; Piotr Tomasik, 2004)

Gum	composition	Main Chain
	Algal Polysaccharides	
Furcelluran	Galactose, D-galactose 2, 4, or 6 sulfated, 3,6-anhydro-D-galactose	Galactan
Carrageenan (*kappa)*	3,6-anhydro-D-galactose, D-galactose-4-sulfate	Galactan
Alginate	Glucoric, mannuronic acid	Guluromannuronan
Agar	D-Galactose,3,6-anhydro-L-galactose	Galactan
	Plant Polysaccharides	
Locust bean gum	Mannose, galactose	Mannan
Fenugreek gum	Mannose, galactose	Mannan
Flaxseed gum	Glucose,xylose, galactose, rhamnose	Xylan
Tara gum	Mannose, galactose	Mannan
Psyllium gum	Xylose, arabinose, rhamnose, galactose	Galactan
Guar gum	Mannose, galactose	Mannan
Pectin	Galacturonic acid, rhamnose	Galacturonan
Tamarind gum	Glucose, xylose, galactose	Glucan
Quince seed gum	Galacotse, arabinose, xylose	Galactose
Cassia gum	Mannose, galactose	Mannan
	Plant Exudates	
Gum tragacanth	Galactose, fructose, xylose, arabinose	Galactan
Karaya gum	Galactose, rhamnose	Galactan
Gum arabic	Xylose, arabinose, rhamnose, glucuronic acid	Galactan
Gum ghatti	Arabinose, galactose	Galactan

2.3.1 Chemical composition, structure and functionality of gums. Gums are structurally different in many ways and are categorized according to their source or their chemical composition. Gums are considered as indigestible part of the dietary fiber. Most of the polysaccharides found in gums can be broken down by microorganisms but are not digestible by the human gut due to lack of appropriate enzyme. A gum is classified according to the percentage of its main monosaccharide composition (Millar, 2003). There are three main types of gums; plant gums, algal gums and plant exudate gums. Different gums were selected for the study because they are the most commonly used gums in food. Additionally, the gums vary in chemical composition and of carbon chain. According to Hernandez-Hernandez et al. (2012) the length of carbon chain in carbohydrates such as galactooligosaccharides affects digestibility. The longer the carbon chain the slower the digestibility which subsequently impact on the growth of probiotics. It is expected to find the same trend in the digestibility of the selected gums due to their composition and structure as well as carbon chain length with their consequent effect on growth and viability (Hernandez-Hernandez et al., 2012).

2.3.2 Algal and microbial gums. Algal gums (carrageenan, alginate, furcellarran) are obtained from edible seaweeds. Xanthan and gellan are extracted through microbial fermentation.

2.3.2.1 Carrageenan. Carrageenan is a group of polysaccharides extracted from seaweeds (Rhodophyta). Carrageenan is made of polysaccharides which are linear sulfated galactose polymers with a size of 400-600 kilodaltons. Like other gums, varieties of carrageenan are grouped according to their composition and functionality. There are three commonly known types of carrageenan, namely: kappa (k), which is made up of about 25% sulfate ester groups and about 34% anhydro-galactose; iota (ι) constitutes about 32% sulfate ester groups and about 30%

anhydro-galactose; and lambda (λ) which comprises 35% sulphate ester groups and no anhydo-galactose (Goff, 2004). According to Bixler (1996), these chemical differences confer varying functionality as well. K-carrageenan is soluble and forms a hard brittle gel that is converted by heat at low temperature. I-Carrageenan, on the other hand, is soluble at lower temperature and forms soft, elastic gels. λ-carrageenan is non-gelling and soluble in cold water (Gibson & Wang, 1994). These three types of carrageenan are extracted traditionally from Irish moss, Chondrus crispus. Of major importance to food are k and λ-carrageenan.

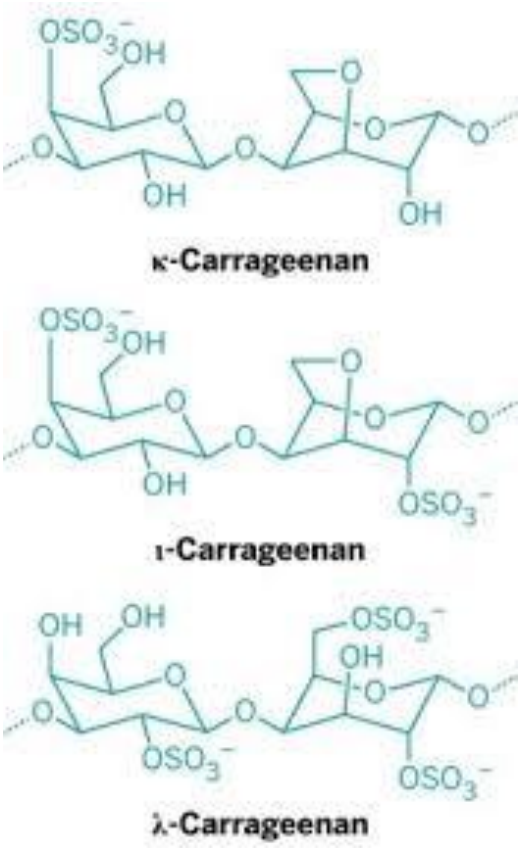

Figure 1. The chemical structure of gums. (www.scielo.cl)

2.3.2.2 Alginates. Alginates are extracted from different seaweeds (*Macrocystis pyrifera, L. digitata,* or *L. saccharina).* They are composed of chains of β-D-mannuronic and α-L-guluronic acids attached with 1→4 linkages (Hekmat & McMahon, 1992). Though insoluble in water, alginates are able to readily absorb water. Alginates are useful as gelling and thickening

agents. The sodium salt of alginic acid, sodium alginate, is used in the food industry to increase viscosity and as an emulsifier. Alginate is used in combination with carrageenan, locust bean, or xanthan and gellan gum in microencapsulation to enhance the viability of probiotics. Alginates are found in food products such as ice cream, mousse, and in slimming aids where they serve as appetite suppressants. Alginates have a wide range of applications in food, drug delivery, tissue engineering, cell encapsulation, and transplantation (Hekmat & McMahon, 1992).

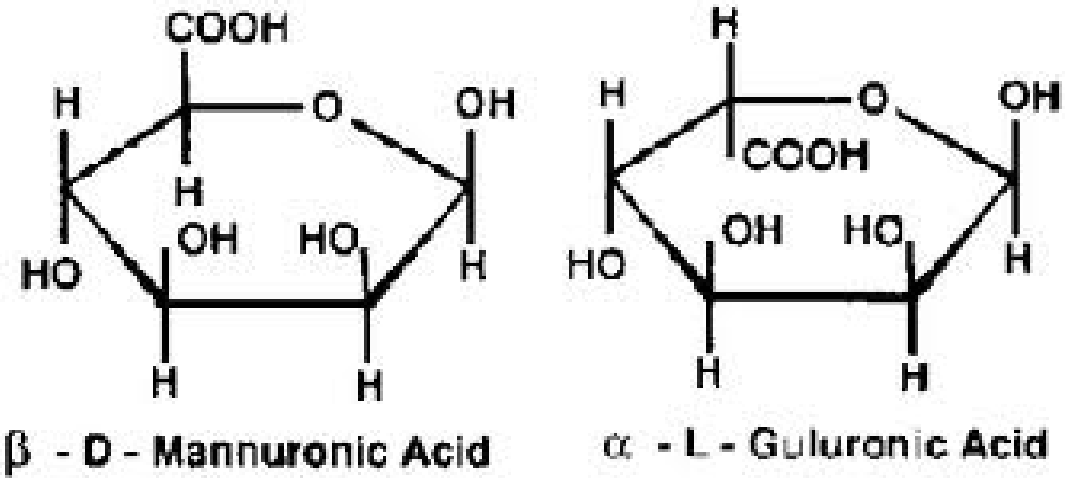

Figure 2. Chemical structure of alginate. (FAO.org)

2.3.2.3 Furcellaran. Furcellaran is an anionic sulphated polysaccharide extracted from seasweed, Furcellaria lumbricalis (Laos, 2005). It has properties similar to both carrageenan and agar composed of repeated unit of alternating 3-linked β-D-galactopyranose and 4-linked α-D-galactopyranose residues, with part of the latter existing as a 3,6-anhydro derivate (Schachat & Glicksman, 1959). Hydroxyl groups in the polysaccharide chain may be sulphated, methylated, or replaced with other monomer residues such as xylose and glucose may be found. It is soluble at 160 ºF and used for gel formation and as a stabilizer in food and drugs. Like carrageenan furcellaran has the ability to form gels in two steps in the presence of specific ions resulting in its change from coil to helix (Laos, 2005).

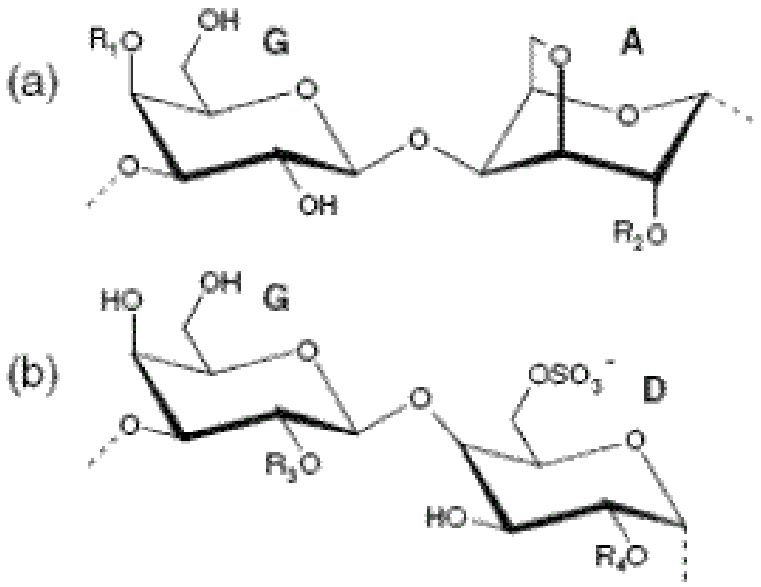

Figure 3. Chemical structure of furcellaran. (estagar.ee)

2.3.2.4 Xanthan. Xanthan is produced through fermentation by microorganisms, Xanthonmonas campestris (Vrese & Schrezenmeir, 2008), found on cruciferous vegetables such as cabbage and cauliflower. Xanthan is made up of a β-D-glucose backbone with every second glucose unit attached to a trisaccharide consisting of mannose, glucuronic acid, and mannose. The mannose closest to the backbone has an acetic acid ester on carbon 6, and the mannose at the end of the trisaccharide is linked through carbons 6 and 4 to the second. Xanthan gum is used extensively in the food industry because it is soluble in cold and hot water, highly viscous at low concentrations, resistant to temperature change, thermally stable, and provides good freeze-thaw stability (Vrese & Schrezenmeir, 2008). The negatively charged carboxyl groups on the side chains cause the molecules to form very viscous fluids when mixed with water. Xanthan gum is used as an emulsifier, a thickener for sauces, prevents ice crystal formation in ice cream, and a fat replacer in food (Jantzen, Göpel, & Beermann, 2013).

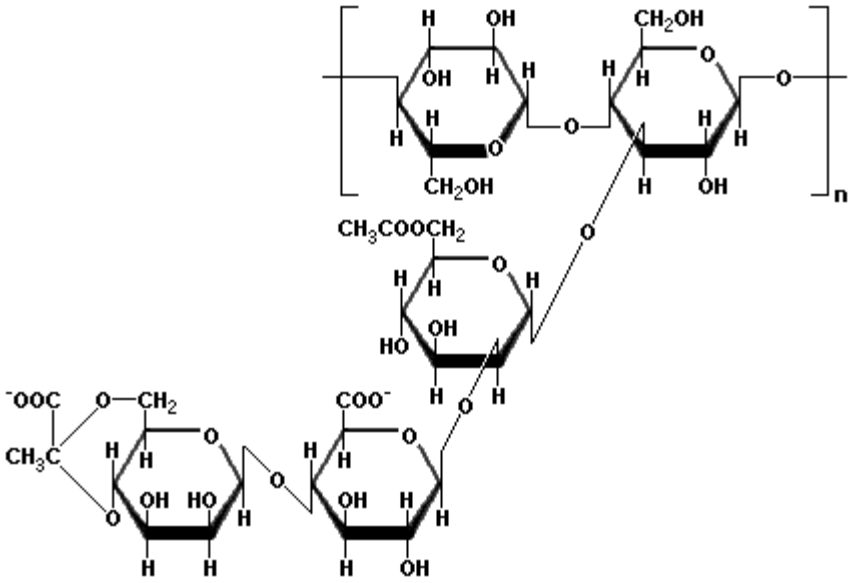

Figure 4. Chemical structure of xanthan. (Scientificpsychic.com)

2.3.2.5 Gellan. Gellan gum is a polysaccharide with a high molecular weight produced as a fermentation product by a pure culture of the microbe *Sphingomonas elodea* which is then recovered with isopropyl alcohol (USDA.gov). Its structure consists of four linked monosaccharides, including one molecule of rhamnose, one molecule of glucuronic acid, and two molecules of glucose (USDA.gov). The molecular formula of gellan gum varies depending on different factors. Gellan gum is a water soluble, off-white powder with molecular weight greater than 70,000 Da. It forms gels when positively charged ions are added. Gellan gum is highly temperature resistant. Its thickening property can be manipulated to give impact on texture by adding potassium, magnesium, calcium, and/or sodium salts. Gellan gum has numerous uses in dairy products, bakery fillings, confections, dessert gels, frostings, icings and glazes, jams and jellies, low-fat spreads, microwavable foods, puddings, sauces, structured foods, and toppings (Sist et al., 2003).

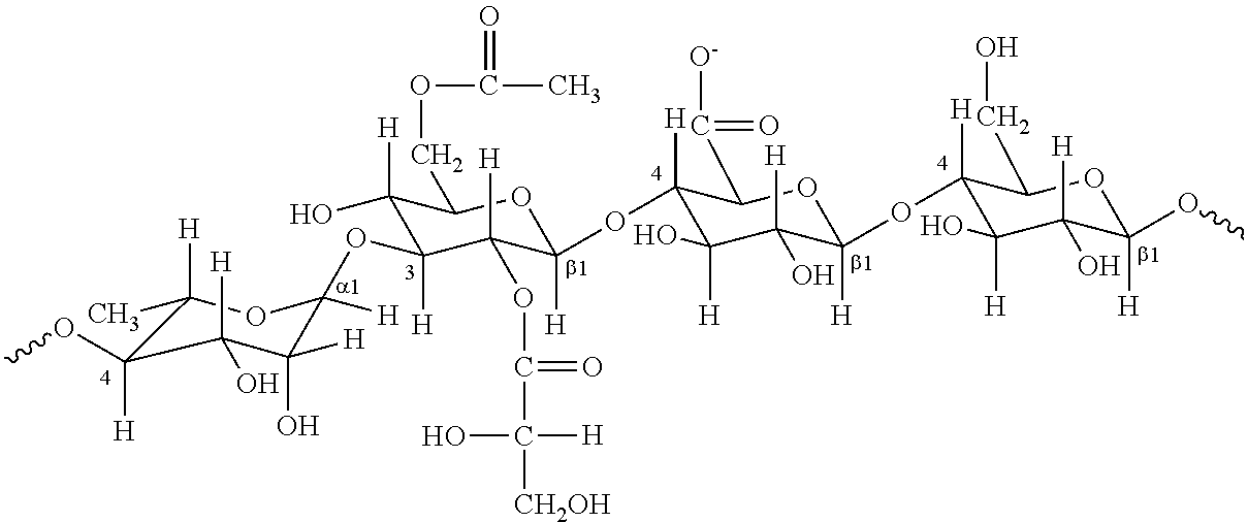

*Figure 5.*Chemical structure of gellan. (en.dsmzk.com)

2.3.2.6 Agar. Agar gum is a water-soluble, gel-forming polysaccharide extract from agarophyte members of the Rhodophyta, composed of repeating agarobiose units alternating between 3-linked β- D-galactopyranosyl and 4-linked 3,6- anhydro-α- L- galactopyranosyl units (Shakerian et al., 2014). This disaccharide regularity may be marked or modified in a number of ways by substitution of hydroxyl groups with sulfate hemiesters and methyl ethers in various combination, and more rarely with a cyclic pyruvate ketal as 4,6-O-[(R)-1-carboxyethylidene] acetal and sometimes by additional monosaccharides.

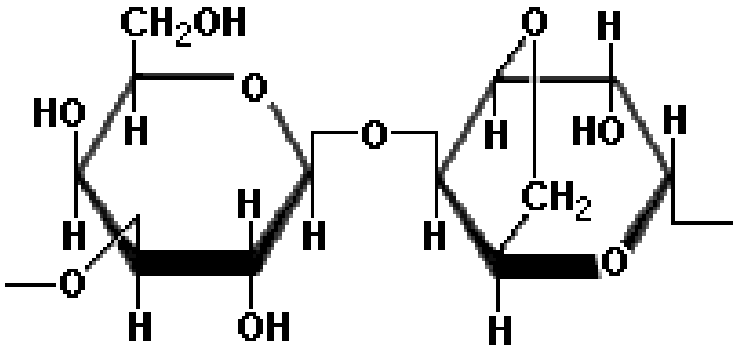

Figure 6. Agar. (http://www.scientificpsychic.com)

2.3.2.7 Curdlan. Curdlan is produced by pure culture fermentation from a nonpathogenic and nontoxicogenic strain of *Agrobacterium biobar* (identified as *Alcaligenes faecalis* var. *myxogenes*) or *Agrobacterium radiobacter*. It is a high molecular weight polymer of glucose, β-(1→3)-glucan. This water-insoluble polysaccharide has an unusual property of forming an elastic gel when its aqueous suspension is exposed to heat (Gänzle & Follador, 2012). The linear structure of curdlan makes it resistant to heat and pH between 2 and 10. Curdlan forms a retortable, freezable gel at both relatively high and low temperatures. At temperatures above 80 °C, this gel is irreversible; however, at temperatures below 60 °C, the gel is reversible. Curdlan powder can be stored for a long period, and, nutritionally, it is an inert dietary fiber (Gänzle & Follador, 2012).

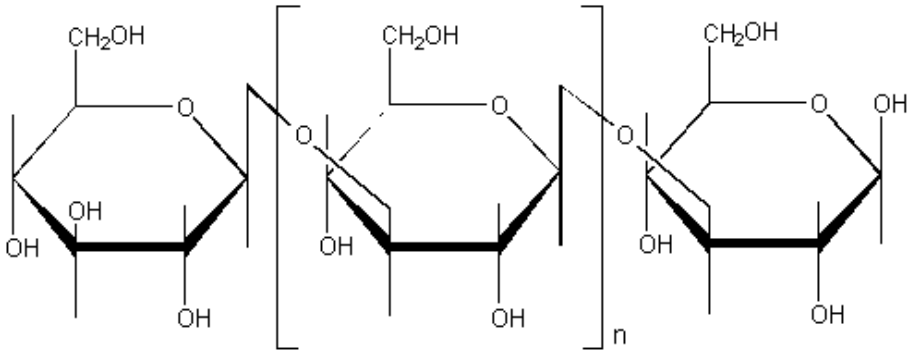

Figure 7. Curdlan. (FAO.org)

2.3.3 Plant exudate gums. Exudate gums are amongst the oldest natural gums which were being used for thickening and stabilizing foods 5000 years ago (Verbeken, Dierckx, & Dewttinck, 2003). These include gum Arabic, gum karaya, and tragacanth. They are produced by many trees and shrubs as a natural defense mechanism, particularly in semiarid regions. When the plants bark is injured, an aqueous gum solution is exuded to seal the wound, preventing infection and dehydration of the plant. The solution dries in contact with air and sunlight, to form

hard, glass-like lumps which can easily be collected. They are water soluble polysaccharides that are used in food, medicine and adhesives industries (Cruz et al., 2013).

2.3.3.1 Gum Arabic. Gum arabic, the oldest and best known of all natural gums, originates from exudate of trees of *Acacia senegal*. Gum arabic is made up of arabinose and galactose in a 1:1 ratio. It is a branched, neutral or slightly acidic, complex polysaccharide obtained as a mixed calcium, magnesium, and potassium salt (Alazzeh, Ibrahim, Song, Shahbazi, & AbuGhazaleh, 2009). The backbone consists of 1,3-linked β-D-galactopyranosyl units. The side chains are composed of two to five 1,3-linked β-D-galactopyranosyl units, joined to the main chain by 1,6-linkages. Both the main and the side chains contain units of α-l-arabinofuranosyl, α-l-rhamnopyranosyl, β-D-glucuronopyranosyl, and 4-O-methyl-b-d-glucuronopyranosyl, the latter two mostly as end-units. It also contains glycoprotein as a minor component (Alazzehet al., 2009). Gum arabic is used as a stabilizer, thickener, and binder in the making of confectionaries, soft drinks, food flavorings, food sweeteners, and drugs.

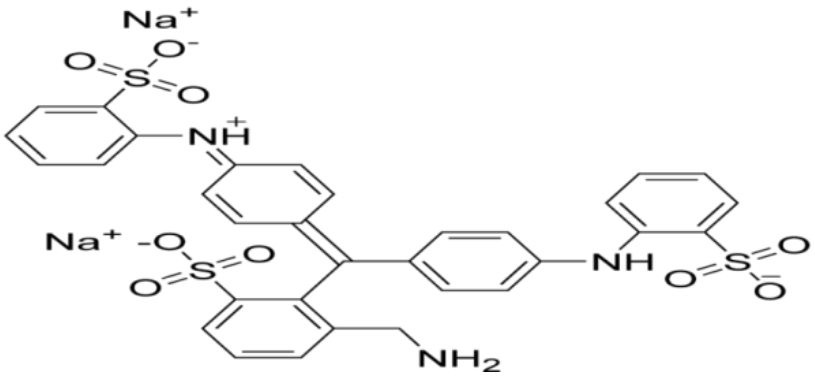

Figure 8. Chemical structure of gum Arabic. (possibilitiesendless.com)

2.3.3.2 Gum karaya. Gum karaya is an exudate gum from the stems and branches of *Sterculia urens roxburgh* and other species of Sterculia or from Cochlospermum gossypium or other species of Cochlospermum (FAO, 1992). It is a complex, partially acetylated polysaccharide obtained as a calcium and magnesium salt. It is branched structure with a high

molecular mass of approximately 16x106 Da (Le Cerf, Irinei, & Muller, 1990). The backbone of the gum consists of α-D-galacturonic acid and a-l-rhamnose residues. Side chains are attached by 1,2-linkage of β-D-galactose or by 1,3-linkage of β-D-glucuronic acid to the galacturonic acid of the main chain. Furthermore, half of the rhamnose residues of the main chain are 1,4-linked to β-D-galactose units (Wang, 2000). Commercial gum karaya contains about 13–26% galactose and 15–30% rhamnose, Gum karaya contains approximately 40% uronic acid residues and 8% of acetyl groups, from which free acetic acid is released on aging. Acetyl nature of gum karaya makes it insoluble and only swells in water (Ghasempour et al., 2012). It is partially soluble in water as only 10% of the native gum was solubilized in cold water and 30% in hot water. Kayara gum is used in mayonnaises and salad dressings. Its main application is medicinal and pharmaceutical.

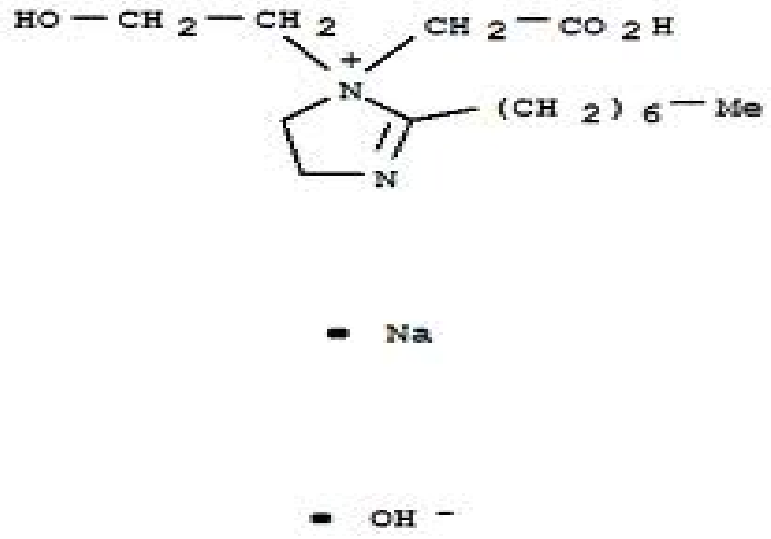

Figure 9. Chemical structure of karaya. (ourchemical.com)

2.3.3.3 Gum tragacanth. Gum tragacanth is an exudate extracted from leguminous shrubs of the genus *Astragalus*. The main chain is formed by 1,4-linked D-galactose residues with side chains of d-xylose units attached to the main chain by 1,3 linkages. The water-soluble tragacanthin is a neutral, highly branched arabinogalactan with a spherical molecular shape. Its

structure probably consists of a core composed of 1,6- and 1,3-linked d-galactose with attached chains of 1,2-, 1,3- and 1,5-linked l-arabinose in a molar ratio of 3: 52: 29: 6: 5: 5 (Liu et al., 2011). Gum tragacanth is less brittle and has a binding strength 8 to 10 times greater than that of gum arabic. It is slightly acidic and composed of tragacanthin, bassorin, starch, and cellulose (Caputo, Festa, Guaragna, Palumbo, & Pedatella, 2003). Gum tragacanth has a large molecular weight of 840,000 and forms strong gel at a lower concentration than gum arabic (Masschelein-Kleiner, 1985). Gum tragacanth solutions become thin at high temperatures but regain viscosity upon cooling; this characteristic indicates the non-degradable nature of the gum. Solutions of gum tragacanth have longer shelf lives than other gums (Caputo et al., 2003).

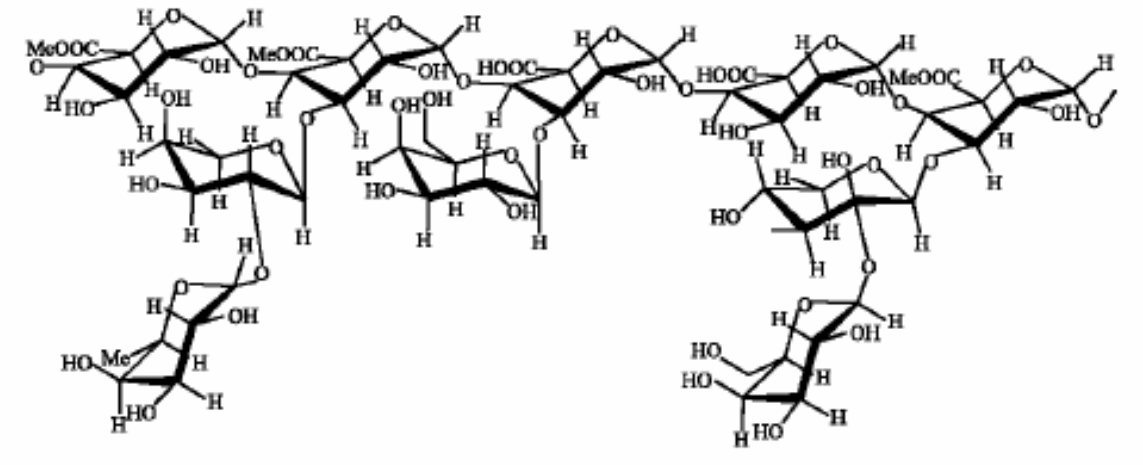

Figure 10. Chemical structure of gum tragacanth. (Khajavi et al., 2007)

2.3.4 Plant gums. These are gums that are obtained mostly from the woody elements of plants (example: Guar, locust bean, gum ghatti, pectin, and konjak), or in seed coatings (example: tara, and tamarind). They can also be found in shrubs (example: gum tracaganth).

2.3.4.1 Guar gum. Guar gum is obtained from the seed of *Cyamopsis tetragonolobus*. It is a straight chain galactomannan with galactose on every other mannose unit. Guar gum contains 1-4-β-D-mannopyranosyl units with every second unit bearing a 1-6-α-D-galactopyrasyl unit and with a molecular weight of about 220,000 (Millar, 2003). It hydrates rapidly in cold

water. It is resistant to changes in pH, neutral in solution, and it is compatible with most foods. Guar gum stops synergism in cheeses, contributes body and chewiness in cheeses, and resists heat shock in ice cream. Guar also increases shelf life in baked products, reduces hygroscopy in icing, and increases viscosity in dressings and sauces.

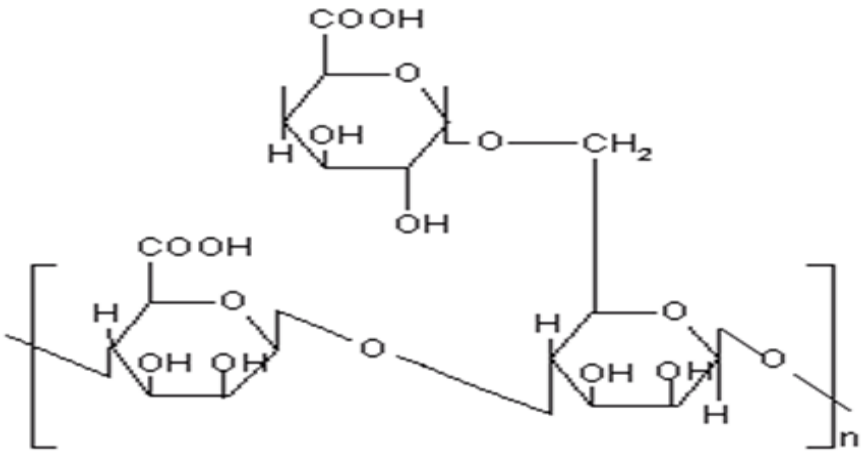

Figure 11. Chemical structure of guar gum. (hbgum.com)

2.3.4.2 Locust bean gum. Locust bean gum, also a galactomannan, is extracted from carob seed, *Cerotonia siliqua*. It has a molecular weight of 400,000 to 1,000,000 and it is comprised of long chains of galactose and mannose. It consists of a D-mannopyranosyl backbone with attached D-galactopyranosyl units in 4:1 ratio giving rise to synergistic activities with carrageenan. Locust bean is similar to guar gum in structure but the uneven side chain distribution makes it less soluble and less viscous, thus forming a weaker gel. Though locust bean gum swells at room temperature, its solubility is enhanced with higher temperature (60 to 90 °C). Locust bean gum contributes water binding property, smoothness, body and chewiness to frozen desserts, speeds up curd formation in cheeses, and acts as a binder in sausages, salami, and bologna (Millar, 2003).

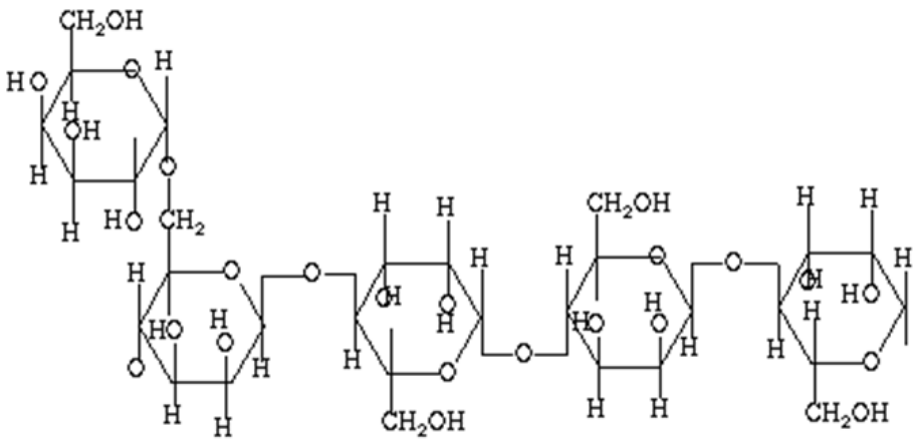

Figure 12. Chemical structure of locust bean. (pietdaas.nl)

2.3.4.3 Gum ghatti. Gum ghatti is a plant gum obtained from *Anogeissus latifolia* with an extremely complex structure. The gum contains free α-Araf-(1→2)-Araf and β-Araf-[β-Araf]n-Ara with n = 4 and 7 which corresponds with 2-O- and 3-O-substituted Araf side-chain structures in the polysaccharide, along with α-Rhap-(1→4)-GlcpA, α-Rhap-(1→4)-β-GlcpA-(1→6)-Gal, and α-Rhap-(1→4)-β-GlcpA-(1→6)-β-Galp-(1→6)-G (Qian & Ge, 2009). The polysaccharide of gum ghatti contain rhamnose, arabinose, galactose and glucose in the molar ratio of 2.3: 72.9: 16.4: 8.46, with 4.6% protein and 2% of uronic acid (Zhou et al., 2010). It is calcium salt of an acidic polysaccharide whose exact molecular structure and weight are not yet fully determined. It is an amorphous, translucent, water soluble gum. However, it can be processed to various other particle sizes, depending on the customer specifications. The impurities are between 1 to 3%, ash content between 3 and 5%, depending on the grade of the material. Gum ghatti has excellent emulsification property and is it is more viscose than gum arabic (Zhou et al., 2010). It is mainly used as an emulsifier for waxes and for flavor oils. It can be used to form both pourable and paste type products (treegums.org). It has excellent emulsifying properties and viscosity and is used for dense pharmaceutical suspensions and emulsions.

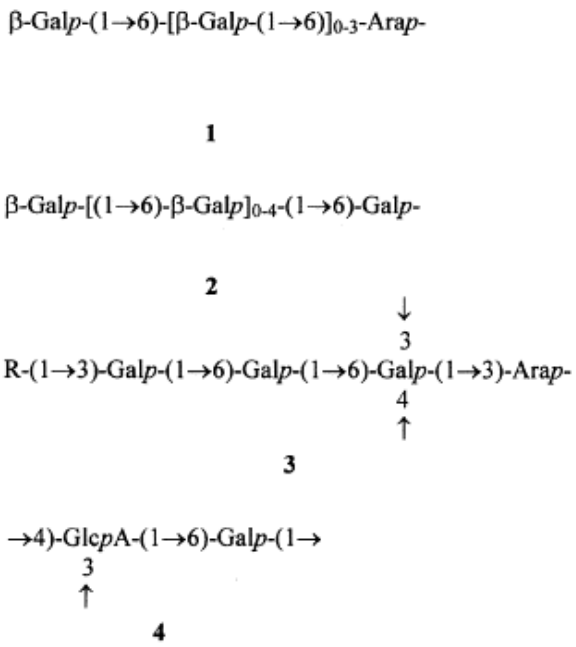

Figure 13. Chemical structure of gum ghatti. (Qian & Ge, 2009)

2.3.4.4 Tara gum. Tara gum is obtained by grinding the endosperm of seeds of *Caesalpinia spinosa*. Tara gum is structurally characterized as a galactomannan, and is similar to guar and locust bean gums, two other galactomannans. It consists of a linear chain of (1→4)-β-D-manna pyranose units with α-D-galacto-pyranose units attached by (1→6) linkages with a ratio of mannose to galactose being 3:1(Ghasempour et al., 2012). Tara gum possesses some unique properties, making it ideal for use in stabilizer blends for dairy foods such as frozen desserts, cream cheese, and cultured products (Yadav, Jain, & Sinha, 2007).

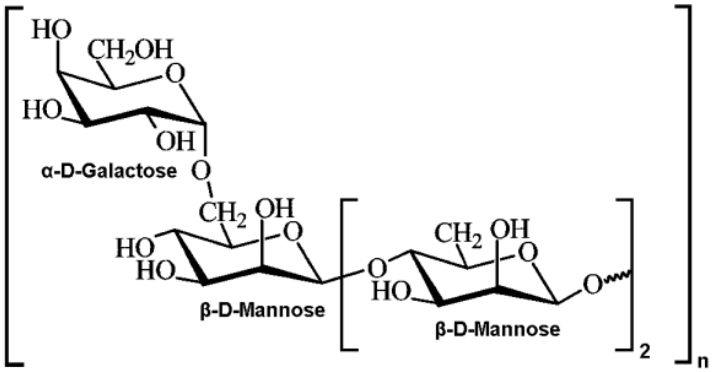

Figure 14. Chemical structure of tara gum. (www.colltec.de)

2.3.4.5 Cacia gum. Cassia is produced from the seeds of leguminous plants (*Cassia tora* and *Cassia obtusifolia*). It consists mainly of high molecular weight (~ 200,000-300,000) polysaccharides composed of galactomannans. The mannose:galactose ratio is about 5:1. Cacia gum is insoluble in ethanol but disperses well in cold water and forms colloidal solutions (Bystrova et al., 2003). Cacia forms a firm viscoelastic gel with xanthan at 40 ºC and with sodium borate at the pH of 9 and above (FAO.org). It is used as a thickening agent in dry soups and seasoning, water retention agent in baked products, and texture improvement in meat and poultry products. It is used as a thickener, emulsifier, foam stabilizer, and texturizing agent.

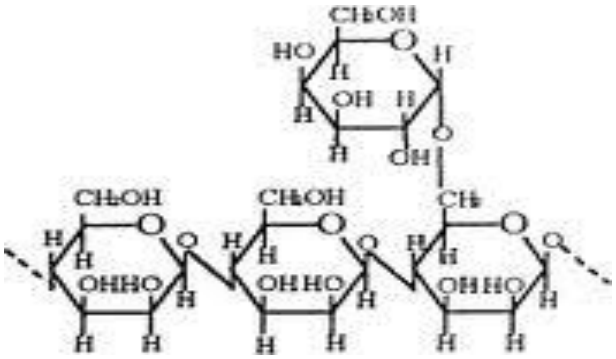

Figure 15. Chemical structure of cacia. (Bystrova et al., 2003)

2.3.4.6 Tamarind gum. Tamarind gum is obtained from the seeds of *Tamarindus indica linn*. It is composed of (1,4)-β-D-glucan backbone substituted with side chains of α-D-xylopyranose and β-D-galactopyranosyl (1,2)-a-Dxylopyranose linked (1,6) to glucose residues (Tomasik, 2003). The glucose, xylose, and galactose units are present in the ratio of 2.8:2.25:1.0. The molecular weight of tamarind gum is within the range of $2.5x10^5$ and $6.5x10^5$. It is soluble in water and forms mucilage upon heating. It also gels at neutral and acidic pH and is used for thickening and stabilizing in food and pharmaceutical industry (Tomasik & Tomasik, 2003).

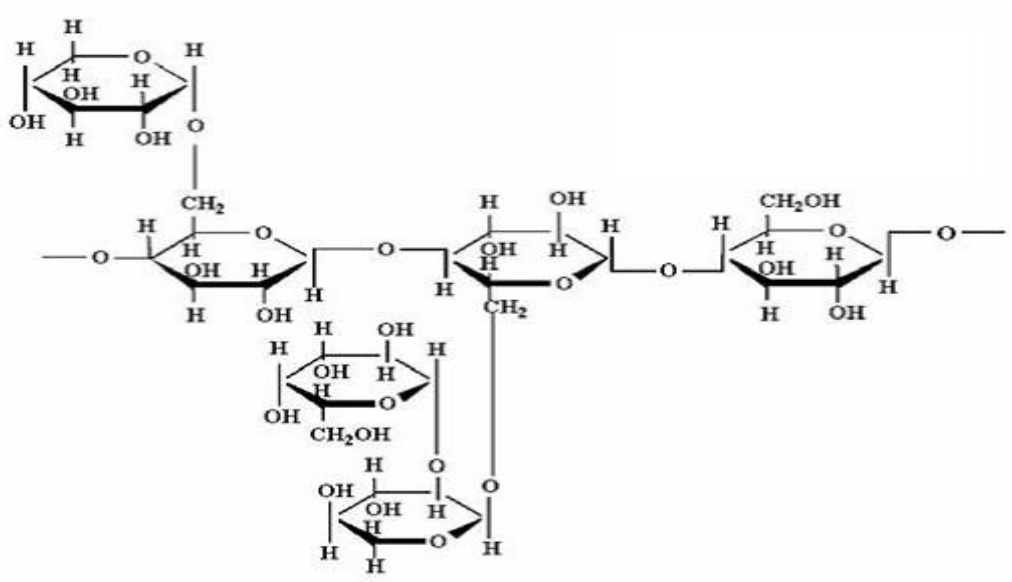

Figure 16. Chemical structure of tamarind gum. (http://journal.ippi.ac.ir)

2.3.4.7 Pectin. Pectin is obtained from the cell walls of plants. Pectin consists of chains of 300 to 1,000 galacturonic acid units joined linearly with 1α→4 linkages. The degree of esterification affects the gelling properties of pectin. The neutral side-chains in the pectin molecule form weak non-covalent bonds and hinder gel formation. The structure has three methyl ester join to every two carboxyl groups; hence it is has a 60% degree of esterification called a DE-60 pectin. Pectin gel formations are complex processes and are generally influenced by factors such as pH, Ca2+ and soluble solids, which vary in their in their effect on gelling process of different pectin types. Pectin is used in fruit preservation, jellies, and jams preparations.

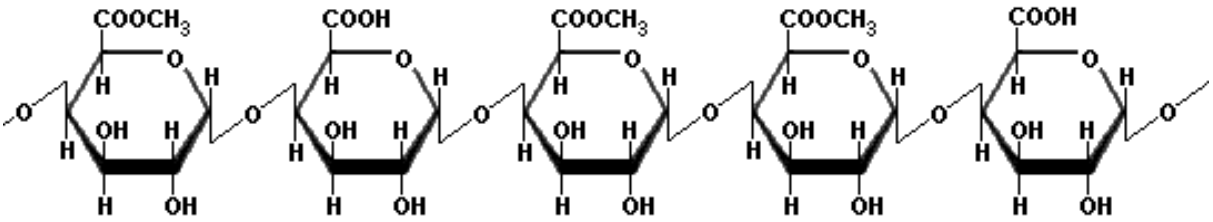

Figure 17. Chemical structure of pectin. (ujungdaun.blogspot.com)

2.3.4.8 Inulin. Inulin is a natural plant-derived polysaccharide obtained mostly from the *Compositae* family including chicory, dahlia, and Jerusalem artichoke (Jacobs, Palm, Zacchi, & Dahlman, 2003). Inulin is also produced by microorganisms including *Streptococcus mutans,* and fungi belonging to the *Aspergillus* family. Chemically described as α-D-glucopyranosyl-[β-D-fructofuranosyl] (n-1)-D-fructofuranoside, inulin is a polymer of fructans consisting of linear carbon chains. Generally, inulin has chain length with 2-200 glucose attached subject to the species. It has much larger degree of polymerization ranging from 10,000 to 100,000 (Jacobs et al., 2003). Biochemically, inulin is inert, non-toxic, and soluble in water. Inulin and fructooligosaccharides are the best studied prebiotics for their bifidogenic activity in the intestine (Gibson et al., 2004). Inulin has β (2-1) glycosidic bonds that make it indigestible by humans but digestible by certain microorganisms living in the gut that have inulinase activity including *Lactobacilli*. The non-digestible nature of inulin in the digestive system makes it a prebiotics which enhances growth of a healthy gut flora that in turn produces bioproducts which suppress colon cancer development. Inulin also has several food and pharmaceutical applications (Jacobs et al., 2003).

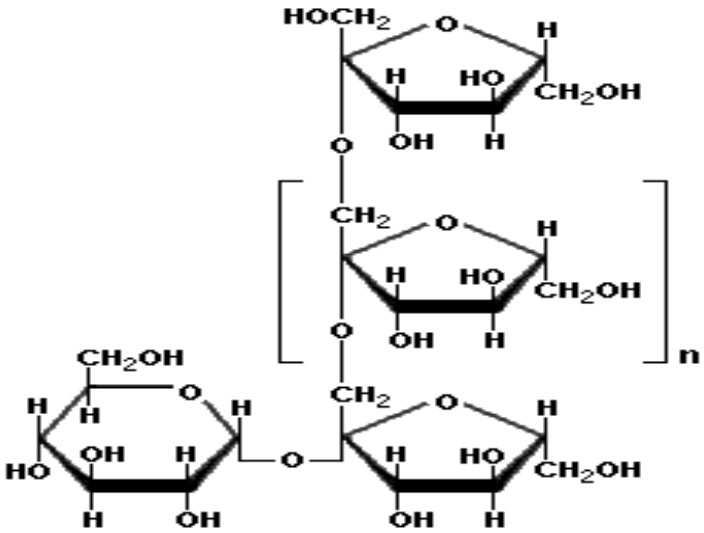

Figure 18. Chemical structure of inulin. (scientificsychic.com)

2.3.4.9 Konjac. Konjac is extracted from the tubers of *Amorphophallus konjac*. Konjac has been cultivated for centuries in Japan. It is a β-(1→4) linked polysaccharide composed of a D-lucosyl and D-mannosyl backbone lightly branched, possibly through *β*-(1→6) glucosyl units. The mannan:galactose ratio is 1.6:1. Its flour is used in the production of noodles and jellies and as a texture modifier and thickener (Miller, 1992). It has a molecular weight of 200,000 to 2,000,000 DA. Konjac swells at room temperature, but shear and heat increase the hydration rate. It is considered a pseudoplastic viscosifier, and yields thermally irreversible gels that are stable at pH 3 to 9 when set with alkali or heat (Yadav et al., 2007).

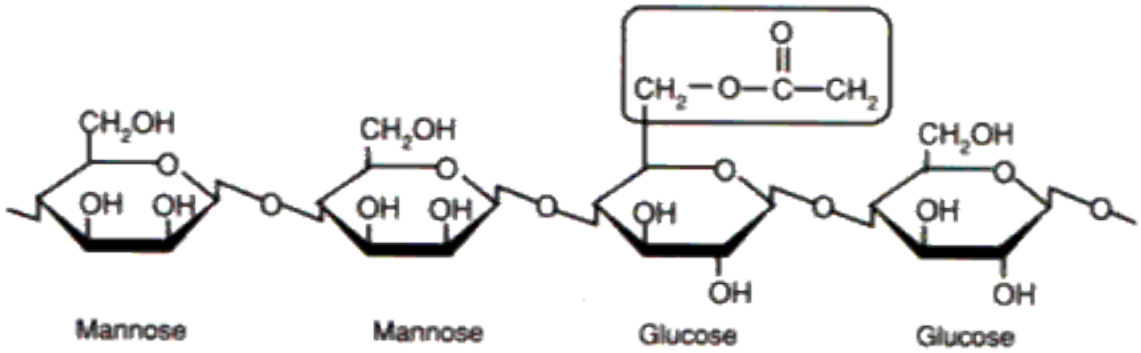

Figure 19. Chemical structure of konjac. (*konjacfoods.com*)

2.4 Application of Gums in Dairy Products

Each gum has specific use in the food industry. Carrageenan is the most extensively used gum in food industry for stabilizing and texturing various food products such as flavored milk, frozen dessert, ready-to eat desserts, soy milk and cottage cheese dressings (Bixler, Johndro, & Falshaw, 2001). Carrageenan is very useful in milk chocolate drinks due to its stabilizing property. Individual mixing a chocolate beverage expect to experience mouthfeel and viscosity within a very short period. The key characteristics required in dairy products specifically dairy beverage is the rate of viscosity formation, which has been estimated as 3-5 minutes in foods supplemented with Kappa-Carrageenan (Bixler et al., 2001). Additionally mixing carrageenan

with other gums such as locust bean gum has been found to increase viscosity, gel strength and elasticity depending on the concentration. Another characteristic of carrageenan is its ability to stabilize milk, which is due to the presence of sulfate groups in the carrageenan which reacts with protein to form a protein-carrageenate complex that is a stable colloid (Whistler & Hymowitz, 1979).

Carrageenan performs other functions in milk products. It prevents precipitation in chocolate milk, serve as stabilizer in milk pudding and egg-free custards, inhibits ice formation in frozen desserts (Kaur, Singh, & Singh, 2009). Carrageenan is commonly used in ice cream as a stabilizer to control "wheyin-off" (Kaur et al., 2009) a defect that gives rise to precipitation of milk proteins from whey in ice cream or syneresis (Goff, 2004). Guar gum, carrageenan, and locust bean gum are combined and used as stabilizers in the preparation of sour cream. Sour cream can be prepared using methylcellulose instead of carrageenan as a stabilizer which is capable of immobilizing water at cold temperatures and thickens it at high temperatures to prevent excessive melting (De Castro et al., 2003). In cream cheese, locust bean gum, xanthan gum, and guar gum act as stabilizers to immobilize water and help smooth the texture while carrageenan is used for moisture control. From all indication gums are used extensively in the dairy products to improve texture mainly viscosity, prevent precipitation and serve as a stabilizer (Bystrova et al., 2003).

Several studies support the use of gums in the dairy industry to improve texture (Anderson, Daubert, & Farkas, 2002; Goff, 2004; Pedersen, 1980). In a study conducted by Soukoulis, Panagiotidis, Koureli, and Tzia (2007) to improve the quality of yogurt four different gums; three milk protein fortifying agents; skim milk powder, whey powder, and milk protein concentrate and four gums κ-carrageenan, xanthan, guar gum, and pectin were incorporated into

whole fat and skim yogurt preparation at 2.0% and 0.01% respectively. These yogurts were heat treated at 80°C for 30 min and 95°C for 10 min. Results indicated that yogurts fortified with xanthan gum pectin became more viscous when the severe heat treatment was applied. Further study of the viscosity data revealed that yogurts were influenced by the interaction of heat treatment and the fat content of milk base. The viscosity of nonfat yogurts containing guar gum, whey protein and milk protein concentrate, and whole fat yogurts with carrageenan and skim milk powder were enhanced when the high temperature-short time heat treatment was implemented. The increase in viscosity when the severe heat treatment was applied was associated with the induced formation of whey protein-casein linkages leading to gelation and texture development (Soukoulis, Panagiotidis, Koureli, & Tzia, 2007). Both incubation time and viscosity development are influenced by heat treatment and based on the same phenomena. Though milk and gums are used extensively as stabilizing agents in the dairy industry, little is known about the role of gum as growth and viability promoting of the probiotics. It is therefore necessary to explore the efficacy of these gums to support the growth and survival of probiotics in milk.

2.5 Health Benefits of Gums

Apart from contributing texture characteristics to foods and serving as substrates promoting the growth of probiotics (inulin and fructooligosaccharides), gums also have numerous health benefits. Guar and pectin have been found by many studies to lower blood cholesterol in individuals with mild to moderate hypercholesterolemia when their diet was supplemented with these gums. In this study, 20 subjects were randomly selected and fed on 20g/day of fiber supplement for 15 weeks and 36 weeks. A total of 125 subjects completed the 15 weeks study. Results of the study showed that that the fiber supplement provides significant

and sustained reduction in LDL-cholesterol without reducing HDL-cholesterol or increasing triglycerides over the 51 weeks treatment period (Kaur et al., 2009).

The high fiber level (80-85%) of guar gum has made it an important ingredient in the development of functional foods capable of reducing plasma cholesterol and glucose levels and subsequently lowering the risk of cardiovascular disease and diabetics respectively (Phillips & Phillips, 2011). One gram of soluble fiber oat products can lower total cholesterol by about 0.045mmol/L and LDL cholesterol by 0.057mmol/L (Steed & Macfarlane, 2009). Diets supplemented with gum arabic showed elevated fecal nitrogen excretion and decreased urea nitrogen concentration in patients with chronic renal failure (Phillips & Phillips, 2011). According to Phillips and Phillips (2011) the mechanism of action was due to increases in bacterial population and activity in the gut. Intestinal microflora produces ureases that convert urea to ammonia and carbon dioxide. The ammonia produced combine with bacterial proteins, which are then excreted through feces leading to increases nitrogen excretion in feces.

A recent study conducted among rats with acute renal failure fed on diet supplement with gum arabic show improvement in renal function. In one study, the diet of normal individuals and a group of diabetic patients with renal disease was supplemented with 25g of supergum for a period of 8 to 12 weeks. Dietary supplementation with supergum resulted in a significant fall in blood pressure for both group and significant fall in systolic blood pressure in normal individuals without hypertension and diabetics (Phillips & Phillips, 2011). There is enough evidence supporting the health benefits of gum (see Table 2) as well as their use as food ingredient but work on their function as non-digestible substrate to increase viability of probiotics is very little. Table 2 shows a summary of health benefits of some gums.

Table 2

Health Benefits of Gums

Gums	Studies	Results/Benefits	References
Guar and pectin	20 subjects were fed on 20g/day of fiber supplement for 15 weeks and 36 weeks.	Reduction in LDL-cholesterol total cholesterol and glucose level	(R. H. Knopp et al., 1999)
Guar	Rats fed on guar gum 3g/100 g for 8 weeks	Reduction in serum cholesterol	(Roberts, 2011; Shahzadi, Butt, Sharif, & Nasir, 2007)
Gum arabic	20 subjects were fed on 50g/day of gum arabic	Significance decrease in serum urea nitrogen	(Bliss, Stein, Schleifer, & Settle, 1996)
Fenugreek	30 subjects fed on 25g/d fenugreek powder for 3 and 6 weeks	Reduces serum total cholesterol, triglyceride and LDL cholesterol	(Moosa, 2006)
Flaxseed	60 patients of Type 2 diabetes were fed on 5 g/day of flaxseed gum for 3 months.	Reduces blood glucose and cholesterol in type 2 diabetic patients	(Al-Ghazzewi, Khanna, Tester, & Piggott, 2007)

2.6 Toxicity and Safety of Gums

The Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) is responsible for evaluating chemical additives in food. JEFCA provides guides Codex on safety on additives (Williams & Phillips, 2003). Gums used in food are screened and approved as a food by, cosmetics or drug additive through "Food and Drug Act" or the FDA in the USA. This is an important criterion for commercially produced gums to be approved as food additives. A decade ago, only three legume-seed gums namely, guar, locust bean and tara gums were recognized as safe to be used in food (trees.org). Currently, most gums are considered safe and are used as stabilizers and fibers in foods through screening by JEFCA.

2.7 Mechanism of Interaction of Gum with Milk Protein

Gums have the ability to produce viscous solutions in water and in milk. This property of gums makes them useful as stabilizers improving texture by inhibiting large ice crystals formation, providing smoothness, uniformity and resisting melting in is dairy products (Schmidt & Smith, 1992). Several approaches have been used to study the mechanism of stabilizers. The relationships have been studied in model systems of hydrocolloids and other components such as milk protein. The relationship between mix viscosity and ice cream coarseness (Schmidt & Smith, 1992) has also been examined. An experiment by Schmidt and Smith (1992) to study the interaction of different gums with whey and casein proteins suggested that gum concentration and protein source affect milk reactivity. Thus, gum and protein concentration must be increased to maximize interaction between hydrocolloid and protein to form a gel, which holds water for microbial activity.

The fat composition of milk impacts directly on efficacy of high pressure homogenization (Riaz & Masud, 2013). In cases where milk is heated before homogenization denaturation of whey proteins occurs binding the fat globules to casein micelles (Schmidt & Smith, 1992). The whey proteins adsorbed to the fat droplets because they form part of the casein micelles. When the milk is homogenized before heating, only the casein micelles are absorbed and subsequent heating attaches some of the whey proteins to these micelles forming an emulsion (Olano-Martin, Gibson, & Rastall, 2002).

Ding et al., (2011) reported lowering of viability of probiotic cultures due to high pressure homogenization. Conversely, Patrignani and others (2009) reported no effect on viability of probiotic cultures, increases in cell load of the starter cultures were observed in high pressure homogenization treatment of milk. Due to the unique relationship between milk-protein

and other polysaccharides and sequence effect on cell viability, it is important to determine the impact of homogenization and gum-milk interaction on probiotics as homogenization could affect survival of probiotic cultures as shown by some studies previously discussed.

2.8 Gum and Mechanism of Action

Gums, like other polysaccharides, contain the necessary food ingredients carbohydrates, sugars, salts and minerals that could support the survival of probiotics organism in food and subsequently in the gut creating gastrointestinal balance impacting intestinal and other health benefits. Figure 20 demonstrates the mechanism by which other polysaccharides become available to microorganisms in the large intestine.

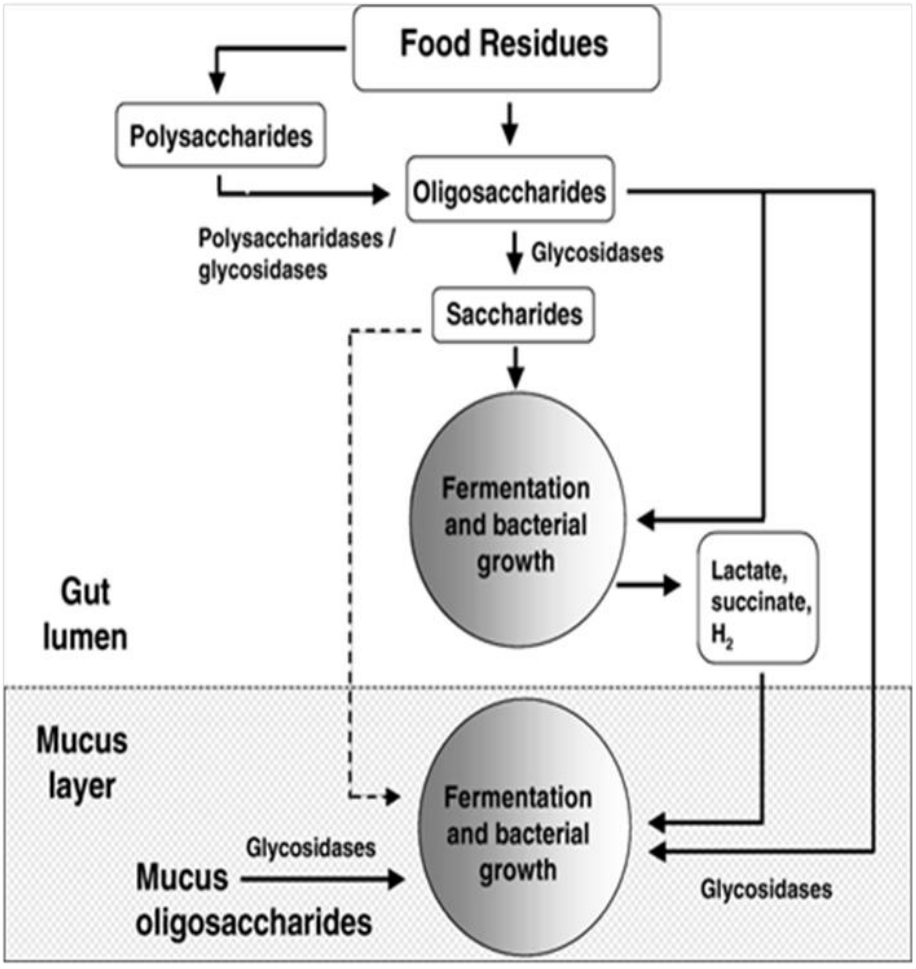

Figure 20. Mechanism of polysaccharides in the large intestine. (Marfarlane et al., 2006)

Although the mechanism for utilization of gums by microflora is unknown it may follow similar patterns as those described in Figure 20 since gums are polysaccharides.Numerous studies have shown that oligosaccharides, which may successfully transit through the GI tract to the lower part of the intestine without being absorbed, can serve as energy source for *Bifidobacteria* leading increased growth and viability of intestinal *Bifidobacteria* (Onishi & Tanaka, 1995). GOS can also serve as growth enhancers of other probiotic intestinal bacteria such as *Lactobacillus acidophilus* and *L. casei*. Gums, being polysaccharides, could equally be used to improve growth and viability of *Lactobacillus* in media and milk thereby improving the quality of functional dairy food ("Graphical Abstracts," 2003).

2.9 Preliminary Results of Effect of Gums on the Growth of *Lactobacillus reuteri*

The impact of different gums on the growth of *Lactobacillus reuteri* was determined (Figure 21). In this study, modified M17 media were prepared with 0.5 % (w/v) of one of the following gums: carrageenan, guar, carrageenan-maltodextrin, locust bean, pectin-dextrose, alginate, pectin, pectin-carrageenan, and inulin, sterilized at 121 °C for 15 minutes and allowed to cool to 42 °C. Sterilized samples were inoculated with *Lactobacillus reuteri* strains at a final inoculum level of 3 log CFU/ml, incubated at 37 °C for 16 h, serially diluted, and plated on MRS agar to obtain final bacterial counts.

Our results showed higher bacterial counts in samples with gums compared to control. Bacterial population in the control sample increased from initial counts of 2.78 log CFU/ml to 7.16 log CFU/ml whereas samples with pectin increased from 3.3 log CFU/ml to 9 log CFU/ml. The bacterial population in samples with carrageenan also increased from 3.1 log CFU/ml to 8.9 log CFU/ml. This study therefore indicates that pectin and carrageenan-maltodextrin could

enhance the growth of *Lactobacillus reuteri* and subsequently improve the quality of functional food.

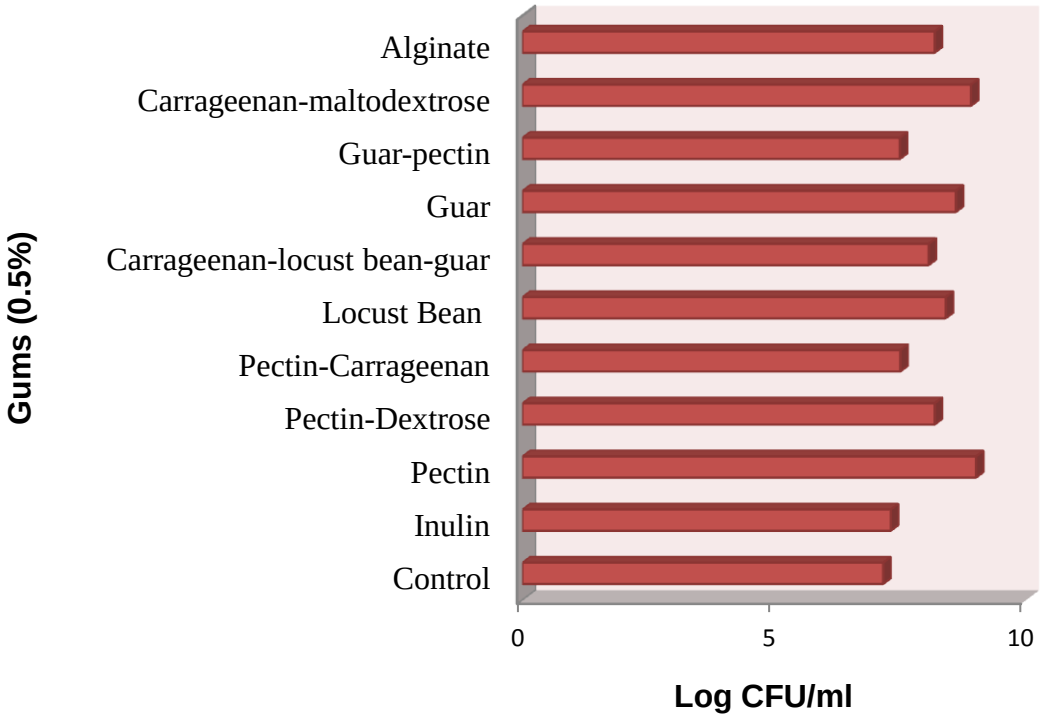

Figure 21. Growth of *Lactobacillus reuteri* in the presence of different gums during incubation at 37 °C for 16 hours.

CHAPTER 3

Activity of Prebiotics Including Gums

3.1 Prebiotics

Prebiotics are food components that undergo selective fermentation by specific microbial groups but are not digested in the stomach and small intestine (Roberfroid, Van Loo, & Gibson, 1998). Food plays an important role in controlling microbial colony in gastrointestinal tract. This is achieved through buffering effect of food on the bacteria through the stomach. Such colonic foods namely non-digestible carbohydrates, peptides and proteins, as well as certain fructose oligosaccharides which promote the growth of favorable bacteria, are referred to as prebiotics. Prebiotics are capable of reaching the large intestine almost intact and are selectively used by gut microflora. Genera *Lactobacilli* and *Bifidobacteria* are the usual target for activity of prebiotics. Human colon is populated with more *Bifidobacteria*, which show preference for oligosaccharides, than *Lactobacilli* as a result changes in *Bifidobacteria* are more visible compared to those in *Lactobacilli (*Slavin, 2013*).* Food ingredients must possess some basic characteristic to be classified as prebiotics, these include (1) resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption; (2) fermentation by intestinal microflora; (3) selective stimulation of the growth and/or activity of intestinal bacteria associated with health and wellbeing (Gibson et al., 2004).

3.2 Sources and Health Benefits of Prebiotics

Well researched prebiotics are oligosaccharides such as lactulose, galactooligosaccharides, inulin, fructooligosaccharides, and some selected food carbohydrates (Oliveira et al., 2009b; Ranadheera et al., 2010). However, prebiotics such as oligosaccharides and inulin are considered as a functional food ingredients (Mousa, Liu, Chen, Zhang, & Chen,

2014) because they able to create balance in intestinal microbiota composition of human gut by suppressing the multiplication of pathogenic mocroorganisms (Chen & Mustapha, 2012), thus improving the host health (Moayednia, Ehsani, Emamdjomeh, & Mazaheri, 2009; Mortazavian et al., 2007). Inulin has often been used in studies to improve the viability of probiotics in stored refrigerated dairy foods (Akalin et al., 2004). In vitro studies have also shown that inulin and oligofructose have a specific pattern of fermentation (Hidaka et al., 1986; Wang & Gibson, 1993). This occurrence has been confirmed by Gibson et al. (1995) in a human trial that evaluated inulin and oligofructose in vivo (Gibson, 1995). The efficacy of inulin as the best prebiotics has been tested in several studies (Roberfroid et al., 2010). Moro et al. (2002) observed an increase in *Bifidobacteria* and *Lactobacilli* in infants who were fed with formula milk supplemented with a mixture of inulin and galactooligosaccharides, thus the use of inulin and galactooligosaccharides may be used in infant formulation as ingredients.

Other health benefits of prebiotics include effect on mineral absorption and effect on the metabolism of lipids. Roberfroid (2000) reported the positive effect of inulin and ologofructose on the absorption and balance of dietary calcium (Roberfroid, 2000). Guar gum is also useful for controlling cardiovascular diseases, diabetics and obesity (Masood Sadiq, Naureen, Mian Kamran, & Muhammad, 2007). The prebiotics activities of these carbohydrates and subsequent health benefits to humans call for more research to identify other prebiotics that can support the growth and viability of probiotic cultures (Anonymous, 1998; Bernet, Brassart, & Nesser, 1993; Bixler, 2010; Bliss et al., 1996; Goldin, 1992; Jack, Tagg, & Ray, 1995; Knopp et al., 1999; Manning & Gibson, 2004; Matsumoto et al., 2006; Rolfe, 2000). Figure 22 summarizes the mechanism of prebiotics in the delivery of health benefits.

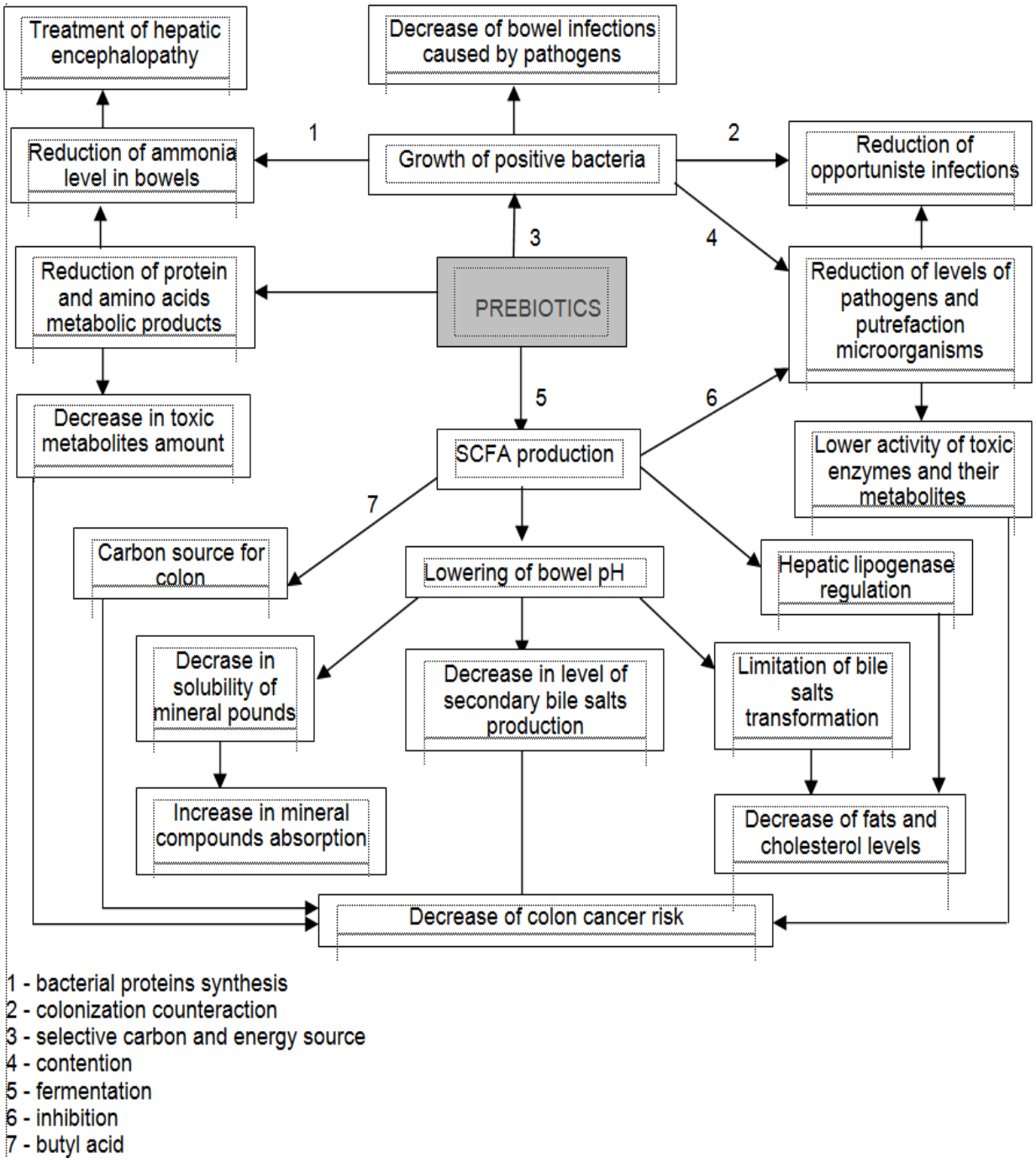

Figure 22. Mechanisms of prebiotics and health benefits.

3.3 Uses of Prebiotics in Dairy Industry

Certain food may contain natural ingredients that may impart prebiotics effects enhancing the activity of probiotics. But others such as dairy, meat products, cereals, beverages and infant

formulates can be fortified with prebiotics during manufacturing process to increase probiotic efficacy (Gibson et al., 2004). Infant formulae have been improved to taste like breast milk by the incorporating prebiotics such as fructo- and galactoologosaccharides (Vrese & Schrezenmeir, 2008) to enhance taste and microbial activity. Food containing prebiotics can now be found easily in the market. These include bread, cereal bar, spread, confectioneries, sauces, infant milk formulae, beverages, and health drink.

Though many fruits and vegetables such as onion, garlic, banana, asparagus, leek, Jerusalem artichoke, and chicory may contain prebiotic oligosaccharides such as fructooligosaccharides (FOS) naturally, their level of bioavailability in such fruits and vegetables is too low to have any significant effect. An amount of 4 g to 8 g of FOS must be consumed daily to increase *Bifidobacteria* in the colon. Considering the amount of FOS that must be consumed, it is important to fortify common food with prebiotics to increase the amount of microflora in the gut. One of the ways to achieve that is to fortify dairy products which are consumed by many people. Such foods include milk, yogurt, cheese, ice creams. Other non-dairy products with probiotics could also be fortified with prebiotics for the same purpose (Manning & Gibson, 2004). Manning and Gibson (2004) claimed the following oligomers are potential prebiotics: lactulose fructo-oligosaccharides, galacto-oligosaccharides, soybean oligosaccharides, lactosucrose isomalto-oligosaccharides, gluco-oligosaccharides, xylo-oligosaccharides and palatinose. In this study, other polysaccharides including: (carrageenan, carrageenan-maltodextrin, guar, locust bean, pectin, pectin- dextrose, pectin-carrageenan, carrageenan-locust bean-guar, inulin, and xanthan were studied for viability and beta-galasctosidase activity.

3.4 Prebiotics and Infants

In the past two decades, prebiotics have been used in making infant formulas in Japan, Europe, and in the U.S. Human milk contains oligosaccharides which are associated with increased levels of *Bifidobacteria* in breast-fed babies compared to bottle-fed infants. GOS also is capable of acting like glycoconjugate receptors on cells surface receptors could lead to increased protection from pathogens (Steed & Macfarlane, 2009). Due to the complex structure of oligosaccharides available for the production of infant formulas, a nonhuman milk alternative oligosaccharides short-chain galacto-oligosaccharides (GOS) and long-chain fructo-oligosaccharides (FOS) have been have been used in combination with probiotic in infant formulas (Salvini et al., 2011). Many clinical studies, aimed at determining the ability of prebiotic to increase fecal levels of *Bifidobacteria,* have been carried out in infants. These clinical trials made it possible to consider as GRAS the addition of 0.8g/dl of a mixture of 10% short chain FOS and 90% long chain into infant formulas (Steed & Macfarlane, 2009).

3.5 Health Benefits of Prebiotics

Supplementation of food with prebiotics has numerous health benefits. Prebiotics are able to prevent invasion of gastrointestinal pathogens thereby promoting food safety. They are believed to increase satiety by suppressing hunger ghrelin, the hunger hormone. This phenomenon can be used in weight loss programs (Lenoir-Wijnkoop et al., 2007). Consumption of food containing prebiotic also correlated with calcium absorption, bone accretion and bone mineral density in adolescents and postmenopausal women (Roberfroid et al., 2010). In one of such studies, the absorption of calcium significantly increases when 40 healthy subjects were fed with 40 g of inulin daily for 28 days (Coudray et al., 1997). However, when 15 g of inulin, FOS and GOS were each fed to 12 healthy subject daily for 28 days, there was no significance

difference in the absorption of calcium and iron (Van den Heuvel, Schaafsma, Muys, & Van Dokkum, 1998; Van den Heuvel, Muys, van Dokkum, & Schaafsma, 1999). On the contrary, there was 10.8 % rise in calcium balance with no significant change in urinary excretion when 12 adolescents were fed on 15 g FOS daily for nine days (Van den Heuvel et al., 1999) .

Another health benefit of prebiotics is its ability to control colon cancer. Although some studies have shown that inulin is capable of controlling some form of colon cancer (Reddy, Hamid, & Rao, 1997), a study conducted by Alles et al. (1999) found no significant changes in *Bifidobacteria* or in markers of carcinogenesis. However, a more recent study shows that inulin-type-fructans may reduce the risk of colorectal cancer when given at the initial stages of cancer development (Pool-Zobel, 2005). Inulin also controls the synthesis of insulin which provides a direct health benefits to individuals with diabetics. Prebiotics such as FOS also regulate blood cholesterol by lowering the synthesis of triglycerides by the liver. The effect of prebiotics on serum cholesterol has also been studied (Delzenne & Kok, 1999). It is therefore expected that gums will improve health in a way similar to prebiotics and probiotics, whilst at the same time being cheaper, and carrying less risk and being easier to incorporate into the diet.

CHAPTER 4

Probiotic Microorganism and Mechanism of Action

4.1 Probiotics

Probiotics are live microorganisms, generally lactic acid bacteria (LAB), that are intended to promote the health of the host by creating favorable intestinal microflora balance when they are ingested in sufficient quantities (Fuller, 1989). The concept of probiotics started as far back as 1907 by a Russian scientist Elie Metchnikoff. According to Elie Metchnikoff, the food one eats controls the kind and amount of bacteria in the gut. He noticed that the consumption of large quantities of fermented milk containing lactobacilli daily increased life span (Quigley, 2010). Currently it is established by some authors that daily intake of probiotics in food promotes intestinal microflora and prevents gut disorders (Billoo et al., 2006; Canani et al., 2007). However, probiotics can only impact such health benefits if they have the following characteristics; survive and multiply in the human body, adhere to the cells of the host, produce antimicrobial products that are antagonistic to pathogenic bacteria, form a balanced, normal microflora, safe to be administered to human beings (non-pathogenic, non-carcinogenic, non-invasive (Reid, 1999; Salminen et al., 1998), and must be consumed in a dose of five billion colony forming units a day ($5x10^9$ CFU/day) for at least five days (Mukerjea & Robyt, 2003).

4.1.1 Sources of probiotics and application in food. Most of the studies relating probiotics for human have been performed on LAB isolated from dairy products (Maurer-Menestrina, Sassaki, Simas, Gorin, & Iacomini, 2003). Traditionally, LAB have been used in the fermentation of various foods. Two decades ago, Lactic acid bacteria used in food manufacturing, such as *lactobacilli, lactococci, streptococci, Leuconostoc, pediococci*, and *Bifidobacteria*, for the characteristic properties including flavor and texture attributes as wells as

their resistance against processing condition(Barrangou & Horvath, 2012). Some of these probiotics have also gained recognition as health enhancers and are included for this purpose primarily in fermented dairy products and dietary supplements. Probiotics have shorter shelf life in food as a result could only remain active in food for about a month.

Probiotics are also taken as dietary supplement. Due to the dry nature of dietary supplements, probiotic will remain dormant increasing their shelf life to up to 24 months, making them more viable when used. Dietary supplements with *L. acidophilus, Bifidobacterium longum, Bifidobacterium infantis, Enterococcus faecium* and others with or without probiotic and fructooligosaccharides (FOS) are also used. Some of the most common probiotic products are *L. acidophilus* with FOS, *L. acidophilus* and *Bifidus longum* with FOS, and *Bifidus infantis* and *L. acidophilus* with FOS (Parvez, Malik, Kang, & Kim, 2006). These probiotic preparations may be presented in the form of powders, tablets, capsules, pastes or sprays depending on the animal or human receiving the supplement and the condition to be treated (Parvez et al., 2006). Probiotic preparations are useful in the treatment of lactose intolerance, immune enhancement, decrease in fecal enzymes and mutagenicity and have an effect on hypocholesterolemic serum (Roberfroid, 2000). However, few of these functional formulations were made with traditional gums whichserve as cheaper alternatives.

4.2 Health Benefits of Probiotics and Mechanism of Action

Gastrointestinal health of individuals is dependent on the equilibrium of gut ecosystem (Panesar & Shinde, 2012). This balance can be achieved by the presence of probiotic bacteria in their right numbers in the human intestinal tract, boosting mucosal resistance to pathogens (Panesar & Shinde, 2012). Evidence supporting the primary role of gut health promotion by probiotics exists and numerous mechanisms have been reported. The mechanisms of action have

not been studied extensively. However, excretion of acids (lactate, acetate), competition for nutrients and gut receptor sites, immunomodulation, and formation of specific antimicrobial agents may be included in the mechanism (Fooks & Gibson, 2002). Lactic acid bacteria such as *Lactobacillus* produce some antimicrobial agents such as organic acids; hydrogen peroxide, bacteriocins and bacteriocin-like agents that inhibit the growth of sensitive strains of bacteria (Jack et al., 1995). Other *Lactobacillus* strains also secrete bacteriocin (see Table 3).

Table 3

Bacteriocin Produced by Lactobacillus Species (Farmer, 2001)

Bacterocin	Bacterial Species
Acidolin	*Lactobacillus acidophilus*
Acidophilin	*Lactobacillus acidophilus*
Lactacin B	*Lactobacillus acidophilus*
Lactacin F	*Lactobacillus acidophilus*
Bulgarin	*Lactobacillus bulgaricus*
Plantaracin SIK-83	*Lactobacillus plantarum*
Plantaracin A	*Lactobacillus plantarum*
Lactolin 27	*Lactobacillus helveticus*
Helveticin J	*Lactobacillus helveticus*
Reuterin	*Lactobacillus reuteri*
Lactobrevin	*Lactobacillus brevis*
Lactobacillin	*Lactobacillus brevis*

Another proposed mechanism is linked to how probiotics block pathogen adhesion sites on intestinal surfaces (Fuller, 1992). This makes adhesion property of some probiotics an

attractive characteristic to be consideration for their use. For instance strains of *Bifidobacterium* isolated from human feces were found to inhibit adhesion of pathogens onto cultured enterocyte-like cells (Bernet, 1993). Evidence shows that probiotics can protect against intestinal disease through stimulating immunity. Ingestion of sufficient amount of *Lactobacillus* sp. GG for the treatment of acute rotavirus diarrhea caused by *Clostridium difficile* is associated with to improved immune response to rotavirus.

Though the mechanism for immune stimulation is not well understood, Rolfe (2000) suggested that the composition of cell wall or cell layers may increase the humoral immune responses (Rolfe, 2000). Similar explanation may be involved in increased resistance to *salmonella* by rats fed *Bifidobacterium*. In a double-blind, placebo-controlled, multicenter study 287 children between the ages of 1–36 months from 10 countries who were admitted to the hospital with moderate-to-severe diarrhea, most commonly due to rotavirus or an unknown pathogen were randomly given oral preparation plus live *Lactobacillus GG* (LGG) cultures and others received hydration solution with no cultures. Patients who were given LGG versus placebo had a shorter duration of diarrhea. In addition, patients treated with LGG were less likely to have persistent diarrhea (Rolfe, 2000). Thus, probiotics impact several health benefits to individuals who ingest them in sufficient amount in their daily diets. However, survival in processed foods; as a result of types of ingredients, acidity and storage condition of the food, and in gastrointestinal tract as a result of enzyme, pH and other stressors affects health benefits. Numerous health benefits of probiotics with associated mechanisms are summarized in Figure 23.

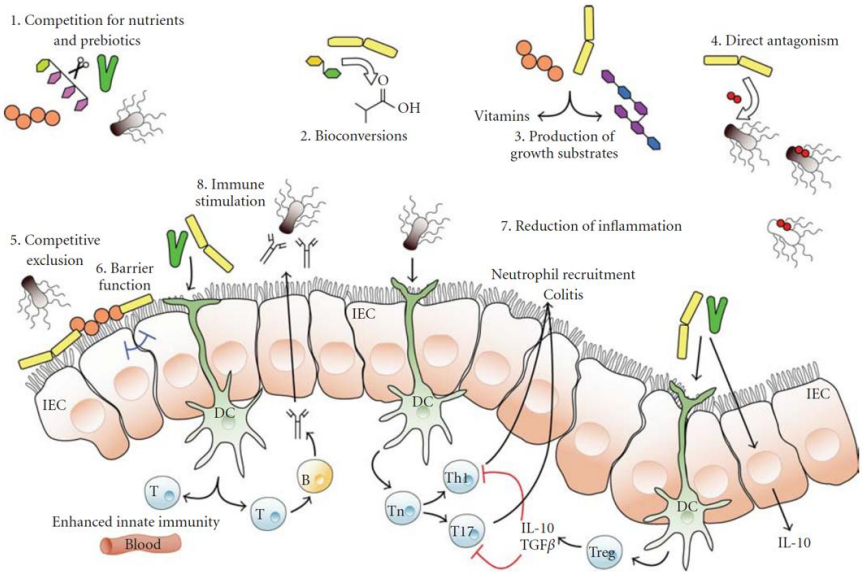

Figure 23. Health benefits and mechanism of action by probiotics. (www.customprobiotics.com)

4.3 Probiotic Microorganisms

The majority of probiotic microorganisms belong to the genera *Lactobacillus* and *Bifidobacterium.* However, other bacteria and some yeast may have probiotic properties as well. *Lactobacillus* species are Gram positive lactic acid-producing bacteria that constitute a major part of the normal intestinal microflora in animals and humans. *Lactobacilli* are non-spore forming rod-shaped bacteria. They have complex nutritional requirements and are strictly fermentative, aerotolerant or anaerobic, aciduric or acidophilic. *Bifidobacteria* are gram-positive anaerobic bacteria. They are colonic bacteria constituting approximately 5% of the total bacteria constitute a major part of the normal intestinal microflora in humans throughout life (Bystrova et al., 2003). They appear in the stools a few days after birth and increase in number thereafter. The number of *Bifidobacteria* in the colon of adults is 10^{10}–10^{11} cfu/gram, but this number decreases with age as a result of ingestion of foods infected with pathogenic microrganims (. Rascon-Diaz, Tejero, Mendoza-Garcia, Garcia, & Salgado-Cervantes, 2012). The most commonly used and reported probiotics include two genera; *Lactobacillus* (*L. acidophilus, L.*

casei, L. bulgaricus) and *Bifidobacterium* (*B. bifidum, B. logum, B. breve, B. infantis, B. animalis,* and other *Bifidobacterium* species). Both genera are found in the normal intestinal flora at relatively low levels in healthy human adults. *Bifidobacteria* exert various beneficial effects on host health by controlling undesirable intestinal bacteria. Among reported beneficial effects of *Bifidobacteri*a, are their inhibitory effect to putrefactive bacteria, reduction of fecal enzymes involved in cancer initiation, and reduction of serum cholesterol (Ibrahim & O'Sullivan, 2000). The health promoting benefits of these microorganisms is related to their ability to adhere and colonize the intestinal tract, a characteristic which is a pre-requisite for consideration as probiotic for use in food (Ibrahim & O'Sullivan, 2000; Petr & Rada, 2000).

4.3.1 Genera *Lactobacilli.* Members of genus *Lactobacillus* are non-sporeforming, catalase negative cocobacilli or rods. They are fermentative in nature and are characterized by low GC (guanine and cytosine) contents up to 59.2 mol%. *Lactobacilli* are aero-tolerant or anaerobic (Salvetti, Torriani, & Felis, 2012). They are found in a variety of habitats where rich, carbohydrate-containing substrates are available, such as human and animal mucosal membranes, on plants or material of plant origin, sewage, fermented milk products and fermenting or spoiling food. *Lactobacillus* has several applications in the food industry from fermentation of beer, meat product and probiotics starter culture in dairy and some non-dairy food. *Lactobacilli* require complex media due their fastidious nature to growth. These include complex nutrient-rich media containing proteins, fermentable carbohydrates, nucleic acid derivatives, fatty acids, vitamins and metal ions, which are generally found in plant material, dairy products, meat and meat products, fish, the oral cavity, gastrointestinal tract and vagina (Kandler, 1986). *Lactobacilli* are able to grow in a pH range between 3 to 8, but growth best at an optimal pH of 5.5 to 6.2 is (Salvetti et al., 2012). The pH of most gums used in this fall with

the optimal pH for Lactobacillus growth that enhancing the interaction between the bacteria and the gums.

Some *Lactobacillus* in the GI tract been associated with health benefits and are considered as probiotic *Lactobacillus*. Such probiotic *Lactobacillus* are capable of impacting characteristic flavors, texture as well as resistance to manufacturing processes are required have specific characteristic to be considered as probiotic (Azaïs-Braesco, Bresson, Guarner, & Corthier, 2010). Meanwhile, *Lactobacilli* are found naturally in most plants including fresh produce (Rodriguez, 2009). *Lactobacilli* are used as probiotic in many commercial products. *L. reuteri, L. rhamnosus, L. acidophilus, L. casie, and L. plantarum,* have been extensively studied and documented as probiotics (Barrangou & Horvath, 2012) .

4.3.2 *Lactobacillus reuteri*. *Lactobacillus reuteri* was previously mistaken for as *Lactobacillus fermentum*. However, further study led to its re-classification in 1965 by Gerhard Reuter, where its name was derived from. *Lactobacillus reuteri* was first isolated from human faecal and intestinal samples (Reuter, 1965). *Lactobacillus reuteri* are natural inhabitans of the GI tract of healthy humans and animals. *Lactobacillus reuteri* is irregular shape, bent rod with rounded ends with a diameter of 0.7-1.0 um, and a length of 2.0-5.0 um. *Lactobacillus reuteri* can be identified from *L. fermentum* through physical test. *Lactobacillus reuteri* growth very fast at 37-49 C and are tolerant in low pH and bile salts of the human gastrointestinal system (Wall et al., 2007). Apart from its probiotic attributes, *Lactobacillus reuteri* is also capable of producing reuterin, antimicrobial agent against Gram-negative pathogens (Gänzle, Hältzel, Walter, Jung, & Kammes, 2000). *L. reuterin* is capable of fermenting a number of carbohydrates including prebiotic fibers such as inulin and dextrin (Stewart et al., 2008). Many *L. reuteri* strains also

produce exopolysaccharides such as glucan reuteran, which could serves as a potential thickener in the food industry.

4.3.3 *Lactobacillus rhamnosus*. *Lactobacillus rhamnosus* is one of the most extensively studied probiotics, recognized for its ability to survive and even thrive in the harsh conditions of the digestive and urinary tracts. *Lactobacillus rhamnosus* is among probiotic bacteria that are available because of their health benefits. *L. rhamnosus* was first isolated from the intestines of a healthy human subject by scientists Barry Goldin and Sherwood Gorbach in 1983 (Conway, Gorbach, & Goldin, 1987). *L. rhamnosus* GG is derived from the last names of the two scientists who isolated it. It is tolerant to high acidic condition in the stomach and digestive tract. Like other probiotics, *L. rhamnosus* has numerous health benefits including reinforcement of gut mucosal immunity, decreased risk associated with mutagenicity and carcinogenicity, alleviation of lactose intolerance, acceleration of intestinal mobility, hypocholesterolemic effects, reduced duration of diarrhea, prevention of inflammatory bowel disease, prevention of colon cancer, inhibition of *Helicobacter pylori* and intestinal pathogens, and treatment and prevention of allergies (Passos & Ribeiros, 2009).

4.4 Technologies for Enhancing Viability of Probiotics

Probiotic microorganisms are not able to survive rigorous processing steps causing decrease in their population in food at the end of processing. As a result, a number of technologies are used to enhance cell viability including the application of sub-lethal stresses during fermentation, the addition of protectants, continuous fermentation and cell protection by microencapsulation (Lacroix & Yildirim, 2007). The technologies are based on the ability of microorganisms to grow and survive as well as their capacity to adapt to changing environments. Adaptation to adverse environments is usually associated with the induction of a large number of

genes, the synthesis of stress-response proteins, and the development of cross-resistance to various stresses (Girdis et al., 2003).

4.4.1 Microencapsulation. The common technology used in the dairy industry to improve viability is microencapsulation. Microencapsulation, is a technology that has been studied (Chen & Mustapha, 2012; Mousa et al., 2014; O'riordan, Andrews, Buckle, & Conway, 2001) for over two decades is used to protect probiotic microorganisms from lethal damage during processing, storage and survival in the GI. Through the process of microencapsulation, longevity of probiotic bacteria is retain by gradual release of probiotic from microcapsules into hostile environment such as the acidity of GI during digestion, freeze and thaw during storage and transportation and mechanical force such as homogenization and grinding. To protect probiotics from fatal damage during food processing, storage and upper gastrointestinal (GI) tract digestion, microencapsulation as a novel technique has been extensively studied for the past two decades (Riaz & Masud, 2013; Totosaus, De Jesus Ariza-Ortega, & De Lourdes Perez-Chabela, 2013).

Though these technologies have been used to enhance cell viability lower levels of probiotic cultures have been detected in yogurt and other fermented dairy product compared to the amount declared on their labels (Ibrahim & Carr, 2006; Moayednia et al., 2009). According to Ibrahim and Carr (2006), most of yogurt products in North Carolina contain viable *Bifidobacteria* culture less than the level declared on the labels. Viability of probiotic cultures in functional food and gastrointestinal tract as well as cell high cell yield is essential to obtaining health benefits. As a result, there is an industrial demand for technologies that will further increase cell yields and ensure viability of probiotic in food. It is therefore important to develop ways to improve the high yield and viability of probiotics in large scale in foods cheaply and

easily. Thus, this study proposes the use of selected gums which are less costly functional ingredients to increase the viability of *Lactobacillus* species through less laborious procedure.

4.5 Mechanism of Immune Modulation by Probiotic Bacteria

This mechanism of probiotics occurs in three levels (see Figure 24): the first being the interference of probiotic bacteria with growth of pathogens in the gut lumen; the second level of action is the mucosal barrier fortification to enhance function and immune system of the mucosa; and the third level of action is the effect of probiotic bacteria on the systemic immune system as well as other cells organs such as liver and brain (Joshi & Kapoor, 2003). The mechanisms of action of probiotics have also been described as specific mechanism by which probiotics modulate humoral and cell-mediated immune responses (Ferrero, Martino, & Zaritzky, 1993). In other mechanisms, probiotics strengthen epithelial barrier function through competitive elimination of pathogenic bacteria along epithelium, modification of local microbial niche and reduction of intestinal inflammation (Ferrero et al., 1993).

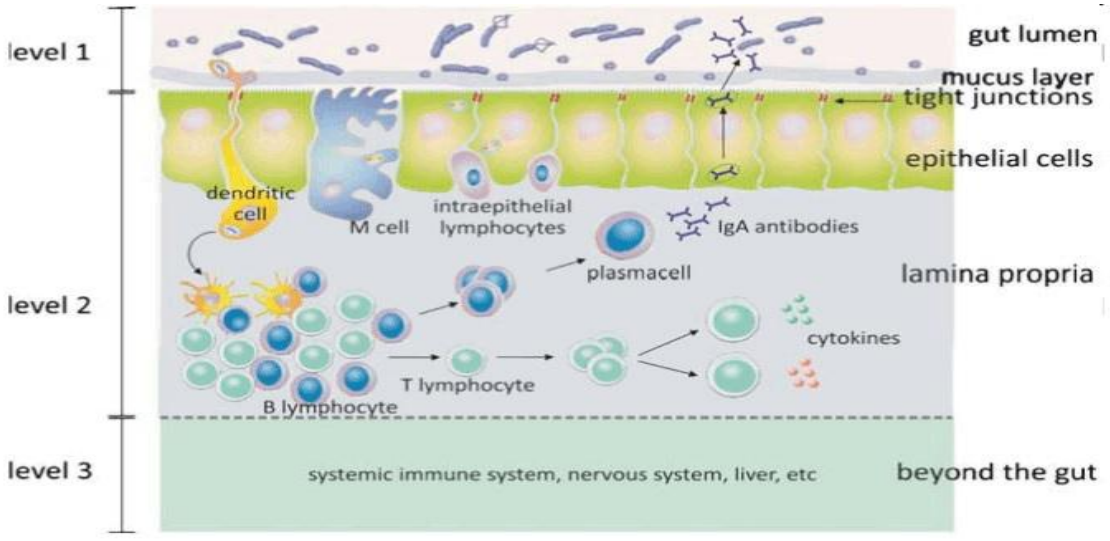

Figure 24. Levels of action by probiotic bacteria in the gum lumen. (Palmquist, 2010)

4.6 Impact of Gums and Other Polysaccharides on the Growth of Probiotics

The functionality of gums as gelling and thickening agent has been studied by many authors as previously mentioned. However very little is known about gums and how they impact the growth and viability of probiotic cultures in food and in the gut. Cherbut et al. (2003) showed the bifidogenic property of gum acacia in their study. Gum acacia is able to ferment milk slowly producing short chain fatty acids that selectively raise the proportion of lactic acid bacteria and *Bifidobacteria* in healthy individuals, and raise fecal digestibility to 95%. Daily consumption of 10g/day of gum acacia acts as prebiotic (Cherbut et al., 2003). In a recent study using a newly discovered gum, zedo gum, could not enhance the viability of *L. acidophilus* and *B. bifidum* in yogurt during storage; however, the counts in all samples were above the minimum therapeutic level (10^6–10^7 cfu/g) (Passos & Ribeiros, 2009).

Lacroix et al., (2004) report the use of various gums including carrageenan, gellan, alginate to enhance cell mass and viability throught immobilization techniques. Other gums (such as pectin, carrageenan, alginate, gellan, xanthan, zedo, konjac, starch, celluslose, chitosan), proteins (casein, whey, gelatin, β-lactoglobulin) and wax have also have been studied as prebiotics to improve the viability of probiotic microorganisms (Corona-Hernandez et al., 2013; Ibrahim et al., 2010, Ibrahim & O'Sullivan, 2000) in various dairy food.

Although very few studies were done using gum to support cell growth and viability, numerous other carbohydrates and polysaccharides have been studied extensively. Further study is necessary to evaluate the use of gum as prebiotics functional ingredients that could enhance viability and improve enzyme activity. A summary of some growth and viability studies using different carbohydrates is demonstrated in Table 4.

Table 4

Beneficial Effect of Probiotic on Growth and Viability of Probiotics in Foods (Ranadheera et al., 2010)

Foods	Prebiotics/gums	Probiotics	Studies	Reference
Yogurt	High maize/resistant starch	*L. acidophilus*, *L. casei*	Increased growth and viability	Dontoh et al., (2007)
	Inulin		Increased growth and viability	Hekmat, Soltani, & Reid (2009), Aryana & McGreww (2007)
	Fructooligosaccharides	*L. acidophilus* *L. casei* *L. Rhamnosus* *L. reuteri* *Bifidobacterium*	Increase viability and fatty acid production	Akalin, Tokusoglu, Gonc & Aycan (2007), Akalin et al. (2004), Capela, Hay, & Shah, (2006).
		L. acidophilus *L. casei, LRhamnosus* *Bifidobacterium B. animalis* *B. longum*		
	Zedo gum	*L. acidophilus*, *B. bifidum*	decreased viability	Ghasempour et al. (2012)
Fermented milk	Polydextrose	*L. acidophilus* *L. rhamnosus* *B. animalis* subsp. *lactis*	Increased growth, viability and fatty acid production	Olivera et al. (2009)
	Oligofructose	*L. acidophilus* *L. rhamnosus* *B. animalis* subsp. *lactis*	Increase growth, viability and fatty acid production	
Cheese and cheese-based products	Oligosaccharides	*L. acidophilus* *B. animalis subsp. Lactis*	growth, viability, sensory and fatty acid production	Cardarelli, Saad, Gibson, & Vulevic (2007)
	Inulin	*L. acidophilus* *B. animalis subsp. Lactis*	Increased viability	Cardarelli et al. (2008)
	Carboxy methyl cellulose	*P. freudenreichii subsp. shermanii*	Increased growth	Cardarelli et al. (2007) Cardarelli et al. (2008) Buriti et al. (2005)

Table 4

(Cont.)

Foods	Prebiotics/gums	Probiotics	Studies	Reference
Ice cream	Inulin	*L. acidophilus*	Increased growth, viability and fatty acid production	Olivera et al. (2009)
		B. lactis	Increased	Akin et al. (2007)

4.7 Economic Importance of Gums

Apart from their health benefits, there is a huge economic benefit in the development and production of functional foods containing polysaccharides such as gums. The income generated from the sale of gums is expected to exceed $167 billion US dollar by 2010 (Just-Food, 2004). The current demand for guar gum exceeds supply and guar is being introduced into new emerging areas. Owing to the enormous functions of guar gum, it has been observed that the U.S. imports more than 96 million pounds of guar gum from India and Pakistan annually (Pszczola, 2003). Table 4 shows current demand for some gums.

The probiotics market consists of food and beverages, dietary supplements and animal feed. This classification is based on the application of probiotics. The market for probiotic bacteria in foods and supplements is the most rapidly growing segment among consumer goods and expected to grow by 10 % each year, and its market value at over 100 Billion Euro per year. This rapid growth of probiotic market in the area of food, beverage and dietary supplements is as a result of increasing awareness of the health benefits of probiotics by consumers. Most of the probiotic bacteria used are *Lactobacillus* with one third being *Bifidobacteria* (Rascon-Diaz et al., 2012 . Tables 5 and 6 show the breakdown of the economic value of LAB.

Table 5

Seaweeds Hydrocolloids Sales Volume: Last Decade Growth (Bixler, 2010)

	1999		2009	
Gums	Amount (tons)	Costs (Million US$)	Amount(tons)	Cost (Million US $)
Agar	7,500	128	9,600	173
Alginates	23,000	225	26,500	318
Carrageenan	42,000	291	50,000	527
Total	72,500	644	86,100	1,018

Table 6

Economic Value of Fermentation Using LAB and Bifidobacteria (Rascon-Diaz et al., 2012)

Product	Global product value	Main bacterial genera
Cheese products	55 Billion	*Lactococcus and Lactobaccillus*
Yoghurt & Fresh Dairy	25 Billion	*Streptococcus and Lactobacillus*
Probiotic Products	20 Billion	*Lactobacillus and Bifidobacterium*

CHAPTER 5

Enzymatic Activity and Mechanism of Action of Gum Including α/β-galactosidase

5.1 α-galactosidase

5.1.1 Alpha-galactosidase. Alpha-galactosidase (EC 3.2.1.22), also named α-D-galactoside galactohydrolase, are hydrolyzing enzymes that catalyzes the hydrolysis of oligo- and polysaccharides hydrolyzes containing α-galactosyl groups. Alpha-galactosidase can be found in bacteria (for example: *Lactobacillus* and *Bifidobactria*), yeast (aspergillus oryzae and saccharomyces cerevisiae), (plants (example: legumes food or gums) and in mammals (Chen & Mustapha, 2012; Qian Ge, 2009). Alpha-galactosidase is intensely used in food processing applications, in the sugar industry, as additive in animal feed, in the paper industry, and in the field medicine (Zhou et al., 2010). Figure 25 shows the structure of *alpha-galactosidase.*

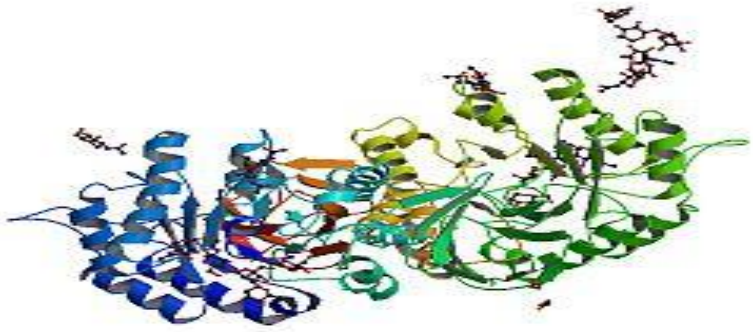

Figure 25. The structure of alpha-galactosidase. (http://www.ebi.ac.uk/pdbsum/1r46)

Alpha-galactosidase is capable of digesting galactomannans in leguminous seeds. Galactomannans consist of a main chain of 1,4-linked β-D-mannopyranosyl residues, most of which are substituted at O-6 with α-D-galactopyranosyl side chains. For example oligosaccharides (e.g., melibiose, raffinose, and stachyose) and galactomannans (e.g., guar gum and locust bean gum) can be digested by *α*-Galactosidase (1,6-*α*-D-galactoside galactohydrolase;

melibiase; EC 3.2.1.22). The α-linked or β-linked oligosaccharides component of α-galactosidase contributes to its use as a biomarker for microbial metabolic activity within the large intestine for potential prebiotics (Holt, Teresi, & Côté, 2008). The breakdown of gums could enhance the α-galactosidase activity of the probiotic microorganism.The enzymatic activity of α-galactosidase from bacterial sources can be affected by storage conditions, pH, temperature, carbohydrate sources, protein sources and presence of metal ions (Osaana N. Donkor, Henriksson, Vasiljevic, & Shah, 2007; Ibrahim et al., 2010b). Activity of α-galactosidase is shown in Figure 26.

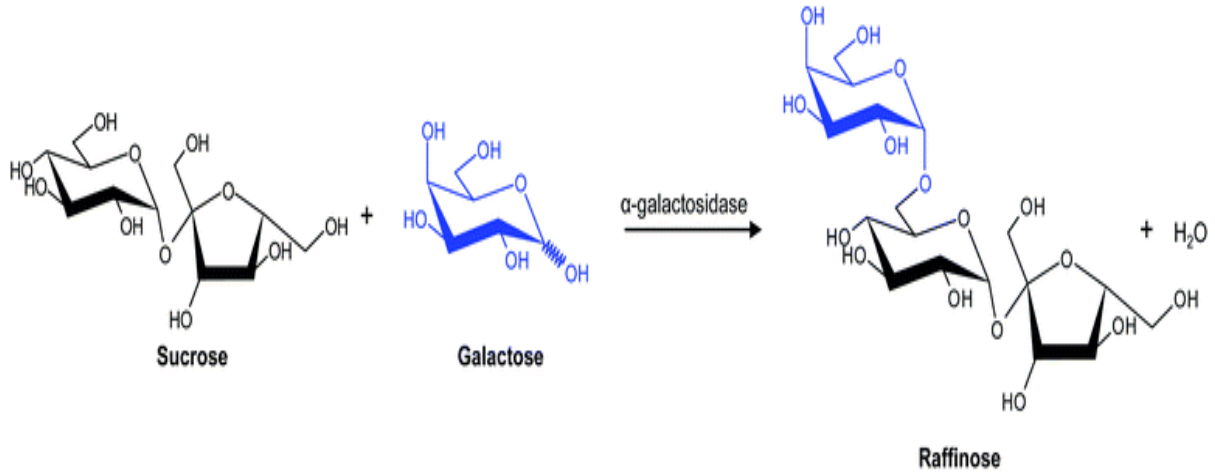

Figure 26. Biosynthesis of raffinose by alpha-galactosidase. (Daude, Remaud-Simeon, & Andre, 2012)

5.1.2 Health benefits of α-galactosidase. Apart from the numerous application of alpha-galactosidase in the food industry, *alpha-galactosidase* has also found several uses in the biomedical industry. Alpha-galactosidase is used in the treatment of Fabry's disease. Anderson-Fabry is a disease that is caused by deficiency of alpha galactosidase A and accumulation of globotriaosyl ceramide (Gb3) in cells throughout the body. It is the systemic deposition of glycosphingolipids caused by faulty X-linked glycolipid resulting from the deficiency of α-Galactosidase (Schiffmann et al., 2001). Fabry disease may manifest in fever of unknown origin and hypohidrosis that frequently lead to decreased exercise tolerance in children. In adolescents,

the disease diarrhea causes abdominal pain which results from eating fatty foods and vascular skin lesions termed angiokeratoma and asymptomatic corneal opacities. More severe effect of Fabry disease is renal failure (MacDermot et al., 2012; Schiffmann, 2009). Though the mechanism by which Fabry disease occurs is not clear some clinical trials has led to the improvement in renal function health of individuals with the disease (Hughes, 2010).

5.2 β-galactosidase

β-galactosidase (EC 3.2.1.23), originally obtained from *E. coli*, is an enzyme of great importance in the food industry (see Figure 27). β-galactosidase is used in the hydrolysis and transgalactosylation of β-D-galactopyranosides such as lactose (Liu et al., 2011). The ability of β-galactosidase to alleviate lactose intolerance is due to whey disposal in human and prevention of lactose crystallization in frozen dairy product.

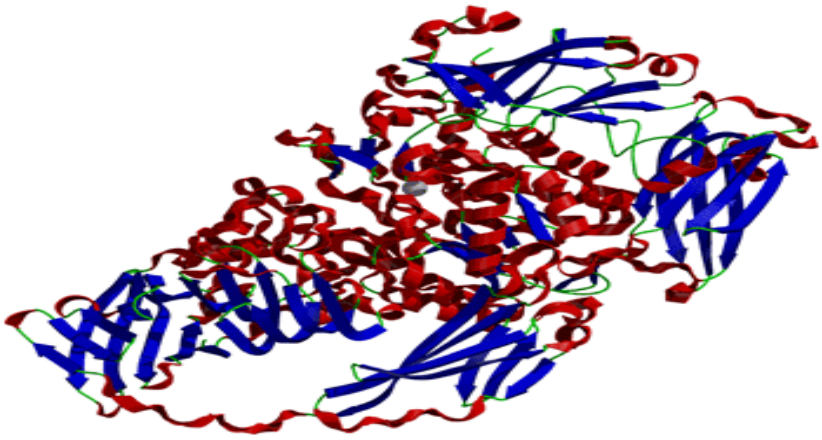

Figure 27. The structure of beta-galactosidase. (biochem.arizona.edu)

Transgalactosylation make it possible for β-galactosidase to catalyze the production of galacto-oligosaccharides (GOS). Lactose donates and accepts galactosyls groups to form GOS containing varying chain saccharide chain length of 2 to 8 monomeric units (Liu et al., 2011). Prebiotics activity of GOS to promote the growth of *Bifidobacteria* and other probiotics microorganisms with their associate health benefits have been studied extensively.

5.2.1 Sources of *β*-galactosidase. *β*-galactosidase is widely distributed and can be obtained from plants and animals. Other sources of *β*-galactosidase include microorganisms such as bacteria, yeast and fungi (Panesar et al.,, 2010; Zárate & Chaia, 2012). *β*-Galactosidases have been commercially produced from various sources, including, bacteria, yeast, fungi , animal organs and plants (see Figure 28). Bacteria source tend to be the most preferable. This is because *β*-galactosidase from bacteria is stable, easily fermentable, higher levels of activity could be attained. Other reason include they are obtained from food grade bacteria with their associated health benefits, the most prominent being management of lactose intolerance (Sriphannam et al., 2012). Table 7 shows a compilation of microbial sources of β-galactosidase.

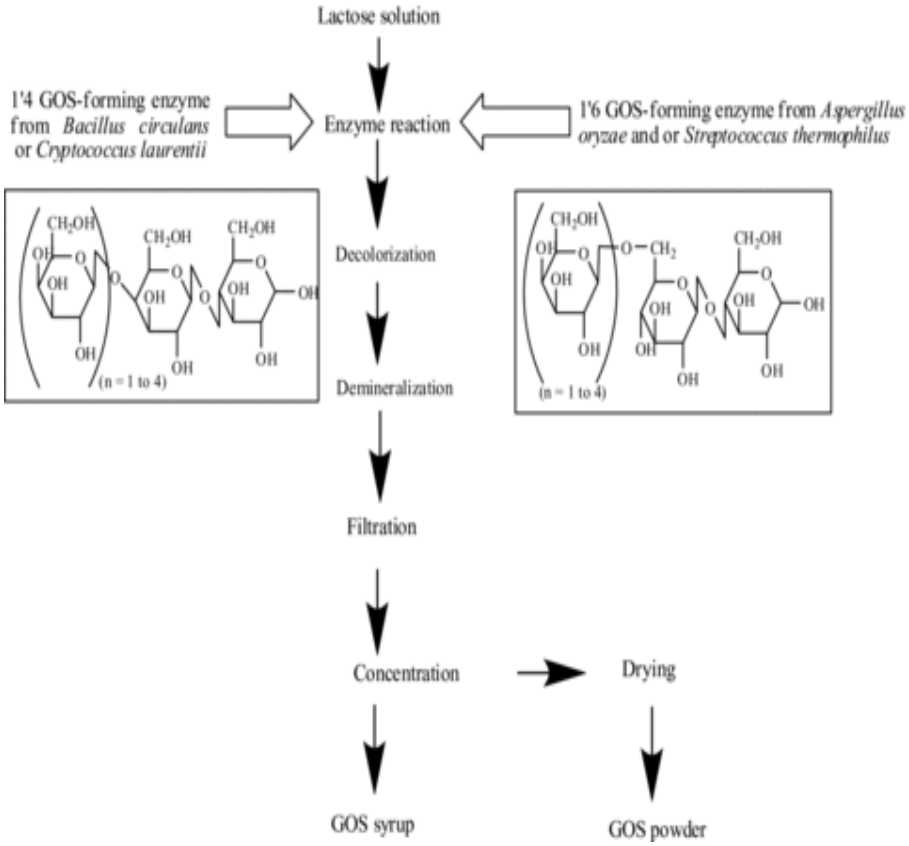

Figure 28. The industrial production of galactooligosaccharides. ("Graphical Abstracts," 2003)

Table 7

Microbial Sources of β-Galactosidases (Panesar et al., 2010)

Bacteria	Fungi	Yeast
Alicyclobacillus acidocaldarius subsp. *rittmannii* *Arthrobacter sp.* *Bacillus acidocaldarius, B. circulans, B. coagulans, B. subtilis, B. megaterum, B. stearothermophilus* *Bacteriodes polypragmatus* *Bifidobacterium bifidum, B. infantis* *Clostridium acetobutylicum, C. thermosulfurogens* *Corynebacterium murisepticum* *Enterobacter agglomerans, E. cloaceae* *Escherichia coli* *Klebsiella pneumoniae* *Lactobacillus acidophilus, L. bulgaricus,L. helviticus, L. kefiranofaciens, L. lactis, L. sporogenes, L. themophilus, L. delbrueckii* *Leuconostoc citrovorum* *Pediococcus acidilacti, P. pento* *Propioionibacterium shermanii* *Pseudomonas fluorescens* *Pseudoalteromonas haloplanktis* *Streptococcus cremoris, S. lactis, S. thermophius* *Sulfolobus solfatarius* *Thermoanaerobacter sp.* *Thermus rubus, T. aquaticus* *Trichoderma reesei* *Vibrio cholera* *Xanthomonas campestris*	*Alternaria alternate, A. palmi* *Aspergillus foelidis, A. fonsecaeus, A. fonsecaeus, A. Carbonarius, A. Oryzae* *Auerobasidium pullulans* *Curvularia inaequalis* *Fusarium monilliforme, F. oxysporum* *Mucor meihei, M. pusillus* *Neurospora crassa* *Penicillum canescens, P. chrysogenum, P. expansum* *Saccharopolyspora rectivergula* *Scopulariapsis sp* *Streptomyces violaceus*	*Bullera singularis* *Candida pseudotropicalis* *Saccharomyces anamensis, S. lactis, S. fragilis* *Kluyveromyces bulgaricus, K. fragilis, K. lactis, K. marxianus*

5.2.2 *β*-galactosidase activity of probiotic and health benefits. Novel application of *β*-galactosidase through the production of galacto-oligosaccharides has also been reported (Albayrak & Yank, 2002). In a study conducted by Santos et al. (1998) β-galactosidases produced by yeasts are mainly used in the fermentation of milk, whey and dairy products with neutral pH. This enzyme is responsible for the hydrolysis of oligo- and polysaccharides with β-D-galactopyranosides, respectively (Alazzeh et al., 2009). Probiotic microorganisms possess α-galactosidase, *β*-galactosidase and β-glucosidase, (Tochikura et al., 1986). These enzymes play a major role in the hydrolysis isoflavone glycosides to the bioavailable aglycones forms. The health benefits of bioactive isoflavone aglycones in post-menopausal women have been well documented. β-galactosidases are also produced commercially from yeasts *Kluyvermyces lactis* and *Kluyvermyces marxianus* and moulds such as *Aspergillus niger* and Aspergillus oryzae (Shaikh et al., 1997; Santos et al., 1998). The activity of the use of *lactobacillus* strains for the production of *α*-galactosidase and *β*-galactosidase has been studied over the years by a few researchers (Chowdhury, Chakraborty, & Raychaudhuri, 2008; Ibrahim et al., 2010a).
The focus of most of such studies was on *Lactobacillus acidophilus, Lactobacillus delbruekii and Lactobacillus fermentum* for the production of galactosidases. Alazzeh et al. (2009) studied how carbohydrate and protein source influenced the induction of α-Galactosidases and β-galactosidases in *Lactobacillus reuteri*. These authors used six strains of *Lactobaccillus reuteri* to induce the production α-galactosidases and β-galactosidases in six carbohydrate sources and four protein sources. They reported raffinose and lactose as the best carbohydrate sources for α-galactosidase and *β*-galactosidase respectively, yeast extract the best protein source for the production of both enzymes and CF2-7F the best producing strain. The stability of these enzymes in food is key to obtaining claimed health benefits. The present study examined the

production and activity of for *α*-galactosidase and *β*-galactosidase *Lactobacillus* species in the presence of some selected gums.

5.2.3 Lactose intolerance and the role of *β*-galactosidase. Lactose intolerance stems from poor absorption of milk sugar referred to as lactose due to malfunction of the hydrolyzing enzyme β-galactosidases. Lactose intolerance can occur under three main conditions; reduction in enzyme activity during childhood due to genetical condition, secondary lactose intolerance caused damage to the intestinal mucosa during treatment of disease, use of certain medication medications , surgery, or radiation and congenital lactose intolerance which is a rare condition of complete absence of the enzyme lactase from birth (Rusynyk & Still, 2001). Milk allergy is different from lactose intolerance. Milk allergy is an abnormal response to milk proteins and not to lactose by the human immune system. It also requires complete elimination of milk and milk products from the diet (Ibrahim & Gyawali., (2013).

Lactose is a disaccharide sugar (see Figure 29) that is found exclusively in mammalian milk). Low levels or absence of intestinal lactase results in poor absorption of lactose. The unabsorbed lactose is metabolized by colonic bacteria to produce gas and short chain fatty acids, causing flatulence, bloating, abdominal cramps and diarrhea (Panesar et al., 2010). Poor absorption of lactose does not always cause lactose intolerance. It is associated with the amount of lactose and rate of lactose available into in the colon. An estimated number of 75 % adult population worldwide experience problems of lactose intolerance. Among this group are 25% of people in Europe; 50-80% of people of Hispanic origin, people from south India, black people, and AshkenaziJews; and almost 100% of people in Asia and American Indians (Bhatnagar & Aggarwal, 2007).

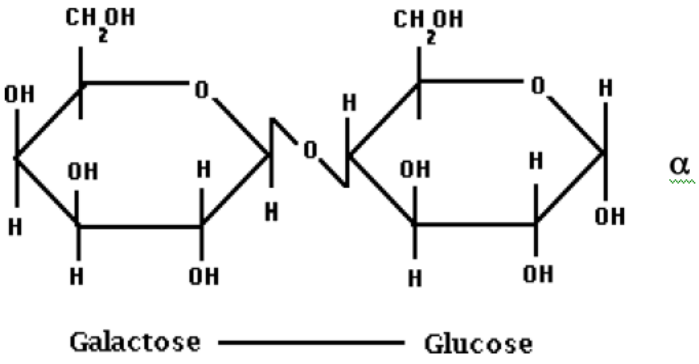

Figure 29. Chemical structure of lactose molecule. (www.uogulf.ca)

Individuals with inadequate enzyme β-galactosidase experience symptom such as abdominal discomfort when they consume milk or milk products. The unabsorbed lactose is metabolized by colonic bacteria to produce gas and short chain fatty acids, causing the clinical syndrome of abdominal cramps, bloating, diarrhea, and flatulence (see Figure 30).

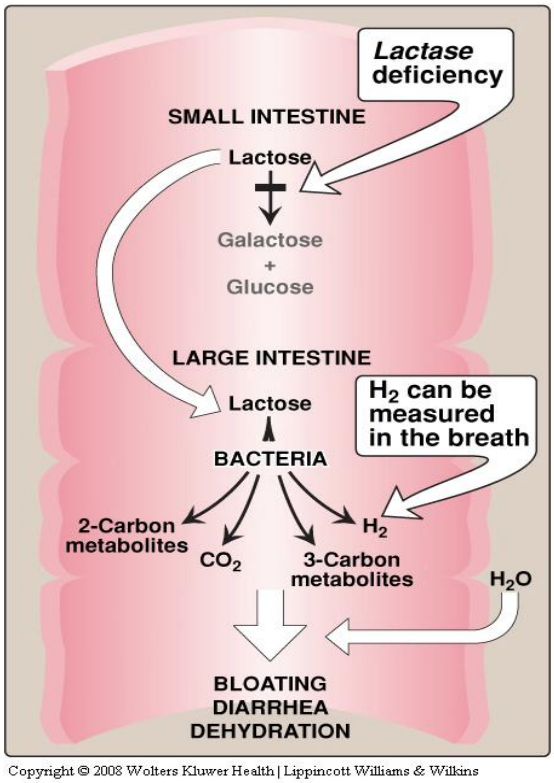

Figure 30. Mechanism of lactose intolerance. (http://chemistry.ewu.edu/jcorkill/biochem/lactose_intest.jpg)

Lactose malabsorption does not always cause lactose intolerance; symptoms depend on the amount and rate of lactose reaching the colon, and the amount and type of colonic flora. Disaccharide lactose, the main carbohydrate in milk requires β-galactosidase which is an enzyme that catalyzes the hydrolysis of lactose in the small intestine to monosaccharides galactose and glucose (Ibrahim et al., 2010a). Lactase activity is high in infants whose main diet is milk and decrease with age. However, in healthy adults lactase activity persists at high level throughout adult life as these individuals are able to digest lactose in fresh milk without complication. Lactose intolerant individuals have much lower lactose digestion capacity which affects their milk consumption. However, these individuals are able to digest fermented products such as yogurt since most of the lactose in the yogurt is converted into lactic acid by the cultures (Swallow, 2003).

To digest lactose in the intestinal tract the probiotics and their *β*-galactosidase must be viable and survive gastrointestinal stresses and processing technology (Totosaus et al., 2013). Thus, the benefits obtained by lactose intolerant individuals are two ways. There is increased lactase activity by probiotic cultures and lowering of lactose concentration in the fermented food (Fudala et al., 2003). Researchers have used different method to enhance β-galactosidase activity. Chowdhury et al. (2008) have fortified yogurt using different types of herbs which showed enhanced β-galactosidase activities in comparison with the control yogurt. Ibrahim et al. (2010a) demonstrated that modifying culture medium with small amount of manganese ion (Mn^{+2}) and certain carbohydrates and protein can promote α and β-galatosidase production in certain *Lactobacilli*. Probiotics have been very effective in the treatment of lactose intolerance. Table 8 show a summary of numerous studies supporting the use of probiotics in the in different

food in the treatment of lactose malabsorption. There is a need to study the effect of gums on β-galatosidase level of *Lactobacillus* strains in milk.

Table 8

Effectiveness of Probiotics for the Treatment of Lactose Intolerance (Ibrahim & Gyawali, 2013)

Experimental treatments	Associated actions	References
Non-fermented milk with yogurt culture	Improved lactose digestion	(Lin et al., 1991)
Fermented dairy products (Butter milk, yogurt, cottage cheese)	Well tolerated by lactose intolerant	(Gallagher et al., 1974)
Ingestion of lactose in milk, water or in yogurt	Lactose in yogurt resulted in about one-third of the H_2 breath excretion compared to water or milk	(Kolars et al., 1984)
Ingestion of yogurt with living bacteria vs. ingestion of heated yogurt	Better lactose digestion after ingestion of living bacteria	(Gilliland & Kim, 1984; Savaiano et al., 1984)
	Fecal β-galactosidase activity increased in lactase deficient subjects who ingested heated yogurt indicating significant amount of lactose in colon	(Pochart et al., 1989)
Yogurt consumed with or without meal	Reduced lactose maldigestion	(Martini et al., 1991a)
Yogurts made with Streptococcus thermophilus and Lactobacillus bulgaricus consumed with or without a meal	Improvement in lactose maldigestion was similar regardless of strains	(Martini et al., 1991b)
Milk with sonicated *Lactobacillus acidophilus*	Improvement of lactose digestion	(McDonough et al., 1987)

Table 8

(Cont.)

Experimental treatments	Associated actions	References
Ingestion of lactase isolated from yeasts and molds in the form of capsules	Increases lactose digestion	(Corazza et al., 1992)
S. thermophillus produces β-galactosidase during its transit in the digestive tract of mice	Reduces the lactose content in digestive tract	(Drouault et al., 2002)
Lactobacillus strains	Lactose digestion improved, decrease diarrhea and symptoms of lactose intolerance	(Marteau et al., 2001)
Lactobacillus plantarum	Reduced bloating, flatulence, and pain in irritable bowel	(Nobaek et al., 2000)
Saccharomyces boulardii (yeast)	Decreased only functional diarrhea, but not any other symptoms of irritable bowel syndrome	(Marteau et al., 2001)
Milk containing *Lactobacillus acidophilus*	Not effective than regular milk in reducing gastrointestinal symptoms in the study of self reported lactose intolerant individuals	(Semenza et al., 2001)
Fermented semi solid milk containing *L. acidophilus, Bifidobacterium* sp. and yogurt bacteria	No differences in the digestibility	(Marteau et al., 1997)

5.3 Mechanism of β-galactosidase in the Production of GOS

Microbial β-galactosidase is very important in transgalactosylation and hydrolysis of lactose to produce forms of galactooligosaccharides. Lactose hydrolysis and transgalactosylation are complex processes involving sequence of reactions which leads to the formation of GOS, and

many intermediate products such as glucose and galactose (Figure 31) ("Graphical Abstracts," 2003; Torres, Gonçalves, Teixeira, & Rodrigues, 2010). The normal function of β-glycosidases is to hydrolyze substrates formed by a monosaccharide coupled by a β bond (β1′4 and β1′6 more common and β1′2 and β1′3 rarely) to another polyol (see Figure 31). However, under certain conditions, the same enzymes also catalyze the transgalactosylation reaction and synthesize GOS. The main problem of oligosaccharide synthesis by these enzymes is that the reaction equilibrium is shifted to favor hydrolysis over synthesis in aqueous systems, which leads to a low yield in GOS production ("Graphical Abstracts," 2003).

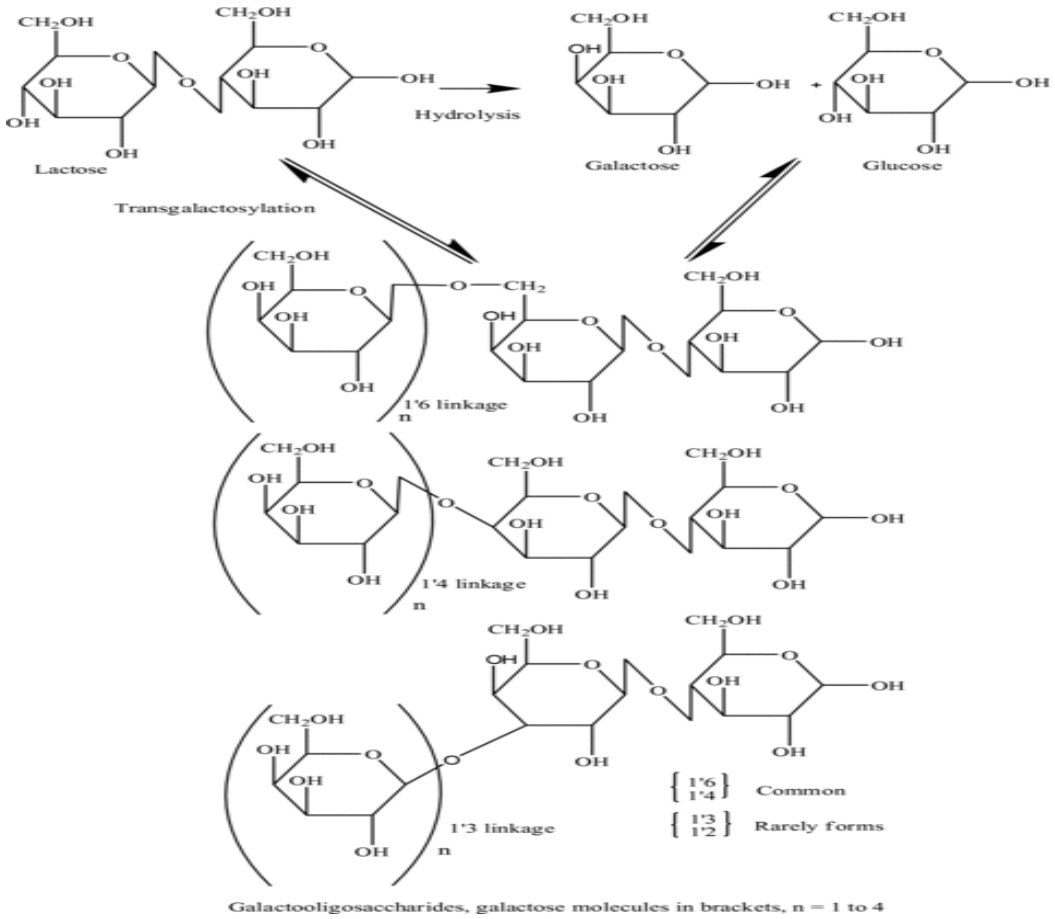

Figure 31. Synthesis of β-galactooligosaccharides from lactose using microbial β-Galactosidases. ("Graphical Abstracts," 2003)

CHAPTER 6

Impact of Selected Gums on the Growth of *Lactobacillus* Strains in Laboratory Media[2]

6.1 Abstract

Probiotics are increasingly being used as dietary ingredients in functional food products. The composition of food contributes to maintaining the growth of probiotics in food products. Gums are polysaccharides used as stabilizers and emulsifiers in foods and could also be used to enhance the growth of probiotics. Thus, the objective of this study was to determine the impact of different gums on the growth of *L. rhamnosus GG* B101, *L. rhamnosus GG* B103, *L. delbrueckii subsp. bulgaricus* SD35, *L. delbrueckii subsp. bulgaricus* SD33, *L. acidophilus* SD 16, *L. acidophilus* EF7, *L. reuteri* DSM20016, *L. reuteri* CF2-2F in laboratory media. Modified basal media was prepared with either 0%, 0.25% or 0.5 % (w/v) of one of the following gums: locust bean, guar-locust bean-carrageenan, guar, xanthan, carrageenan, carrageenan-maltodextrin, pectin, pectin-carrageenan, and inulin, pectin-dextrose. Samples with dextrose served as positive control and media without gum is the negative control. Modified basal medium was prepared and divided into 12 portions. Batches of 200 ml samples with either 1, or 0.5 g of each gum were dispensed at 10 mL and sterilized at 110 °C for 10 min. Sterilized samples were allowed to cool to 42 °C and then inoculated with *Lactobacillus* culture at a final inoculum level of 3 log CFU/ml. The inoculated samples were incubated at 37 °C for 12 h, serially diluted, and plated on MRS agar to obtain final bacterial counts. Results showed that guar at 0.5% concentration stimulated growth of strains (*L. rhamnosus* GG B101, *L. rhamnosus* GG B101, *L. delbrueckii* subsp. *bulgaricus* SD35, *L. delbrueckii* subsp. *bulgaricus* SD33, *L. acidophilus* SD 16, *L. acidophilus* EF7, *L. reuteri* DSM20016, *L. reuteri* CF2-2F) during storage

[2] Parts of this chapter were adopted from: Karlton-Senaye, B. D., & Ibrahim, S. A. (2013). Impact of gums on the growth of probiotics. Agro FOOD Industry: Functional food and Nutraceuticals, 24:4.

at 37 °C in laboratory media stored for 12 hours at 37 °C. Generally, bacterial populations of samples with gums were significantly ($p < 0.05$) higher than those of the control without gums except for *L. delbrueckii* ssp. *bulgaricus* SD 33 *and L. acidophilus* EF7, which showed no significant differences in the presence of the gums. Addition of all tested gums led to increases of 0.94 to 1.31 log CFU/mL in the population of *L. rhamnosus* GGB101 over the control. Among tested gums, guar induced the highest (1.31 log CFU) increase in growth of *L. rhamnosus* GGB101 (8.45 ± 0.042 Log CFU/mL) compared to the control (7.14 ± 0.056 log CFU/mL) whereas *L. acidophilus* EF7 exhibited the least population (5.78 ± 0.014 Log CFU/mL) in the presence inulin. The population of *L. rhamnosus* GGB101 in the samples containing gums were significantly ($p < 0.05$) higher than the control without gums. *L. rhamnosus* GGB101 was the best strain in terms of growth in the media. Populations of *Lactobacillus* of all strains were > 6 log10 cfu/mL, the required level for health benefits, except for *L. acidophilus* EF7, that were slightly lower. Addition of gums at 0.5% led to higher bacterial population compared to 0.25% gum. Longer incubation period of 12 hour resulted in higher bacterial population compared to 6 hours on incubation. Titratable acidity and pH of all samples containing different concentration of gums were similar to the control. In another studies in which bacterial population in milk was compared to those in modified basal media, population of the *L. rhamnosus* GGB101, *L. rhamnosus* GGB103 strains were higher in milk than in the media, whereas *L. reuteri* DSM20016 were higher in the media than in milk. Xanthan induced highest growth of *L. rhamnosus* strains in milk whereas carrageenan-maltodextrin led to highest growth of *L. reuteri* DSM 20016 in the media. Findings from this study could help the dairy industry to include guar, xanthan and carrageenan-maltodextrin as probiotic ingredients to improve the quality of functional food.

6.2 Introduction

Gums are complex polysaccharides extracted from sources such as the endosperm of plant seeds, plant exudates, sea weeds, bacterial and animal sources (MacDermot et al., 2012; Zárate & Pérez Chaia, 2012). Gums are also polymers with hydrophilic ability due to the presence of a hydroxyl bond. The composition and structure of gums enable them to imbibe large amounts of water, forming a gel. It is this hydrophilic characteristic that makes gums useful in the food industry. They are used as stabilizers to improve viscosity and texture by preventing "wheying off" (De Castro et al., 2003). Gums also contribute fiber, enhance sensory qualities of foods (Heydari et al., 2011), and promote growth in probiotics (Karlton-Senaye & Ibrahim, 2013). In addition, gums are used in other industries, namely as thickeners in the pharmaceutical, cosmetic, paint, inks, paper, color and adhesive industries (Heydari et al., 2011).

A prebiotic is defined as a ‘‘non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health’’(Gibson et al., 2004). Techniques that combine both probiotics and prebiotics are called synbiotics. Symbiosis is a combination of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth of probiotics and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare (Ferrero et al., 1993; Maurer-Menestrina et al., 2003). Many natural ingredients have been approved as prebiotics that promote the growth and viability of lactic acid bacteria (LAB) in dairy products (Ranadheera et al., 2010). However, inulin and fructooligosaccharide (FOS) have been the most extensively studied prebiotics (Akalin et al., 2004; Donkor et al., 2007; Gibson et al., 2004; Oliveira et al., 2009b).

Probiotic microorganisms promote a healthy gastrointestinal tract. These health benefits include reinforcement of gut mucosal immunity, decreased risk associated with mutagenicity and carcinogenicity, alleviation of lactose intolerance, acceleration of intestinal mobility, hypocholesterolemic effects, reduced duration of diarrhea, prevention of inflammatory bowel disease, prevention of colon cancer, inhibition of intestinal pathogens, and treatment and prevention of allergies (Maganha et al., 2013). However, it must be noted that these microorganisms are vulnerable to various stressful physical and physiological conditions to which they are subjected during fermentation, storage, and digestion processes. The survival of probiotic bacteria is also dependent on the composition and by-products of the fermentation of food and the interaction between different strains of bacteria (Yeo & Liong, 2010). To create the required microbial balance and promote the health functionality of probiotic bacteria, there is a need to consume and maintain a minimum amount of 10^6 CFU per ml of probiotic cultures in food daily (Quigley2010). To attain this level, the consumption of 100 g of products containing 10^6–10^7 live cells has been suggested (Yeo & Liong, 2010). Gums, like other polysaccharides, contain the necessary food ingredients of carbohydrates, sugars, salts and minerals that could support the survival of probiotic organisms in food and subsequently in the gut. This level of probiotics creates gastrointestinal balance, positively impacts intestinal health and provides other health benefits. Limited studies have shown the impact of gums on probiotics. For example, one study conducted by Ghasempour et al. (2012) showed that zedo gum did not improve viability of probiotic bacteria although minimum therapeutic levels of 10^6–10^7 cfu／g were maintained (Ghasempour et al., 2012). In several other studies, inulin, being a recognized probiotic, was reported to enhance the growth and viability of probiotic bacteria (Donkor et al., 2007; Ranadheera et al., 2010). Though gums are known to improve the texture of food, limited

studies have been done using gums to enhance the growth and viability of probiotics bacteria. The aim of this work was to study the impact of gums of *Lactobacillus* strains in a laboratory medium.

6.3 Materials and Methods

6.3.1 Culture activation and preparation. *Lactobacillus* strains in table 9 were obtained from the culture collection of the Food Microbiology and Biotechnology Laboratory at North Carolina Agricultural and Technical State University and were activated in MRS broth (Neogen Corporation, Lansing, MI) by transferring 100 µL of cultures to 10 mL of sterile MRS broth and incubating at 37 C for 24 h. Activated strains were then stored at 4°C. Prior to each experiment, each individual strain was streaked plated onto MRS agar and incubated for 48 h at 37 °C.

Table 9

Lactobacillus Strains and Their Sources of Origin

Strains	Species	Original source
Lactobacillus delbrueckii	subsp. *bulgaricus* SD33	Yogurt culture
	subsp. *bulgaricus* SR35	Yogurt culture
Lactobacillus acidophilus	SD 16	Commercial source
	EF7	Commercial source
Lactobacillus reuteri	DSM20016	Mother's milk
	CF2-2F	Mother's milk
	SD2112	Mother's milk
Lactobacillus rhamnosus GG	B103	Child fecal isolate
	B101	Child fecal isolate

6.3.2 Preparation of MRS broth and agar. *Lactobacillus* MRS (DeMan Rogosa Sharp) broth was prepared by dissolving 55g of MRS and 0.5g of L-cystein in 1 L of distilled water.

MRS agar was prepared by adding 14 g agar. After complete dissolution, the media was sterilized for 15 min at 121 ºC.

6.3.3 Preparation of modified basal media. Modified basal media (Salvini et al., 2011) was prepared by dissolving Proteose peptone (1.0 g), Yeast extract (1.0g), Tween 80 (0.5 ml), Dextrose (0.5 g), L-cysteine (0.35 g), Arginine (0.25g), Sodium dihydrogen phosphate Na_2HPO_4 (1 g), Sodium acetate (4 g), $MgSO_4$_7H2O (0.05 g), $MnSO_4$_5H2O (0.025 g) in 1L of distilled water and sterilized at 110 ºC for 10 min.

6.3.4 Maintenance of culture. All strains were preserved in a frozen form and stored at -80ºC in MRS (DeMan Rogosa Sharp). The glycerol stocks of samples were prepared by mixing 0.5 ml of active cultures and 0.5 ml of sterile glycerol. Active cultures were streaked plated and stored at 4°C. Before use, single colony of each strain were activated separately in freshly prepared MRS broth and anaerobically incubated for 16 h at 37°C. Each activated train was serially diluted three times and 1 ml was aseptically withdrawn from the appropriate dilution and inoculated into each of the sterilized samples.

6.3.5 Sample preparation and treatment. Two liter of modified basal media was prepared and divided into 10 ml portions. Each 10 mL batch of modified basal media with different gums; Tic Pretested® Pectin AM 800 (pectin-destrose, PD), Ticaloid® 710H Powder (carrageenan, CA), Ticaloid® 750 (carrageenan-maltodextrin, CM), Tic Pretested® Pectin 1400 (pectin, PE), Tic Pretested® locust bean gum POR/A2 Powder (locust bean, LB), FASTir® Xanthan EC (xanthan, XA), Dairyblend YG SP (pectin-carrageenan, PC), Dairyblend SC FT Powder (guar, locust bean, carrageenan, GL), Pre- Hydrated® Guar Gum 8/24 Powder (guar-GU) and Pretested Inulin LV 110 Powder (inulin, I) (TIC GUMS INC, Maryland, GA). was sterilized at 110°C for 10 min (Filippo Salvini & Giuseppe Banderali, 2011). Sterilized samples was

allowed to cool to 42°C and inoculated with *Lactobacillus* strains. One milliliter (1mL) of each suspended culture was transferred to 9 mL peptone mixed by vortex and serially diluted. Aliquots (1 mL) from the third dilution tube were added to fresh modified basal (10ml) containing different concentrations of gums. Inoculated samples were plated on MRS agar and anaerobically incubated at 37 ºC for 48 h to determine initial bacterial counts. Inoculated samples were incubated at 37 °C. After incubation, serially diluted aliquots were aseptically withdrawn at 6 and 12h and plated on MRS agar to obtain final bacterial counts. Sample with dextrose served as positive control and negative control was sample without gum.

6.3.6 Bacterial enumeration. Samples were serially diluted and 100 µl of appropriate dilution was plated on MRS agar. Plates were incubated anaerobically at 37 °C for 48 h and plates having 25-250 colonies were counted to determine bacterial population.

6.3.7 Determination of pH. Each sample was withdrawn after incubation to measure pH. The pH meter (Accumet basis, AB15/15+, Fisher Scientific) was calibrated with pH standard buffers 4.0 and 7.0. After calibration, sample pH was taken and recorded. Electrode was rinsed with distilled water between different samples.

6.3.8 Determination of titratable acidity. Samples were titrated with pH meter (Accumet basis, AB15/15+, Fisher Scientific) according to AOAC method (AOAC, 1995). Prior to the determination of titratable acidity the pH meter was standardized using pH 7 and pH 4 standard buffers. The pH of a100 mL distilled water was adjusted to 8.2 using 0.1N sodium hydroxide (NAOH) solution. Five milliliter (5mL) samples were added to the distilled water followed by the gradual addition of 0.1 N NaOH to reach pH 8.2. The volume of NaOH was recorded and titratable acidity was calculated according to the method by Vargas and Ohashi (1996).

$$\text{Titratable acidity} = \frac{\text{Volume (mL) of NaOH (N of NaOH) (7.5)}}{\text{Sample volume (mL)}} \quad \text{(Eq. 1)}$$

6.3.9 Statistical analysis. Experiments were replicated in a randomized block design to evaluate the growth of *Lactobacillus* strains in modified basal media containing gums compared to the control without gums. Statistical analysis of data was performed using R-Project for Statistical Computing version R-2.15.2 (http://www.r-project.org website). Means for all variables were calculated and one way ANOVA (analysis of variance) was used to determine significant ($p < 0.05$) differences. Letters a, b, c, d, and e indicates that results within the same determinations are significantly differences.

6.4 Results and Discussion

6.4.1 Growth of *Lactobacillus* strains in modified basal media. In this study, ten different gums (CA, CM, GU, LB, PE, PD, PC, CL, GP, and XA and dextrose; positive control) were added to media to enhance the growth of eight *Lactobacillus* (*L. rhamnosus GG* B103, *L. rhamnosus GG* B101, *L. delbrueckii subsp. bulgaricus* SD35, *L. delbrueckii subsp. bulgaricus* SD33, *L. acidophilus* SD 16, *L. acidophilus* EF7, *L. reuteri* DSM20016, *L. reuteri* CF2-2F) strains at three different concentrations of 0%, 0.25%, 0.5% during 12h and 6h incubation at 37 °C (see Appendixes A to H). Samples without gums served as megative control. Titratable acidity and pH of the basal media containing different concentrations of gums were also studied.

6.4.1.1 Growth of Lactobacillus strains in modified basal media at 0.5% gum stored at 37 °C for 12h. Appendix A shows the growth of *L. rhamnosus* GG B103, *L. rhamnosus* GG B101, *L. delbrueckii subsp. bulgaricus* SD35, *L. delbrueckii subsp. bulgaricus* SD33, *L. acidophilus* SD 16, *L. acidophilus* EF7, *L. reuteri* DSM20016, *L. reuteri* CF2-2F in the presence of gums at 0.5% during 12h incubation at 37 °C. The average population of all tested *Lactobacillu*s strains varied from an average of 5 to 8 log CFU/mL. Generally, bacterial

populations of samples with gums were significantly ($p < 0.05$) higher than those of control without gums except for *L. delbrueckii* ssp. *bulgaricus* SD 33 *and L. acidophilus* EF7. Only a slight difference among bacterial population of sample with gums compared to the control was observed. *L. acidophilus EF7* had the least population between the range of 5 and 6 log CFU/mL. The population of all the tested *Lactobacillus* strains grown in the presence of dextrose and guar were slightly higher than those in the control. Addition of dextrose led to the highest population of *L. rhamnosus* GG B101 (8.47 ± 0.06 log CFU/mL), an increase of 1.33 log CFU/mL, over the control (7.14 ± 0.06). This observations were expected as *Lactobacillus* are able to metabolized sugar such as dextrose which is more readily available carbon source for bacterial metabolism due to their lower molecular weight compared to branched, long carbon chain and higher molecular weight polysaccharides such as gums. Among tested gums, guar exhibited the highest (1.31 log CFU) increase in growth of *L. rhamnosus* GGB101 (8.45 ± 0.042 Log CFU/mL) compared to the control (7.14 ± 0.056 log CFU/mL) whereas *L. acidophilus* EF7 showed the least population (5.78 ± 0.014 Log CFU/mL) in the presence of inulin (a well-known prebiotic). Addition of tested gums led to increases of 0.94 to 1.31 log CFU/mL in the population *L. rhamnosus* GGB101 over the control portraying *L. rhamnosus* GGB101 as the best strains in terms of growth. Guar showed slightly higher (1.31 log CFU/mL) growth among all treated strain over the control. The population of *L. rhamnosus* GGB101 in the samples containing gums were significantly ($p < 0.05$) higher than the control without gums. According to Hernandez-Hernandez et al. (2012) the length of carbon chain in carbohydrates such as galactooligosaccharides affects digestibility. The longer the carbon chain the slower the digestibility which subsequently impact on the growth of probiotics. Gums, being polysaccharides may exhibit similar trend (Hernandez-Hernandez, 2012). Guar impacted more

growth due to its relatively shorter chain length resulting in faster digestibility and making nutrients available for probiotic bacteria within the short incubation period.

Similar bacterial population of *Lactobacillus* strains observed in gums and dextrose is an indication that gums could serve as good replacers of dextrose and possibly other sugars in food as a healthier alternative due to the numerous health benefits of gums apart from their fiber contents (Al-Ghazzewi et al., 2007; Moosa, 2006; Roberts, 2011). Many studies on the growth of *Lactobacillus* have been reported (S. Hekmat et al., 2009; Oliveira, Perego, Converti, & De Oliveira, 2009a). The findings in this study are in agreement with a research conducted by Olivera et al., (2009) who found out that polydextrose and olygosaccharides have enhanced the growth of *L. acidophilus, L. rhamnosus* and *B. animalis* subsp. *Lactis.* In our study we found out that inulin stimulated the least growth of *L. acidophilus* EF7 in the media. Contrary to our findings, inulin remarkably stimulated the growth of *L. rhamnosus, L.acidophillus and L. Lactis* in a mono culture and in a co-culture with *S. thermophillus* (Ranadheera et al., 2010).

6.4.1.1.1 The pH profile of modified basal media containing 0.5% gum at 37°C for 12h. The average initial pH of samples ranged from 5.93 to 7.38 (data not shown). No marked ($p < 0.05$) difference in population of *Lactobacillu*s strains was found in the treated samples and the control. However, the pH values of media containing tested strains were slightly lower varying from 4.85 to 5.67 (Appendix B). The lower pH of the media containing gums could be due to slightly higher metabolic activity of the *Lactobacillu*s strains due to the introduction of more nutrients by tested gums. The low pH of treated samples corroborated with the slightly higher *Lactabacillus* population observed in the presence of the gums and dextrose. This is because most *Lactobacillus* strains grow within a pH range of 5 and 6. A few, however, could grow at a pH of 4.4 (Von Wright & Axelsson, 2011). Only a slight decrease in the pH values was

observed. Slight decrease in pH was expected due to short incubation period of 12 hours resulting in decrease in metabolism and acid production. Generally *Lactobacillus* grow relatively slowly and thus low metabolic activity within twelve hours of incubation resulting in low acidity with its associated low levels of organic acid was expected. Lower acidity observed in the treated samples also related to the slight increase in the bacterial population in the treated samples compared to the control. This is because acidity could be evaluated as an indirect characteristic of the growth of the tested *Lactobacillus* strains.

6.4.1.1.2 Titratable profile of modified basal media containing 0.5% gum at 37°C for 12h. Generally, the TA levels of samples during 12 hour of incubation at 37 °C ranged from 0.08% to 0.5%. Similar levels of TA values were observed in all samples with no significant ($p < 0.05$) difference in TA of samples containing gums and the control during the 12 hour incubation period corroborating the lower pH. Low TA values were observed in all samples. However, the TA values of dextrose were slightly higher. The low titratable acidity was expected due to relatively stable pH values. The amount of lactic acid produced could be evaluated as indirect indication of growth of *Lactobacillus* strains. TA showed the same pattern as the pH indicating no buffering effect.

6.4.1.2 Growth of Lactobacillus strains in modified basal media at 0.25% gum at 37 for 12h. The population of *Lactobacillus* strains in the presence of gums at 0.25% during 12h incubation at 37 °C is shown in Appendix C. The population of tested strains varied 5.92 to 8.35 log CFU/mL with locust bean stimulating the highest (8.41 log CFU/mL) growth of *L. reuteri* DSM20016. Generally, the population of tested *Lactobacillus* strains observed in the samples with 0.25% gums was slightly lower than those in samples containing 0.5% of gum (see Figures 32 and 33).

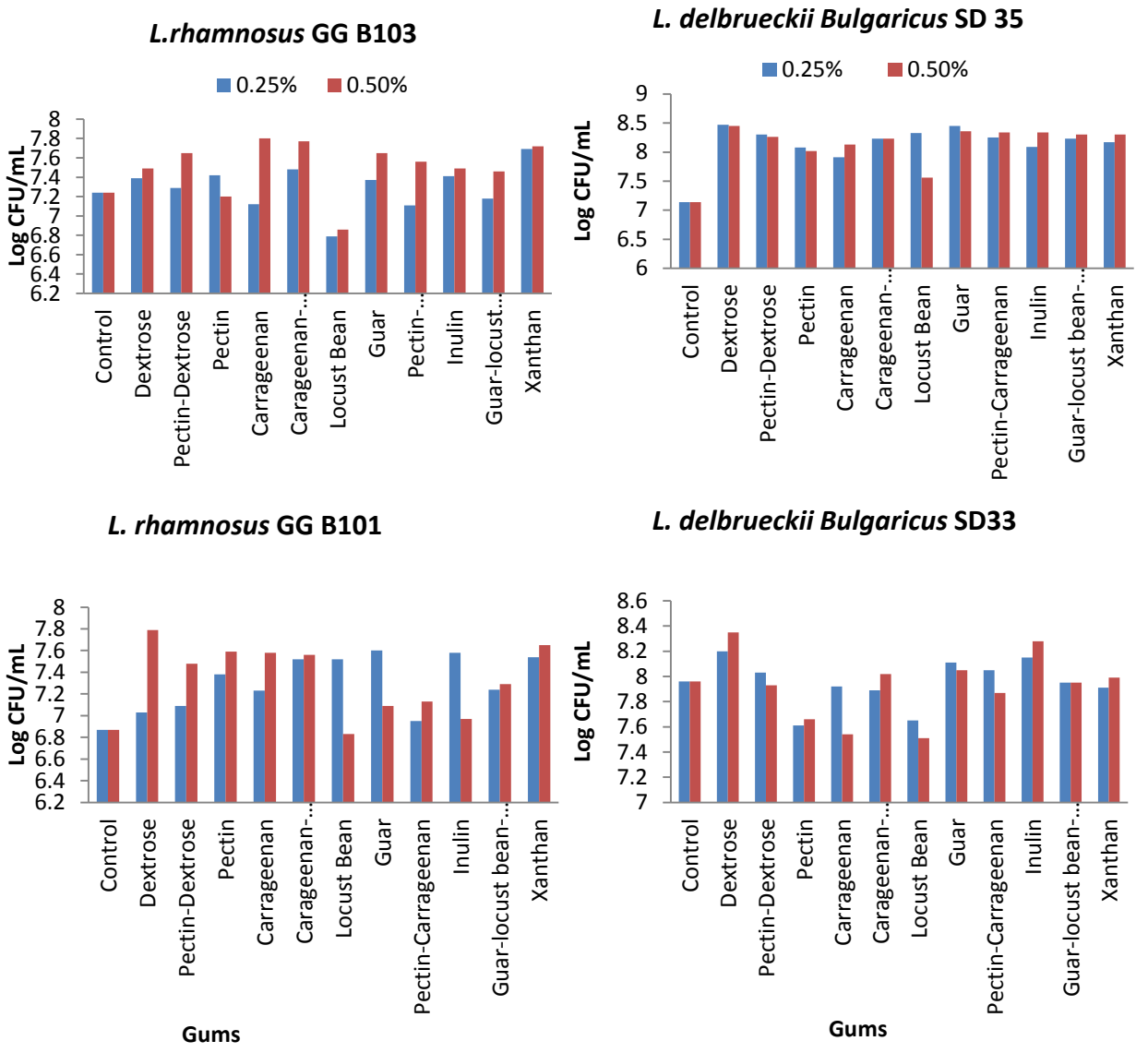

Figure 32. Population of Lactobacillus in modified basal media containing different gums incubated at 37 °C for 12 h.

However, guar and locust bean induced more growth at 0.25% concentration in the bacterial population for *L. rhamnosus* GG B101, *L. delbrueckii* subsp. sulgaricus SD35, *L. delbrueckii* subsp. *sulgaricus* SD33, *L. reuteri* DSM20016 in laboratory media at 0.5% concentration of the gums. This is an indication that guar and locust bean could be impact growth of probiotics such as *L. rhamnosus* GG B101, *L. delbrueckii* subsp. *sulgaricus* SD35, *L.*

delbrueckii subsp. sulgaricus SD33, *L. reuteri* DSM20016 at very low concentration. This information may be useful and appealing to the dairy industry as it may cut down on cost of ingredients. The ability of guar gum to enhance growth (above 7 log CFU/mL) of the probiotic bacteria at a very low concentration levels could be due to its digestibility as a result of shorter chain and high nutrient content.

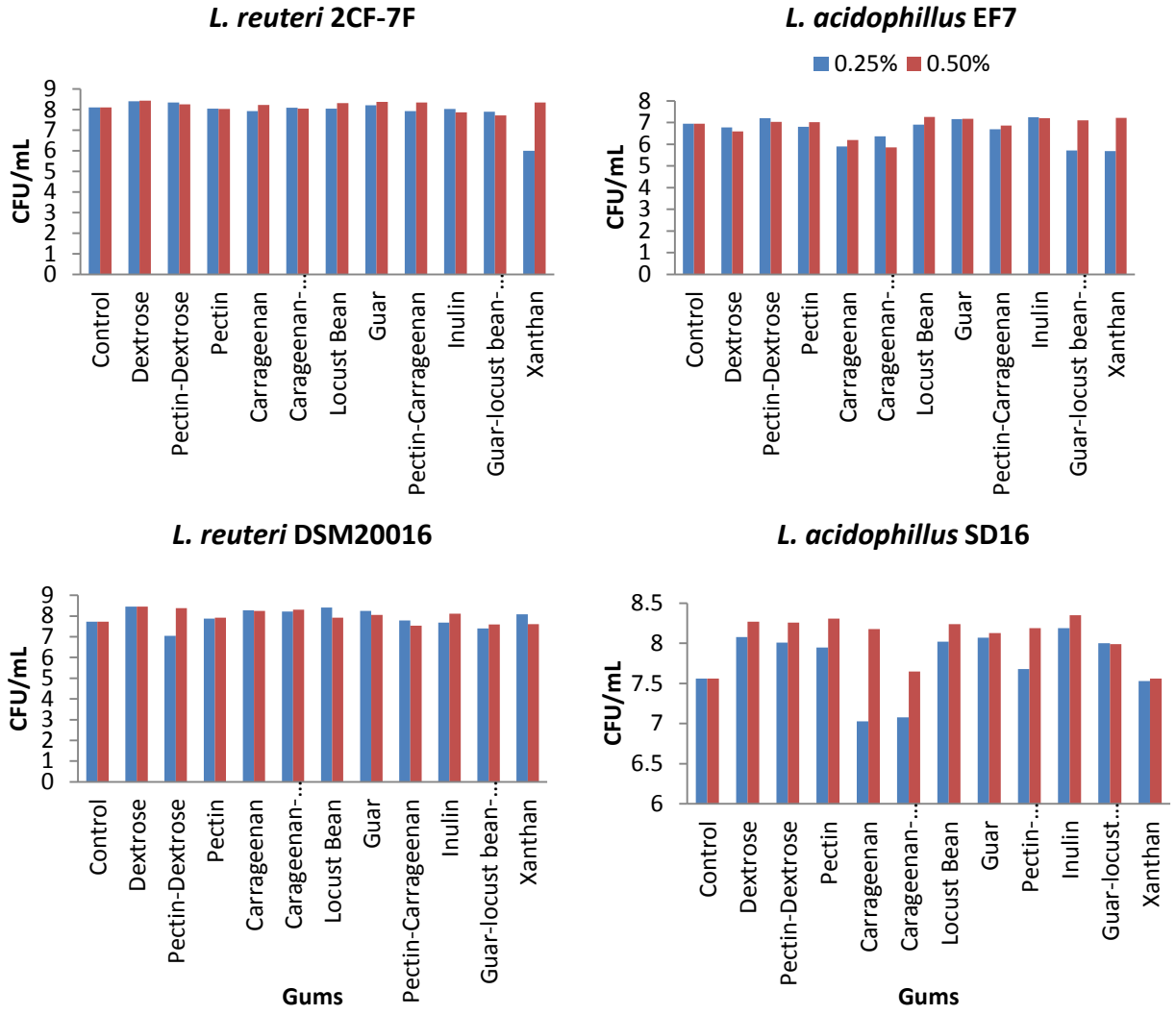

Figure 33. Population of Lactobacillus in modified basal media containing different gums incubated at 37 °C for 12 h.

These observations are expected because more gums mean more nutrients for bacterial growth. The availability of less gums resulted in slightly lower population of *Lactobacillus*

strains. The addition of gums was found to have lesser influence on the population of *L. acidophilus* EF7 resulting in lower population. Generally, the population of tested strains in the presence of gums was slightly more than the control. Addition of dextrose showed similar growth levels across all strains compare to samples with gums, indicating that gums could serve as healthier replacers of dextrose in probiotic food product.

6.4.1.2.1 The profile pH values of modified basal media containing 0.25% gum stored at 37 °C for 12h. The pH of the sample ranged from 5.27 to 7.69 (Appendix D). Lower pH values were observed with *L. reuteri* strains, which accounts for the high population of this strains. Slightly higher pH was observed in sample containing gum compared to the control. This could be due to alkaline nature of most gums. Low pH is associated with high bacterial growth.

6.4.1.2.2 Titratable acidity of modified basal media containing 0.25% gum stored at 37 °C for 12h. TA values ranged from 0.08% to 0.25%. Similar TA values were observed in all samples. Higher TA values were observed in samples fermented with *L. reuteri* which led to lower pH values in these samples. It was observed that TA was not influenced by the higher (0.5%) concentration of gums resulting in similar TA values in sample with 0.25% or 0.5% of gums.

6.4.1.3 Growth of Lactobacillus strains in modified basal media at 0.5% gums stored at 37 for 6h. The growth of *Lactobacillus* strains in the presence of gums at 0.5% during 6h incubation at 37 °C is depicted in Appendix E. Lower *Lactobacillus* population were observed in samples incubated for 6 hours compared to those incubated for 12 hours. Average growth among all strains ranged from 4 Log CFU/mL to 7 log CFU/mL. The addition of pectin-dextrose led to the highest (1 log increase) growth in *L. delbrueckii* subp. *bulgaricus* SD33 (7.26 Log CFU/mL Log CFU/mL) compared to control 6.26 Log CFU/mL, whereas *L. rhamnosus* GG

B103 showed the least growth (4.00 Log CFU/mL) in the presence of CM. The addition of dextrose and inulin (a well-known prebiotic) led to similar levels of growth among all strains.

6.4.1.4 Growth of Lactobacillus strains in modified basal media at 0.25% gums stored at 37°C for 6h. Appendix F shows the growth of *Lactobacillus* strains in the presence of gums at 0.25% during 6h incubation at 37 °C. The population of tested strains ranged from 4.94 to 6.71 log CFU/mL. The population of *L. delbrueckii bulgaricus* and *L. acidophilus* were slightly higher. Lower population of *Lactobacillus* strains were observed in samples incubated at 6 hour. The addition of pectin-dextrose led to the highest (1 log CFU/mL) increase growth in the population of *L. rhamnosus* GGB101 (6.01 Log CFU/mL) compared to those in the control (5.03 Log CFU/mL). GL supported the least population of *L. rhamnosus GG* B103 (4.94 Log CFU/mL. The addition of dextrose and inulin (a well-known prebiotic) led to similar levels of growth among all strains. The population of tested trains was similar in all samples. Population of *Lactobacillus* strains in samples containing 0.25% during incubation at 37 °C for 6 hours were very similar to samples containing twice the concentration of gums at 0.5% (Figures 34 and 35). However, the bacterial population showed slight increase at 0.5% concentration of gums compared to a lower concentration of 0.25% during incubation for 12 hour. The observation indicates the amount of gums had little impact on the bacterial population during 6 hours of incubation, whereas bacterial growth was positively impacted during 12 hours of incubation. This could be due to minimal use of available nutrients in the gums by the strains within the short period of time. The population of *Lactobacillus* strains tested was found to be directly proportional to the concentration of gums used in the growth stimulation. Higher concentration of gums (0.5%) led to slightly higher population of the tested *Lactobacillus* strains. Incubation

time also affected the probiotic population. Longer incubation period of 12 hour resulted in higher bacterial population compared to 6 hours on incubation.

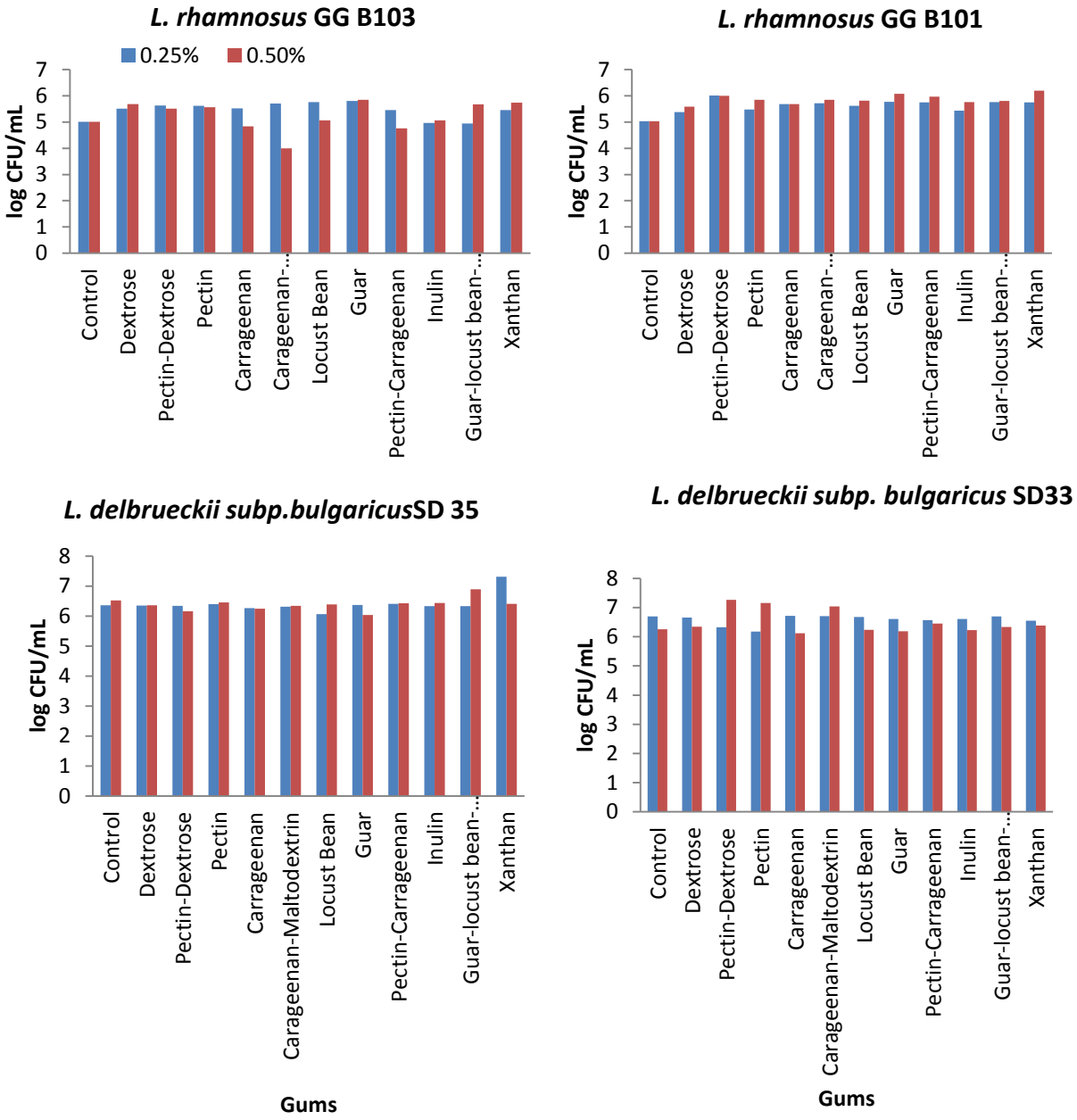

Figure 34. Population of *Lactobacillus* in modified basal media containing different gums incubated at 37 °C for 6 h.

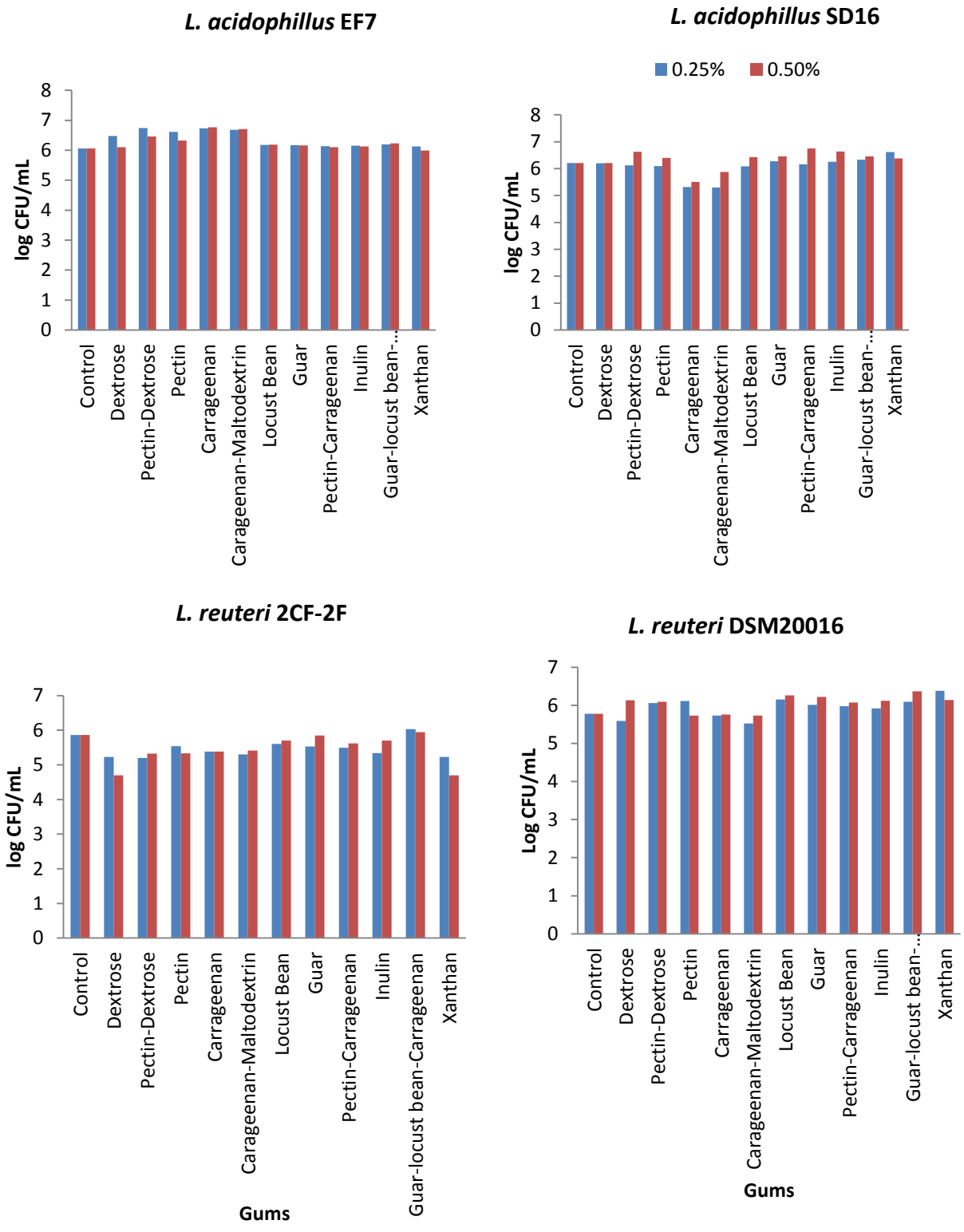

Figure 35. Population of *Lactobacillus* in modified basal media containing different gums incubated at 37 °C for 6 h.

TA and pH of all samples show similar trend. Generally, addition of gums led to slightly high population of the tested strains in the samples with gums compared to the control. *L. rhamnosus* strains were the best strain in terms of growth. Ding and Shah (2009) conducted a study to determine the acid and bile tolerance of *Lactobacillus rhamnosus, Bifidobacterium longum, L. salivarius, L. plantarum, L. acidophilus, L. paracasei, B. lactis* type Bl-04, *B. lactis* type Bi-07, HOWARU *L. rhamnosus*, and HOWARU *B. bifidum*, which were microcapsulated in alginate, xanthan gum, and carrageenan gum. Result showed that encapsulated probiotic bacteria survived better than unencapsulated probiotics when exposed to acidic conditions and bile salts (Ding & Shah, 2009).

Our result of higher bacterial population in samples containing 0.5% gums compared to 0.25% gums. Our finding is similar to a study by Hamim and others (2010). In this experiment, the prebiotic potentials crude polysaccharides from G. *lucidum* were studied in 10 mL basal Trypticase Phytone Yeast medium supplemented with various concentrations (0.5%, 1.0%, 1.5% and 2.0%) of Crude polysaccharides from *G. lucidum.* Trypticase Phytone Yeast medium supplemented with glucose and inulin were used as comparison. Viable cell counts of *Bifidobacterium longum* BB536, *Bifidobacterium pseudocatenulatum* G4, *Lactobacillus acidophilus* and *Lactobacillus casei* and the pH of the medium were determined during anaerobic incubation period of 0, 12, 24 and 48 h at 37 °C (Mohd Hamim, 2010). In the presence of carbohydrate source, cultures showed various degree of growth increment due to different concentration of the probiotic used (source of the polysaccharides).

6.5 Comparison of Growth of *Lactobacillus* Strains in Media and Milk

Figure 36 shows the effect of gums on three *Lactobacillus* strains in basal media and in milk during incubation at 37 °C for 12 hours. Generally, the population of the strains was higher in milk than in the media.

The population of *L. rhamnosus* GG101 was higher in milk compare to the media in the presence of all tested gums, with xanthan inducing the highest (8.81log CFU/mL) growth in milk among all the three strains. Population *of L. rhamnosus* GG103 in milk and media were higher than the control in the presence of all tested gums. Similarly, higher population of *L. rhamnosus* GG103 was observed in milk compared to media samples in the presence of gums, except in the guar and pectin-carrageenan, where slightly higher population of *L. rhamnosus* GG103 was found (see Figure 36). Addition of gums exhibited slightly higher population of *L. rhamnosus* GG103 compare to the control in milk during the incubation period. The presence of xanthan led to the highest population of *L. rhamnosus* GG101 (8.32 log CFU/mL) and in milk during the incubation period. The population of *L. rhamnosus* GG103 was slightly lower in the presence of pectin and locust beans during incubation in media, whereas all other gums showed positive impact on the growth of *L. rhamnosus* GG103 in the media. Xanthan stimulated the highest growth of *L. rhamnosus* GG101 (8.81log CFU/mL) and *L. rhamnosus* GG103 (8.32log CFU/mL) in milk during incubation at 37 C for 12h.

Higher population of *L. reuteri* DSM20016 was found in the media containing carrageenan, carrageenan-maltodextrin, guar, inulin and locust bean than in the media, whereas pectin-carrageenan, pectin, guar-locust bean-carrageenan and xanthan promoted slightly higher population *of L. reuteri* DSM20016 in milk than in media (Figure 36).

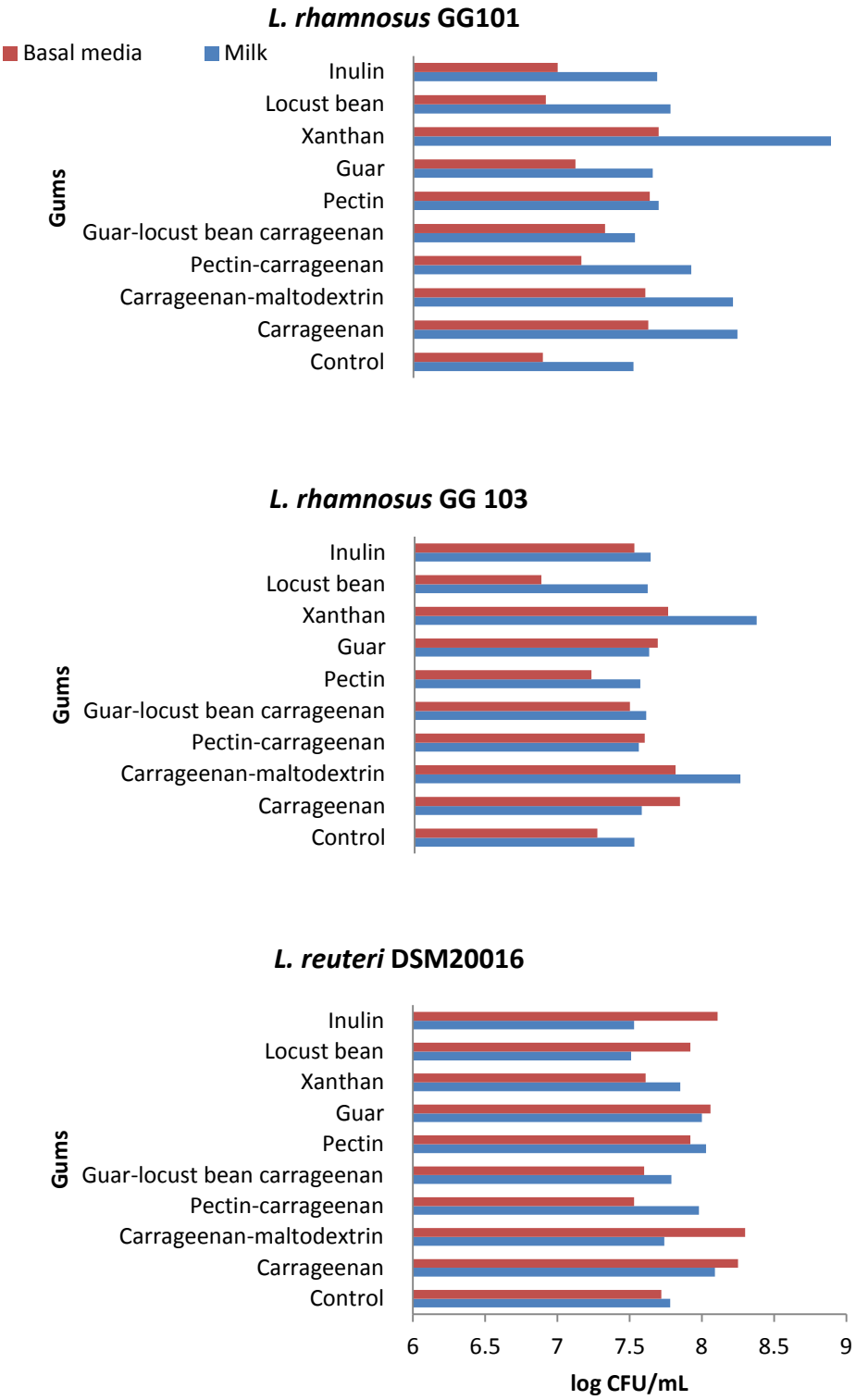

Figure 36. Comparing the population of *Lactobacillus* in modified basal media and milk containing 0.5% gums incubated at 37 °C for 12 h.

The presence of carrageenan-maltodextrin led to the highest population of *L. reuteri* DSM20016 (8.3 log CFU/mL) in the modified basal media during the incubation period. It was interesting to found out that carrageenan, carrageenan-maltodextrin, guar, inulin and locust bean which promoted higher growth in the media compare to milk, also impacted higher growth in treatment than in the control. Similarly, gums (pectin-carrageenan, pectin, xanthan, guar-locust bean-carrageen and carrageenan) that led to higher population of strains in treated sample than in the control samples grew better in milk. This is an indication that the metabolic activity of the *Lactobacillus* strains was affected directly by the gums and indirectly by the medium of growth. This could be due to the interaction between the gums and the strains. *L. rhamnosus* GGB101 grew best in milk compared to the other strains.

6.6 Conclusion

Addition of guar gum led to the highest population of *L. rhamnosus* GGB101. *L. rhamnosus* GGB101 was the best strain in terms of growth. The population of *Lactobacillus* strains tested was directly proportional to the concentration of gums used in the growth stimulation. Population *L. rhamnosus* GGB101 was significantly higher in the presence of all tested gums than the control. Samples containing 0.5% gums showed slightly higher bacterial population for all strains than those in samples containing 0.25% of gums. Longer incubation period of 12 hour resulted in higher bacterial population compared to 6 hours on incubation. TA and pH of all samples show similar trend. Samples maintained similar pH and acid production levels in the treated samples and the control. Guar stimulated the highest increase in growth in all strains at 0.5% concentration of gums during 12 h incubation at 37 °C. Population of the *L. rhamnosus* GGB101, *L. rhamnosus* GGB103 strains were higher in milk than in the media, whereas *L. reuteri* DSM20016 were higher in the media than in milk. Xanthan showed enhanced

growth of *L. rhamnosus* strains whereas carrageenan-maltodextrin led to highest growth of *L. reuteri* DSM 20016. Industries could explore guar, xanthan and carrageenan-maltodextrin as functional ingredient for enhanced growth of *Lactobacillus* strains for quality functional food.

CHAPTER 7

Effect of Selected Gums on the Growth, Viability and Enzyme Activity of *Lactobacillus* spp. in Milk during Refrigerated Storage (4 °C)[3]

7.1 Abstract

The viability and β-galactosidase activity of four *Lactobacillus* strains were assessed in milk containing either 0.5% of gums or no gums during 28 days of refrigerated storage at 4 °C. The impact on four different gums on the growth of three *Lactobacillus* strain in milk during incubation at 37 °C was for 12h was also studied. Our results showed that the presence of gums led to a slight increase in the population of *L. rhamnosus* GG B103 compared to the control. Addition of xanthan led to the highest counts (8.31 ± 0.01 log CFU/mL) in the population *L. rhamnosus* GG B103 8.31 ± 0.01 Log CFU/mL over the control (7.49 ± 0.01). GL supported the least growth (7.47 ± 0.03 Log CFU/mL) in *L. reuteri* DSM20016. The addition of carrageenan-maltodextrin led to the highest viable counts (8.76 ± 0.03 log CFU/mL) of *Lactobacillus rhamnosus* GGB103 during storage. The bacterial population of *Lactobacillus rhamnosus* GGB101 and *Lactobacillus rhamnosus* GGB103 were maintained at an average of 8 log CFU, whereas *Lactobacillus reuteri* DSM20016 and *Lactobacillus reuteri* SD2112 decreased slightly 0.15-0.64 log CFU. The highest decrease during storage was observed in *L. reuteri* 20016 (42 Millier/units) in the presence of pectin-carrageenan and the lowest decrease was 4 Miller units for *L. rhamnosus* GGB101 and *L. reuteri* 2112 in the presence of PC and GL, respectively. Viable counts were retained at 7-8 log CFU with no significant difference in the control versus treated strains. Guar-locust bean-carrageenan led to significantly ($p < 0.05$) higher levels of *β*-gal activity of *L. rhamnosus* GGB103 (1287 ± 4.24 Miller units/mL) compared to the control (78 ±

[3] Parts of this chapter were adapted from: Karlton-Senaye, B. D., Tahergorabi, Reza, Valerie, Giddings., Ibrahim, S. A. (2014). Effect of gums on the viability and *β*-galactosidase activity of *Lactobacillus* spp. in milk during refrigerated storage. International Journal of Food Science and Technology. (manuscript accepted).

2.83 Miller units/ mL). Xanthan, carrageenan and locust bean serve as growth enhancers of *L. rhamnosus* GG B103, *L. rhamnosus* GG B101and *L. reuteri* DSM20016, respectively in milk during 12 h incubation at 37 °C. Marked ($p < 0.05$) differences were observed in milk samples containing gums compared to the control. Thus, the inclusion of both carrageenan-maltodextrin and guar-locust bean-carrageenan in milk could enhance the level of β-galactosidase activity. As a result, industries could explore gums as functional ingredients for enhanced levels of β-galactosidase in order to alleviate lactose intolerance.

7.2 Introduction

Recently, there has been a growing interest in the consumption of functional foods with probiotic microorganisms. FAO/WHO defined probiotics as "Live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO-WHO, 2002). The viability of probiotic microorganisms in the gut is associated with numerous health benefits such as reinforcement of gut mucosal immunity, decreased risk associated with mutagenicity and carcinogenicity, alleviation of lactose intolerance, acceleration of intestinal mobility, hypocholesterolemic effects, reduced duration of diarrhea, prevention of inflammatory bowel disease, prevention of colon cancer, inhibition of Helicobacter pylori and intestinal pathogens, and treatment and prevention of allergies (Passos & Ribeiros, 2009; Wagar et al., 2009).

Dairy products remain good carriers of probiotic bacteria. Lactobacillus species are among the probiotics being incorporated into dairy functional foods to attract claimed health benefits. For *Lactobacillus* to provide such health benefits, a daily dose of more than $>10^6$ viable cells per gram of yogurt must be consumed (Akalin et al., 2004). Despite the importance of viability of *Lactobacillus* in yogurt, low viability of *Lactobacillus* in yogurt products has been reported (Giuliano Garisto Donzelli et al., 2003, Fudala et al., 2003, Ibrahim & Carr, 2006,

Moayednia et al., 2009). Several factors have been shown to have a negative effect on the viability of probiotic bacteria in yogurt including storage temperature, acidity, pH, organic acid concentration etc. during processing, and storage (Akalin et al., 2004).

One of the claimed health benefits associated with the ingestion of probiotic yogurt is alleviation of lactose intolerance which is prevalent in 70% of the adult population worldwide (Ibrahim & Gyawali, 2013). Lactose intolerance is a condition that is caused by the malfunction of the hydrolyzing enzyme β-galactosidase (Wagar et al., 2009, Passos & Ribeiros, 2009). *β*-galactosidase (EC3.2.1.23) is a functional enzyme that catalyzes the hydrolysis of oligo and polysaccharides containing terminal β-D -galactopyranosidase. To digest lactose in the intestinal tract, probiotics and their *β*-galactosidase must be viable and survive gastrointestinal stresses and processing technology (Totosaus et al., 2013). *β*-galactosidase can alleviate symptoms associated with digestion of lactose crystallization in milk and whey disposal (Alazzeh et al., 2009).

A prebiotic is defined as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" (Gibson et al., 2004). Inulin, oligosaccharides, isomatooligosaccharides, lactose, raffinose, sorbitol, xylitol, paratinose, lactosucrose (Gibson et al., 2004, Gibson, 1995; Tomasik & Tomasik, 2003, Akalin et al., 2004) are among the most studied prebiotics. Other polysaccharides gums (such as pectin, carrageenan, alginate, gellan, xanthan, zedo, konjac, starch, celluslose, chitosan), proteins (casein, whey, gelatin, β-lactoglobulin) and wax have also have been studied as prebiotics to improve the viability of probiotic microorganisms (Corona-Hernandez et al., 2013; Ibrahim et al., 2010, Ibrahim & O'Sullivan, 2000).

Gums are non-starch, water soluble polysaccharides found in plants (land or marine) or formed during microbial fermentation processes (BeMiller, 2008). Gums have been used as stabilizing ingredients in dairy products for more than two decades. As polyhydroxy substances, gums are able to bind and hold water to form a gel, a property that makes them useful as gelling and thickening agents to improve viscosity, texture and the organoleptic qualities of dairy foods such as yogurt, cheese, milk and ice cream (Williams & Phillips, 2003). Gums have also been used as growth enhancers for probiotic bacteria (Karlton-Senaye & Ibrahim, 2013). Gums, like other polysaccharides, are capable of enhancing viability and enzyme hydrolysis (Corona-Hernandez et al., 2013). However, very few studies have been carried out using gums as prebiotics to enhance the viability and β-galactosidase activity of probiotic microorganisms (Rascon-Diaz et al., 2012, Totosaus et al., 2013). It is important to identify gums that could be used as functional ingredients to improve viability and enhance β-galactosidase levels in order to support the alleviation of lactose intolerance. Therefore, the objective of this study was thus to investigate the impact of gums on the viability and β-galactosidase activity of *Lactobacillus* spp in milk during refrigerated storage.

7.3 Materials and Methods

7.3.1 Culture activation. Pure cultures of *L. reuteri* DSM20016, *L. reuteri* SD2112, L. rhamnosus GG B101, and *L. rhamnosus* GG B103 strains were obtained from the stock culture collection of the Food Microbiology and Biotechnology Laboratory at North Carolina A&T State University (Greensboro, NC, USA). Bacterial cultures were activated by transferring 100 µl of stock culture to 10 mL of MRS broth (Neogen, Lansing, MI) and incubating at 37 °C for 24h

7.3.2 Gum preparation and inoculation. Active bacterial cultures were mixed and centrifuged at 5,000 rpm for 10 min using Thermo Scientific* Sorvall RC 6 Plus Centrifuge

(Thermo Scientific Co., Asheville, NC, USA). The pellets were suspended in 2 mL 1% fat milk and incubated at 37 °C for 1 h. A 100 mL batch of 1% fat liquid milk was warmed to 70 °C, and 0.5% (w/v) of each gum (carrageenan (CA), carrageenan-maltodextrin (MC), pectin-carrageenan (PC) and guar-locust bean-carrageenan (GL), Maryland, GA, USA) was gradually dissolved in 100 mL of 1% fat milk. Samples without gums served as the negative control. Milk samples were then sterilized in a water bath at 65 °C for 30 min, cooled to 42 °C and inoculated with active 2 mL active bacterial culture. Inoculated samples were then refrigerated at 4 °C and aliquots were aseptically withdrawn at 1, 7, 14, 21, and 28 d to determine bacterial population, pH, and titratable acidity.

7.3.3 Growth study. Active bacterial cultures were mixed and centrifuged at 5,000 rpm for 10 min using Thermo Scientific* Sorvall RC 6 Plus Centrifuge (Thermo Scientific Co., Asheville, NC, USA). The pellets were suspended in 2 mL 1% fat milk and incubated at 37 °C for 1 h. A 100 mL batch of 1% fat liquid milk was warmed to 70 °C and 0.5% (w/v) of each gum (carrageenan (CA), carrageenan-maltodextrin (MC), pectin-carrageenan (PC) and guar-locust bean-carrageenan (GL), Maryland, GA, USA) was gradually dissolved in 100 mL 1% fat milk. Samples without gums served as the negative control. Milk samples were then sterilized in a water bath at 65 °C for 30 min, cooled to 42 °C and inoculated with active 2 mL active bacterial culture. Samples were incubated at 37°C and plated on MRS agar to determine bacterial population.

7.3.4 Bacterial enumeration and cell viability. Bacterial populations were determined by plating appropriate dilutions using sterile 0.1% peptone (Bacto peptone, Becton Dickinson, Sparks, MD, USA). One hundred µl of appropriate dilution were surface plated on MRS agar

(Neogen Corporation, Lansing, MI, USA). Plates were then incubated anaerobically at 37 °C for 48 h, and plates having between 25 to 250 colonies were counted at the end of incubation.

7.3.5 Determination of pH values. The pH values of samples were measured at one week intervals for up to four weeks using a pH meter (Acumet Basic, AB15/15+, Fisher Scientific, Pittsburgh, PA). The pH meter was calibrated with fresh pH 4 and 7.0 standard buffers. The pH electrode was rinsed with distilled water between each measurement.

7.3.6 Determination of titratable acidity. Samples were titrated with a pH meter (Accumet basis, AB15/15+, Fisher Scientific) according to method by (AOAC, 1995). After standardization of the pH meter, the pH of 100 mL of distilled water was adjusted to 8.2 using 0.1N. Five milliliter (5mL) samples were added to the distilled water followed by the gradual addition of 0.1 N NaOH to reach pH 8.2. The volume of NaOH was recorded and titratable acidity was calculated.

7.3.7 β-galactosidase assay. β-galactosidase was determined according to the method described by (Miller, 1992). Mid log bacterial growth was determined by measuring optical density at 610 nm using Thermo Scientific Genesys 10S UV-Vis spectrophotometer (Thermo Fisher Scientific Co., Madison, WI, USA). A 100-µL of starter culture was added to 900 µL of Z buffer (0.06 M Na2HPO4; 0.04 M NaH2PO4; 0.01 M KCL; 0.001 M MgSO4.7H20) to make a final volume of 1 mL. Ten microliter (10 µL) of chloroform were added to each sample which was then shaken for 30 min in the incubator at 37 °C. Reactions were started by adding 200 µL of o-nitrophenyl-β-D-galactopyranoside substrate (4 mg/mL in 0.1M phosphate buffer) to the samples. Reaction times until a strong yellow color (Appendix J) developed were recorded and reactions were stopped by adding 5 µL of 1 M Na_2CO_3. Optical density at 420 nm and 550 nm were recorded. Units of β-gal were calculated as described by (Miller, 1992).

1 Miller Unit = 1000* (OD_{420}-1.75* OD_{550}/t*.v* x OD_{600})

Where

OD_{420} is the absorbance of the yellow o-nitrophenol,

OD_{550} is the scatter from cell debris. It is multiplied by 1.75 to approximate the scatter observe at 420nm.

t = reaction time in minutes,

v = volume of culture cell density

OD_{600} reflects cell density.

7.4 Statistical Analysis

Each experiment was conducted twice in a randomized block design. The mean values and standard deviations were calculated from the duplicate tested samples. R-Project for Statistical Computing, version R-2.15.2 (http://www.r-project.org website) was used to determine significant differences in the effect of different gums on the growth, viability and β-galactosidase activity of *Lactobacillus* strains in milk using one way ANOVA (analysis of variance) at significance level of $p < 0.05$. Letters a, b, c, d, and e indicates that results within the same determinations are significantly differences.

7.5 Results and Discussion

7.5.1 Growth of *Lactobacillus* strains in milk. Table 10 shows the bacterial populations of *L. rhamnosus* GG B103, *L. rhamnosus* GGB101, and *L. reuteri* DSM20016 during 12 h of incubation at 37 °C. Generally, the population of *Lactobacillus* strains was slightly higher in the milk samples with gums compared to the control. Bacterial population in the control samples ranged from 7.49 ± 001 to 8.31 ± 0.01Log CFU/mL.

The population of *L. rhamnosus* GGB103 ranged from 7.49 ± 0.001 to 8.31 ± 0.01 log CFU/mL (see Table 10). The population of *L. rhamnosus* GGB103 in the presence of tested gums was slightly higher than in the control. Addition of xanthan led to the highest population *L. rhamnosus* GG B103 from 7.49 ± 0.01 to 8.31 ± 0.01 Log CFU/mL, whereas pectin-carrageenan supported the least growth. The presence of carrageenan-maltodextrin, guar, inulin and xanthan led to significant ($p < 0.05$) increase in the population of *L. rhamnosus* GG B103 in milk. The other gums led to a slight increase in the population of *L. rhamnosus* GG B103. However, the increase was not significant.

Table 10

Population of Lactobacillus Strains in Milk Containing Gums Incubated at 37 °C for 12h

Treatments	*L. rhamnosus GG* B103	*L. rhamnosus GG* B101	*L. reuteri* DSM20016
Control	7.49 ± 001^{a}	7.81 ± 0.04^{bc}	7.54 ± 0.08^{a}
Pectin	7.52 ± 0.01^{ac}	8.03 ± 0.01^{de}	7.64 ± 0.02^{a}
Carrageenan	7.53 ± 0.02^{ac}	8.09 ± 0.01^{e}	8.12 ± 0.02^{a}
Carageenan-Maltodextrin	8.21 ± 0.01^{d}	7.71 ± 0.04^{ab}	8.14 ± 0.01^{a}
Locust Bean	7.55 ± 0.04^{ac}	7.53 ± 0.02^{a}	8.27 ± 0.76^{a}
Guar	7.56 ± 0.02^{bc}	7.89 ± 0.15^{be}	7.52 ± 0.03^{a}
Pectin-Carrageenan	7.51 ± 0.02^{ab}	7.97 ± 0.02^{cde}	7.86 ± 0.01^{a}
Inulin	7.60 ± 0.01^{c}	7.51 ± 0.03^{a}	7.56 ± 0.01^{a}
Guar-locust bean-Carrageenan	7.55 ± 0.04^{ac}	7.81 ± 0.03^{bd}	7.47 ± 0.03^{a}
Xanthan	8.31 ± 0.01^{e}	7.84 ± 0.01^{bd}	7.59 ± 0.03^{a}

Results expressed as means ± standard deviation ($n = 2$) Means in the same column with different letters are significantly ($p < 0.05$) different.

The bacterial population for *L. rhamnosus* GG B101 ranged from 7.51 ± 0.03 to 8.09 ± 0.01 Log CFU/mL. Addition of carrageenan led to highest population (8.09 ± 0.0101Log CFU/mL) of *L. rhamnosus* GG B101. The presence of inulin stimulated the least (7.51 ± 0.03 Log CFU/mL) showing the ineffectiveness of inulin in enhancing the growth of *L. rhamnosus*

GG B101 in milk. Locust bean and carrageenan-maltodextrin also induced less growth of *L. rhamnosus* GG B101 compared to the control. Pectin and carrageenan exhibited significant ($p < 0.05$) higher growth *L. rhamnosus* GG B101 compared to the control.

The population of *L. reuteri* DSM20016 varied from 7.47 ± 0.03 to 8.27 ± 0.76 Log CFU/mL. Locust bean led to the highest growth (8.27 ± 0.76 Log CFU/mL) of *L. reuteri* DSM20016. The presence guar-locust bean-carrageenan and guar were ineffective as growth enhancers for *L. reuteri* DSM20016. Addition of gums showed no significant increase in the population of *L. reuteri* DSM20016 in milk.

The results of this study suggests that xanthan, carrageenan and locust bean could serve as growth enhancers of *L. rhamnosus GG* B103, *L. rhamnosus GG* B101and *L. reuteri* DSM20016, respectively in milk and other dairy products. The bacterial growth observed in this study showed some variation which depended on the strain and gums used. The growth of bacterial is strain specific and it is influenced by the composition of the growth media. Inulin, a well know prebiotic, supported the least growth in *L. rhamnosus* GG B101 compared to the control in this study, suggesting inhibitory effect of inulin. Inulin also showed inhibitory effect in media during incubation at 37 °C. This finding is contrary to findings in other studies in which inulin supported the growth of *Lactobacillus* stains and *Bifidobacteria* in ice cream and yogurt (Hekmat et al., 2009); (Oliveira et al., 2009a).

7.5.2 Changes in the population of *L. reuteri* and *L. rhamnosus* spp. in milk. Figure 37 shows the changes in the viable counts of *L. reuteri* and *L. rhamnosus* spp. in milk during refrigerated storage. The viability of *L. reuteri* strains remained generally stable during the first two weeks of storage and decreased slightly after the third week. Viable counts of *L. reuteri* strains showed significant ($p < 0.05$) increase in the presence of carrageenan-maltodextrin,

pectin-carrageenan and guar-locust bean-carrageenan in the second week. After 28 days of storage, the counts of *L. reuteri* DSM20016 and *L. reuteri* SD2112 decreased significantly ($p < 0.05$) by 0.15 to 0.64 log CFU/mL (Figure 38). *L. reuteri* DSM20016 showed the least decrease (0.15 log CFU) in the presence of carrageenan-maltodextrin, indicating that the presense of carrageenan-maltodextrin retained more *L. reuteri* strains than the other gums during the storage period. In a similar study, the decrease in counts of *L. acidophilus* and *L. Lactis* in milk sample containing inulin was less than those of the control sample at the end of storage period (Akin, 2007).

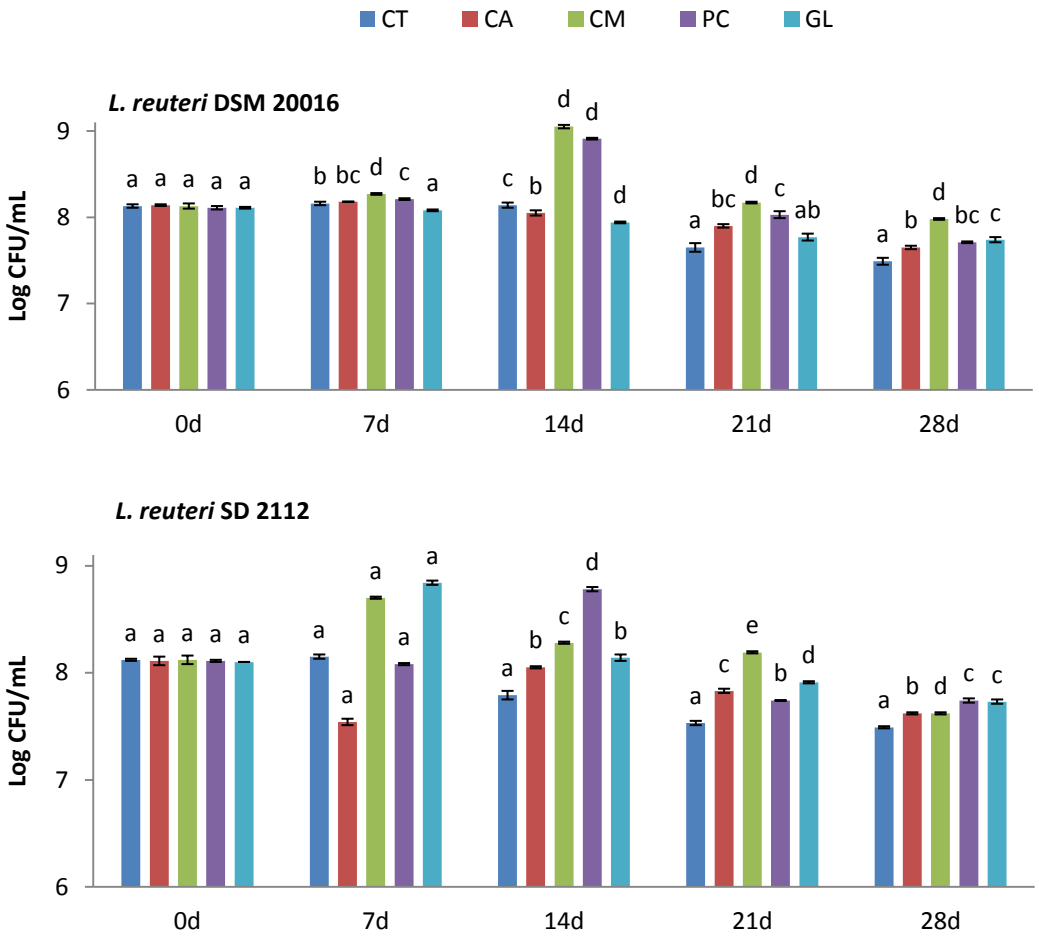

Figure 37. Population of Lactobacillus spp. in milk containing gums stored at 4 °C for 28 days.

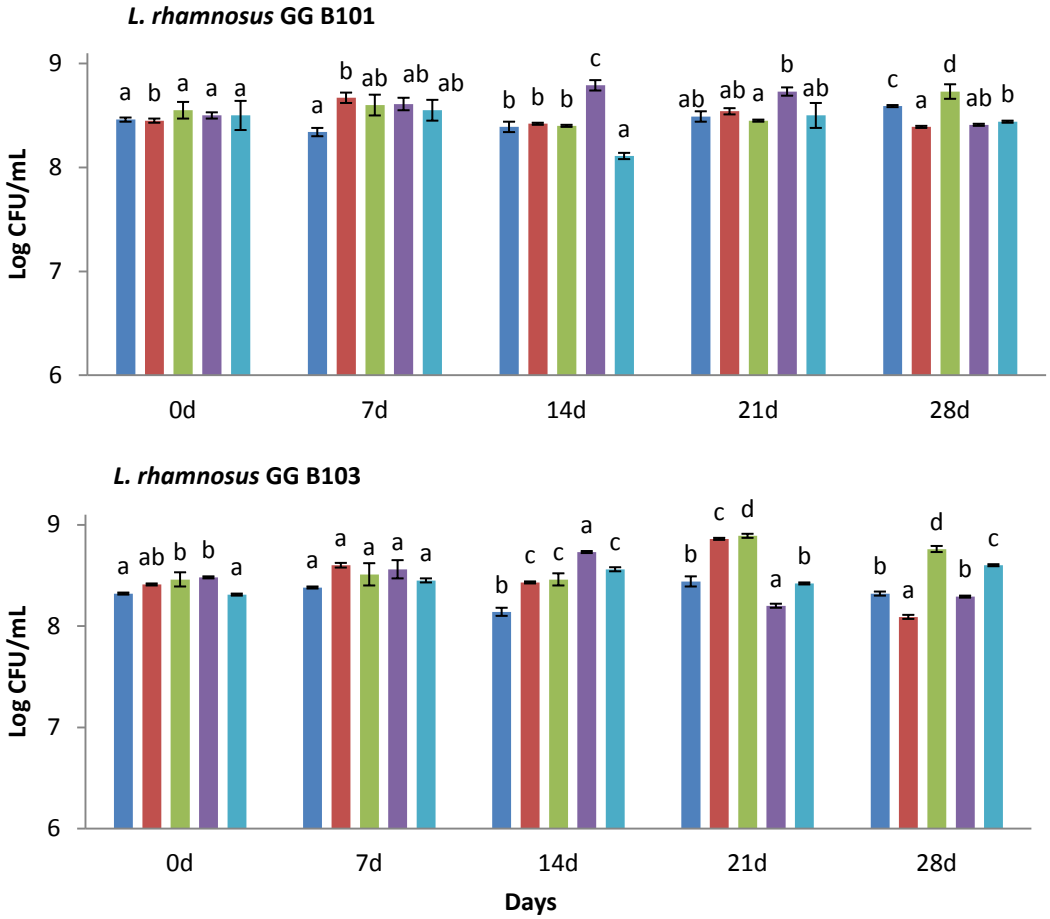

Figure 37. (Cont.)

Viability of *L. rhamnosus* GG B101 and *L. rhamnosus* GG B103 strains in all milk samples remained stable during refrigerated storage (see Figure 37). However, marked ($p < 0.05$) difference was observed in viable counts of *L. rhamnosus* strains in the presence of carrageenan-maltodextrin compared to the control during the refrigerated storage period. Viable count of *L. rhamnosus* GGB103 increased by 0.33 log CFU/mL (see Figure 38) in milk samples containing carrageenan-maltodextrin during storage. On average, the survival rate of *L. rhamnosus* was better than that of *L. reuteri* strains.Higher viable counts of *L. rhamnosus* and *L. reuteri* strains were observed in the presence of carrageenan-maltodextrin.

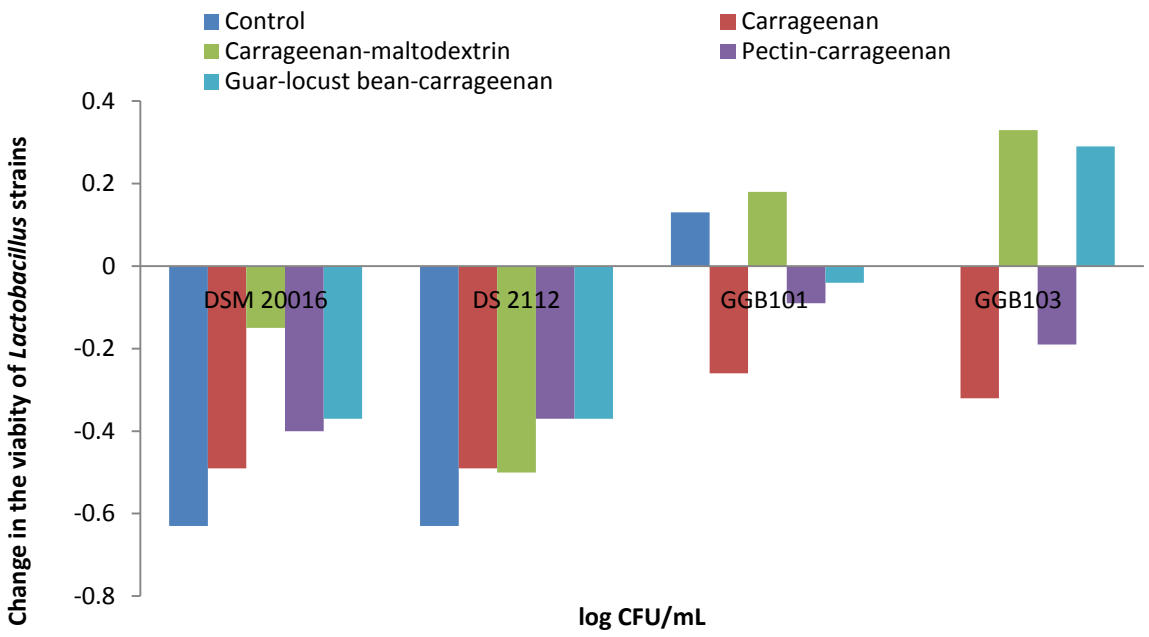

Figure 38. Changes in the viability of *Lactobacillus* strains during storage at 4 °C for 28 days.

The viable counts of *Lactobacillus* spp. in all milk samples remained above 7 log CFU/mL, while 6 log CFU/mL is the level required for adequate therapeutic health benefits in humans (Shah et al., 2007). The positive effect of gums on the counts of *Lactobacillus* strains may be attributed to nutrient availability in gums and the buffering capacity of gums as a result of acid and enzymatic hydrolysis (Al-Ghazzewi et al., 2007). The observation could also be due to the formation of a cross-link matrix between the gums and the whey protein in milk. This matrix could provide protection to bacterial cells by forming a gel that reduces the negative effects of excess water to bacterial growth and enhance survival during storage. The charges on gums could also contribute to the survival of probiotic bacteria. For example, microencapsulation with alginate increases the survival of several species from the genera *Bifidobacteria* and *Lactobacillus* by up to 80% to 95% due to its anionic/acidic diheteroglycan bond (Corona-Hernandez et al., 2013). Hebrard and others (2010) found that Alginate combined with whey

protein produces a highly cross-linked matrix that protects microparticles from acid digestion which supports the survival of *Bifidobacteria* (Picot & Lacroix 2004; Hebrard et al., 2010).

In this study, carrageenan-maltodextrin significantly ($p < 0.05$) improved the viability of *L. rhamnosus* GGB103 by 0.5 log CFU/mL over the control during refrigerated storage. Similarly, the survival of *L. acidophilus, L. casei, L. rhamnosus* and *Bifidobacterium* spp. was evaluated in yoghurt and freeze-dried yoghurt after processing and storage. Microencapsulation of the strains with alginate improved viability of combined selected probiotic organisms by 0.31 log CFU/mL in freeze-dried yoghurt stored at 21 °C (Capela, Hay, & Shah, 2006). In a study carried out by Yeo and Liong (2010), *L. acidophilus, L. casei and Bifidobacterium longum* showed viability exceeding 7 log10 CFU/mL in soymilk supplemented with maltodextrin and pectin.

The findings of our study contradict those from a study by Ghasempour and others in which zedo gum showed no significant effect on the viability of *L. acidophilus* and *B.bifidum* during refrigerated storage for 29 days though viable counts were between 6-7 log/g (Ghasempour et al., 2012). Contrary to our findings, microbial viability was enhanced significantly by using pectin as an encapsulating agent (Rascon-Diaz et al., 2012). However, in a separate study, alginate combined with gellan gums improved the viability of lactic acid bacteria through microencapsulation (Totosaus et al., 2013)

7.5.3 Changes in pH of milk containing gums during refrigerated storage. The initial pH values for milk samples with *L. rhamnosus* GG B101 and *L. rhamnosus* GG B103 ranged from an average of 6.55 to 6.60 (see Table 11). Viable counts of *L. rhamnosus* strains were not affected significantly as the bacterial population remained stable during storage. This stability could be due to the fact that the pH of the milk samples also remained stable throughout the 28

days of storage. Similar pH values were observed in all milk samples with no significant difference in the control and treatment during storage. *Lactobacilli* are able to grow in a wide pH range between 3 to 8, but growth best at an optimal pH 5.5 to 6.2 (Salvetti et al., 2012). The pH of most gums used in this study fall within the optimal pH for *Lactobacillus* growth that could contribute to enhancing the interaction between the bacteria and the gums. The initial pH values for the different milk samples ranged from an average of 6.48 to 6.68 for *L. reuteri* DSM 20016 and *L. reuteri* SD 2112 (see Table 11). The pH of all samples decreased slightly during storage. The pH of milk samples with *L. reuteri* strains decreased sharply from an average of 6 to 4. The drop in pH was similar for all milk samples, showing no significant ($p < 0.05$) difference between the pH of milk containing gums and the control during storage. The slight decrease in the viability of *L. reuteri* DSM 20016 and *L. reuteri* SD 2112 in the third week of storage could be due to decline in pH of milk samples with *L. reuteri* spp. Shah (2000) also observed a similar decrease in pH values in dairy foods during storage (Shah, 2000). No significant ($p < 0.05$) differences were observed between the pH values of samples with gums and the control during the storage period.

Table 11

Average pH Values of Milk Containing Different Gums Stored at 4 °C for 28 Days

	pH			
Days/Samples	*Lactobacillus reuteri* DSM 20016	*Lactobacillus reuteri* SD 2112	*Lactobacillus rhamnosus* GG101	*Lactobacillus rhamnosus* GG103
Day 1				
Control	6.48 ± 0.01^{a}	6.58 ± 0.01^{a}	6.56 ± 0.01^{a}	6.59 ± 0.01^{b}
Carrageenan	6.53 ± 0.01^{b}	6.66 ± 0.07^{a}	6.63 ± 0.01^{b}	6.58 ± 0.01^{b}
Carrageenan-maltodextrin	6.63 ± 0.01^{d}	6.68 ± 0.01^{a}	6.61 ± 0.01^{b}	6.59 ± 0.01^{b}
Pectin-carrageenan	6.57 ± 0.01^{c}	6.63 ± 0.01^{a}	6.55 ± 0.01^{a}	6.57 ± 0.01^{b}
Guar-locust bean carrageenan	6.62 ± 0.01^{d}	6.66 ± 0.01^{a}	6.60 ± 0.01^{b}	6.55 ± 0.01^{a}

Table 11

(Cont.)

Days/Samples	pH			
	Lactobacillus reuteri DSM 20016	*Lactobacillus reuteri* SD 2112	*Lactobacillus rhamnosus* GG101	*Lactobacillus rhamnosus* GG103
Day 7				
Control	6.22 ± 0.01[b]	6.26 ± 0.01[a]	6.76 ± 0.01[b]	6.76 ± 0.01[a]
Carrageenan	6.27 ± 0.01[b]	6.39 ± 0.01[b]	6.84 ± 0.01[c]	6.79 ± 0.01[a]
Carrageenan-maltodextrin	6.09 ± 0.01[a]	6.15 ± 0.07[a]	6.86 ± 0.01[c]	6.83 ± 0.01[a]
Pectin-carrageenan	6.07 ± 0.01[a]	6.24 ± 0.01[a]	6.72 ± 0.01[a]	6.84 ± 0.01[a]
Guar-locust bean carrageenan	6.11 ± 0.02[a]	6.20 ± 0.01[a]	6.78 ± 0.01[b]	6.72 ± 0.01[a]
Day 14				
Control	5.40 ± 0.01[d]	5.56 ± 0.01[c]	6.82 ± 0.01[a]	6.76 ± 0.00[a]
Carrageenan	4.72 ± 0.01[b]	4.47 ± 0.01[a]	6.91 ± 0.01[c]	6.94 ± 0.01[c]
Carrageenan-maltodextrin	4.62 ± 0.01[a]	4.95 ± 0.01[b]	7.04 ± 0.01[d]	6.96 ± 0.01[c]
Pectin-carrageenan	5.25 ± 0.01[c]	5.55 ± 0.07[c]	6.93 ± 0.01[c]	6.88 ± 0.01[b]
Guar-locust bean carrageenan	5.37 ± 0.01[d]	5.46 ± 0.01[c]	6.85 ± 0.01[b]	7.07 ± 0.01[d]
Day 21				
Control	4.50 ± 0.14[a]	4.40 ± 0.07[a]	6.86 ± 0.01[c]	6.82 ± 0.01[c]
Carrageenan	4.49 ± 0.01[a]	4.42 ± 0.01[a]	6.36 ± 0.01[a]	6.75 ± 0.01[b]
Carrageenan-maltodextrin	4.34 ± 0.01[a]	4.38 ± 0.01[a]	6.60 ± 0.01[b]	5.89 ± 0.01[a]
Pectin-carrageenan	4.39 ± 0.01[a]	4.51 ± 0.01[a]	6.50 ± 0.07[b]	6.71 ± 0.01[b]
Guar-locust bean carrageenan	4.39 ± 0.01[a]	4.89 ± 0.01[a]	6.52 ± 0.01[ab]	6.74 ± 0.01[b]
Day 28				
Control	4.35 ± 0.01[c]	4.30 ± 0.01[b]	6.84 ± 0.01[b]	6.83 ± 0.01[b]
Carrageenan	4.21 ± 0.01[a]	4.52 ± 0.01[c]	6.88 ± 0.01[b]	6.78 ± 0.01[b]
Carrageenan-maltodextrin	4.27 ± 0.01[b]	4.27 ± 0.01[b]	5.90 ± 0.07[a]	5.45 ± 0.01[a]
Pectin-carrageenan	4.32 ± 0.01[c]	4.22 ± 0.01[a]	6.85 ± 0.07[b]	6.86 ± 0.01[b]
	4.34 ± 0.01[c]	4.27 ± 0.01[b]	6.77 ± 0.01[b]	6.82 ± 0.01[b]

Note. Results expressed as means ± standard deviation (n = 2). Means in the same column with different letters within the same storage day are significantly (p<0.05) different.

7.5.4 Changes in titratable acidity of milk during refrigerated storage. Table 12 shows the titratable acidity of milk samples. TA values varied from an average 0.08 ± 0.01 to 0.76 ± 0.02% in milk with *L. reuteri* DSM20016. An increasing trend in TA profile was observed in *L. reuteri* strains. Significant (p < 0.05) differences in TA values were observed in milk samples containing gums compare to the control, except for *L. reuteri* SD2112 strains, which showed no significant difference during the 28 days of refrigerated storage. The TA of *L.*

reuteri strains increased with decreasing pH whereas that of *L. rhamnosus* strains were relatively stable. This indicates that *Lactobacillus* strains produced no buffering effect in the presence of the gums in milk samples. The findings of this study are in agreement with that of Guler-Akin and Akin (2007), who reported a lower count of viability of *L. acidophilus* due to increased acidity and inhibition by hydroperoxide production (Guler-Akin, 2007).

7.5.5 β-galactosidase activity of *Lactobacillus* spp. in milk. We also evaluated the effect of gums on the β-galactosidase activity of *L. rhamnosus* and *L. reuteri* spp. in milk containing carrageenan (CA), carrageenan-maltodextrin (CM), pectin-carrageenan (PC) and guar-locust bean-carrageenan (GL) during 21 day refrigerated storage (see Table 12). The levels of β-gal activity of *L. rhamnosus* GG B101 in milk samples varied from 59 ± 1.41 to 1228 ± 2.83 Miller units/mL and from 61 ± 2.83 to 1208 ± 2.12 Miller units/mL on days 1 and 21, respectively. *L. rhamnosus* GG B101 treated with GL showed the highest ($p < 0.05$) increase (21 fold) in β-gal activity at 1228 ± 2.83 Miller units/mL compared to the control at 59 ± 1.41Miller units/mL on the first day of refrigerated storage. *L. rhamnosus* GG B101 treated with other tested gums led to higher ($p < 0.05$) β-gal activity ranging from 568 ± 4.24 to 933 ± 4.24 Miller units/mL (see Table 12). It was observed that all tested gums led to a significant ($p < 0.05$) increase in β-gal activity of *L. rhamnosus* GG strains B101on the first day of storage. On day 21, higher ($p < 0.05$) levels of β-gal activity of *L. rhamnosus* GG B101 were found in milk samples containing GL at 1208 ± 2.12 Miller units/mL compared to the control at 61 ± 2.83 Miller units/mL. Higher ($p < 0.05$) levels of β-gal activity of *L. rhamnosus* GG strains B101 varying from 560 ± 2.12 to 961 ± 1.41 Miller units/mL were observed in milk samples containing the other tested gums compared to the control. Treatment of *L. rhamnosus* GG B101 strains with the tested gums led to higher ($p < 0.05$) levels of β-gal activity compared to the control.

Table 12

Titratable Acidity Profile of Milk Containing Different Gums under Refrigerated Storage (4 °C) for 28 Days

Days /Samples	*L. reuteri* DSM20016	*L. reuteri* SD 2112	*L. rhamnosus* GG B101	*L. rhamnosus* GGB103
Day 1				
Control	0.09 ± 0.01[a]	0.12 ± 0.01[a]	0.12 ± 0.02[a]	0.10 ± 0.01[a]
Carrageenan	0.08 ± 0.01[a]	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.11 ± 0.02[a]
Carrageenan-maltodextrin	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.08 ± 0.01[a]	0.09 ± 0.02[a]
Pectin-carrageenan	0.09 ± 0.01[a]	0.10 ± 0.01[a]	0.12 ± 0.02[a]	0.12 ± 0.01[a]
Guar-locust bean carrageenan	0.12 ± 0.01[a]	0.11 ± 0.01a	0.11 ± 0.01[a]	0.12 ± 0.01[a]
Day 7				
Control	0.13 ± 0.01[a]	0.17 ± 0.01[a]	0.13 ± 0.01[a]	0.13 ± 0.01[a]
Carrageenan	0.15 ± 0.01[a]	0.16 ± 0.01[a]	0.08 ± 0.01[a]	0.11 ± 0.01[a]
Carrageenan-maltodextrin	0.22 ± 0.01[b]	0.22 ± 0.02[b]	0.10 ± 0.01[a]	0.09 ± 0.01[a]
Pectin-carrageenan	0.16 ± 0.01[a]	0.18 ± 0.01[ab]	0.12 ± 0.01[a]	0.13 ± 0.01[a]
Guar-locust bean carrageenan	0.17 ± -0.01[a]	0.19 ± 0.01[ab]	0.13 ± 0.01[a]	0.13 ± 0.01[a]
Day 14				
Control	0.38 ± 0.02[a]	0.35 ± 0.02[a]	0.16 ± 0.01[a]	0.22 ± 0.01[b]
Carrageenan	0.53 ± 0.01[c]	0.67 ± 0.02[b]	0.15 ± 0.01[a]	0.15 ± 0.01[a]
Carrageenan-maltodextrin	0.70 ± 0.02[d]	0.73 ± 0.02[b]	0.18 ± 0.02[a]	0.14 ± 0.01[a]
Pectin-carrageenan	0.44 ± 0.02[b]	0.39 ± 0.01[a]	0.18 ± 0.02[a]	0.16 ± 0.01[a]
Guar-locust bean carrageenan	0.38 ± 0.01[a]	0.36 ± 0.01[a]	0.17 ± 0.01[a]	0.14 ± 0.01[a]
Day 21				
Control	0.66 ± 0.01[a]	0.61 ± 0.01[b]	0.25 ± 0.01[b]	0.25 ± 0.01[ab]
Carrageenan	0.74 ± 0.01[b]	0.53 ± 0.01[a]	0.16 ± 0.01[a]	0.19 ± 0.01[a]
Carrageenan-maltodextrin	0.73 ± 0.01[b]	0.61 ± 0.01[b]	0.23 ± 0.01[b]	0.24 ± 0.01[a]
Pectin-carrageenan	0.64 ± 0.01[a]	0.47 ± 0.01[a]	0.38 ± 0.01[c]	0.31 ± 0.01[bc]
Guar-locust bean carrageenan	0.66 ± 0.02[a]	0.76 ± 0.01[c]	0.34 ± 0.01[c]	0.32 ± 0.03[c]
Day 28				
Control	0.65 ± 0.01[a]	0.68 ± 0.01[a]	0.19 ± 0.01[a]	0.18 ± 0.01[ab]
Carrageenan	0.67 ± 0.02[ab]	0.80 ± 0.02[a]	0.36 ± 0.01[c]	0.32 ± 0.01[c]
Carrageenan-maltodextrin	0.76 ± 0.02[c]	0.70 ± 0.02[a]	0.36 ± 0.01[c]	0.34 ± 0.01[c]
Pectin-carrageenan	0.74 ± 0.02[bc]	0.73 ± 0.01[a]	0.24 ± 0.01[b]	0.19 ± 0.01[b]
Guar-locust bean-carrageenan	0.69 ± 0.01[ab]	0.62 ± 0.01[a]	0.12 ± 0.02[a]	0.10 ± 0.01[a]

Note. Results expressed as means ± standard deviation (n = 2). Means in the same column with different letters within the same day are significantly different.

Table 13

Average β-galactosidase Activities Values of Lactobacillus spp. in Milk Containing Different Gums during Refrigerated Storage at 4 °C for 28 Days

Storage period/gums	β-galactosidase activity (miller unit/mL of cells)			
	L. rhamnosus GGB101	*L. rhamnosus* GGB103	*L. reuteri* DSM 20016	*L. reuteri* SD 2112
Day 1				
Control	59 ± 1.41[a]	69 ± 2.83[a]	74 ± 4.94[b]	103 ± 3.54[b]
Carrageenan	933 ± 4.24[d]	941 ± 1.41[d]	214 ± 2.83[c]	204 ± 2.12[c]
Carrageenan-maltodextrin	568 ± 4.24[b]	570 ± 3.54[b]	242 ± 2.83[d]	404 ± 5.66[e]
Pectin-carrageenan	748 ± 2.83[c]	828 ± 2.83[c]	335 ± 2.83[e]	318 ± 4.24[d]
Guar-locust bean carrageenan	1228 ± 2.83[e]	1308 ± 2.83[e]	57 ± 2.83[a]	54 ± 1.41[a]
Day 21				
Control	61 ± 2.83[a]	78 ± 2.83[a]	62 ± 4.24[a]	89 ± 2.12[b]
Carrageenan	961 ± 1.41[d]	972 ± 2.83[d]	192 ± 2.83[b]	213 ± 4.24[c]
Carrageenan-maltodextrin	560 ± 2.12[b]	564 ± 2.83[b]	223 ± 4.24[c]	373 ± 3.54[e]
Pectin-carrageenan	744 ± 1.41[c]	819 ± 2.12[c]	293 ± 2.83[d]	302 ± 2.21[d]
Guar-locust bean carrageenan	1208 ± 2.12[e]	1287 ± 4.24[e]	50 ± 2.12[a]	50 ± 1.41[a]

Note. Results expressed as means ± standard deviation ($n = 2$).[b-e] Means in the same column with different letters within the same storage day are significantly different ($p < 0.05$).

The levels of β-gal activity for *L. rhamnosus* GG B103 varied from 69 ± 2.83 to 1308 ± 2.83 Miller units/mL and from 78 ± 2.83 to 1287 ± 4.24 Miller units/mL on days 1 and 21 of refrigerated storage (see Table 4). The addition of GL led to a significant ($p < 0.05$) increase (18 fold) in the levels of β-gal activity for *L. rhamnosus* GG B103 of the control from 69 ± 2.83 Miller units/mL to 1308 ± 2.83 Miller units/mL on the first day. Significant ($p < 0.05$) levels ranging from 570 ± 3.54 to 828 ± 2.83 Miller units/mL of β-gal activity for *L. rhamnosus* GG B103 were found in other tested gums on the first day. On day 21, GL led to the highest (1287 ± 4.24 Miller units/mL) level of β-gal activity for *L. rhamnosus* GGB103. Results showed that all tested gums led to significantly higher levels of β-gal activity for *L. rhamnosus* strains compared to the control.

The levels of β-gal activity for *L. reuteri* DSM 20016 ranging from 57 ± 2.83 to 335 ± 2.83 Miller units and from 62 ± 4.24 to 293 ± 2.83 Miller units/mL on days 1 and 21 of storage are shown in Table 12. Higher ($p < 0.05$) β-gal levels were observed in *L. reuteri* DSM 20016 treated with gums, except for GL, which led to decreased b-gal levels compared to the control on day 1 of storage. Similarly, except for GL, all tested gums showed higher ($p < 0.05$) levels of β-gal activity in the milk samples treated with gums compared to the control on day 21.

Table 4 shows β-gal levels of *L. reuteri* SD 2112 during 21 days of refrigerated storage. β-gal activity for *L. reuteri* SD 2112 ranges from 54 ± 1.14 to103 ± 3.54 Miller units/mL and from 50 ± 1.14 to 89 ± 2.12 Miller units on days 1 and 21of storage, respectively. Except for GL, all tested gums exhibited higher ($p < 0.05$) β-gal activity varying from 204 ± 2.12 to 404 ± 5.66 Miller units/mL and from 213 ± 4.24 to 373 ± 3.54 Miller units/mL on days 1 and 21 of storage, respectively. The addition of GL led to a reduction in β-gal activity from 62 ± 4.24 to50 ± 2.12 Miller units/mL for *L. reuteri* DSM 20016 and from 89 to 50 ± 1.41 Miller units/mL for *L. reuteri* SD2112 during 21 days of storage, suggesting an inhibitory effect of GL on *L. reuteri* strains. Results show that the addition of CA, CM and PC to milk led to significantly ($p < 0.05$) higher levels of β-gal activity in *L. reuteri* strains during 21 days of refrigerated storage, whereas β-gal activity of *L. reuteri* stains were inhibited in the presence of GL. All tested gums led to significant ($p < 0.05$) higher levels of β-gal. CM and GL exhibited the highest levels of β-gal activity for *L. reuteri* and *L. rhamnosus* strains, respectively, during the storage period.

Significant ($p < 0.05$) higher levels of β-gal activity of *Lactobacillus* strains in the presence of tested gums could be due to the hydrolysis of 3,6-anhydro-D-galactose and D-galactose-4-sulfate in the gums and the interaction between the gums and the *Lactobacillus* strains due to the breakdown of galactose. Transgalactosylation make it possible for β-

galactosidase to catalyze the production of galacto-oligosaccharides (GOS). Additionally, GL supported the highest β-gal level. This could be due to the presence of guar gum in this sample. Guar contains 1-4-β-D-mannopyranosyl units with every second unit bearing a 1-6-α-D-galactopyrasyl unit (Millar, 2003). The α-linked or β-linked oligosaccharides component of α-galactosidase contributes to its use as a biomarker for microbial metabolic activity within the large intestine for potential prebiotics (Holt, Teresi, & Côté, 2008). Hence the higher β-galactosisdase activity of the strains in the presence of the gums could be due to the breakdown of β and α-linkages in gums by the probiotic microorganisms. The presence of lactose in the milk probably triggered β-gal activity by the Lac operon mechanism (Ibrahim et al., 2010), which contributed to higher β-gal levels.

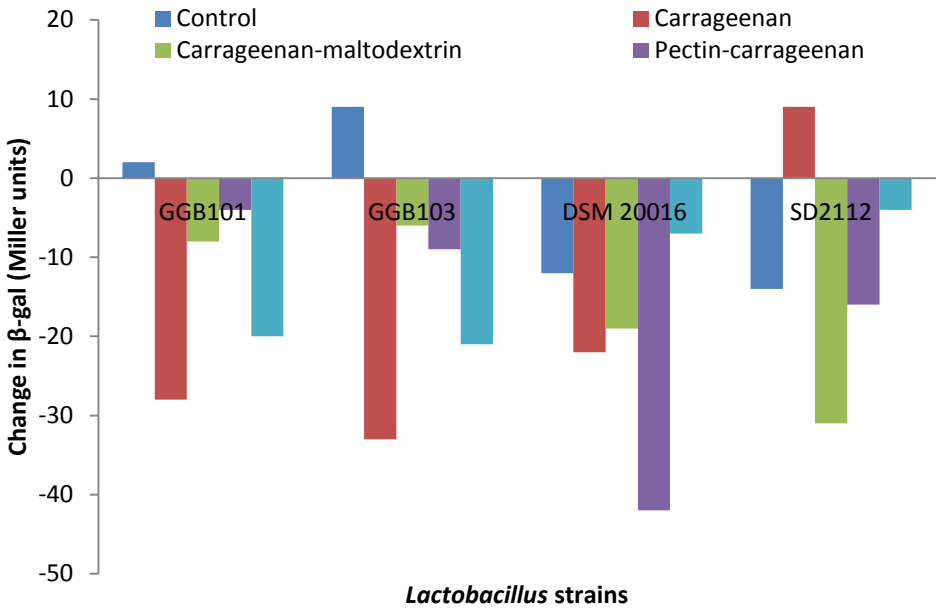

Figure 39. Change in β-galactosidase activity of Lactobacillus strains in the presence of gums after 21 days of refrigerated storage.

Generally, beta-galactosidase activity of the strains decreased slightly from 4 to 42 Miller units (represented by 0.5 to13%) after 21 day of refrigerated storage (see Figure 36). The highest

decrease was observed in *L. reuteri* 20016 (42 Miller/units) in the presence of pectin-carrageenan and the lowest decrease was 4 Miller units in *L. rhamnosus* GGB101 and *L. reuteri* 2112 in the presence of PC and GL, respectively. Other strains showed decreases in beta-gal activity varying from 6 to 33 Miller units. *L. rhamnosus* strains without gum showed slight increase during 21 day storage. *L. reuteri* also exhibited a slight decrease beta-gal in the presence of carrageenan during the refrigerated storage. The slight decrease in expected due to the decrease in viable counts of the strain during the storage period. The result suggests that probiotic milk containing gum should be consumed as early as possible to obtain the full health benefits of lactose alleviation as longer periods of storage tend to decrease enzymatic activity. These findings are in agreement with a study by Hekmat and McMahon (1992). Hekmat and McMahon studied the survival of the *L. acidophilus* and *B. bifidum*, as well as β-galactosidase activity, was monitored at 5 day interval during 17 week of frozen storage in probiotic ice cream. Bacterial counts were 1.5×10^8 CFU/mL for *L. acidophilus* and 2.5×10^8 CFU/mL for *B. bifidum*. Seventeen weeks after freezing, these viable counts had decreased to 4×10^6 and 1×10^7 CFU/mL (decrease of 2 and 1 log cycle), respectively. During the same period, *β*-galactosidase activity decreased from by 31% from 1800 to 1300 units/mL and by 8% during 30 d of refrigerated storage. Mashayekh and Brown observed 20% of β-galactosidase activity in yogurt containing *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus salivarim* ssp.*rhennophilus* was lost after 30 d of refrigeration (Hekmat & Donald, 1992).

As evident in literature, dairy products remain good carriers of probiotic flora.The benefits associated with the consumption of probiotic functional foods are well documented (Gibson et al., 2004; Grandy et al., 2010, Guandalini, 2008; Chow, 2002). Among these health benefits, the alleviation of lactose intolerance by β-gal produced by probiotic bacteria in

combination with prebiotic ingredients is increasingly in demand among the adult population (Ibrahim & Gyawali, 2013). Studies have shown that the use of different food ingredients enhances the β-gal activity of probiotic microorganisms. Alazzeh et al. (2009) used six carbohydrate and four protein sources to induce the production β-galactosidases of six strains of *Lactobaccillus reuteri*. They reported lactose as the best carbohydrate source for *β*-galactosidase, yeast extract as the best protein source for the production of both enzymes, and CF2-2F as the best producing strain.

Carbohydrates such as dextrose, galactose, melibiose and sucrose could be replaced by much healthier, cheaper and more available alternative ingredients such as gums to enhance the production of *β*-gal. Gums are commonly used in food as stabilizers. Exploring the use of gums as prebiotic functional ingredients to increase β-gal levels may improve the quality of dairy food and contribute to alleviating lactose malabsorption.

7.6 Conclusions

Gums have been used in the food industry for over two decades as stabilizing agents that prevent syrenesis and enhance the sensory qualities of foods. However, few studies have focused on gums as growth, viability and *β*-gal enhancers. The composition of gums, their health benefits, low cost and availability qualifies gums as a functional food that enhances bacterial stability and enzyme activity. In this study, we tested the effect of gums on the growth, viability and *β*-galactosidase activity of four *Lactobacillus* strains under refrigerated storage in milk. Addition of xanthan led to the highest population *L. rhamnosus* GG B103. The presence of guar-locust bean-carrageenan supported the least growth in *L. reuteri* DSM20016. The presence of carrageenan-maltodextrin led to a significant ($p < 0.05$) increase in the viable counts of *L. rhamnosus* GG B101, *L. rhamnosus* GG B103 *L. reuteri* DSM 20016 and *L. reuteri* SD 2112

compared to the control. The population of *L. reuteri* strains decreased slightly during the storage period, whereas those of *L. rhamnosus* strains remained relatively stable during the same period. Xanthan was found to be the best growth enhancer, whereas carrageenan-maltodextrin best gum in terms of viability. *L. rhamnosus* strains were remained relatively stable during the storage and are therefore found to be the best strains in terms of viability. Three (CA, CM and PC) out of the four tested gums significantly ($p < 0.05$) enhanced the β-galactosidase activity of *Lactobacillus* strains.Guar-locust bean-carrageenan (GL) performed as the best β-galactosidase activity enhancer in *L. rhamnosus* GG B101 and B103 and as an inhibitor in *L. reuteri* stains. Our results showed that including both CM and GL in milk could achieve an enhanced level of β-galactosidase activity in *L. rhamnosus* GG B101*and L. rhamnosus* GG B103. The information gleaned from this study could help improve the quality of probiotic functional foods and thereby contribute to the alleviation of lactose malabsorption.

CHAPTER 8

Summary and Recommendation

Guar was found to be the best gum to enhance growth of *L. rhamnosus* GG B101, *L. rhamnosus* GG B103, *L. delbrueckii* subsp. *bulgaricus* SD35, *L. delbrueckii* subsp. *bulgaricus* SD33, *L. acidogphilus* SD 16, *L. acidogphilus* EF7, *L. reuteri* DSM20016, *L. reuteri* CF2-2F at 0.5% concentration during storage at 37C in laboratory media. Xanthan stimulated the highest growth of *L. rhamnosus* GG B101 and *L. rhamnosus* GG B103 in milk during 12 h incubation at 37 °C. Marked ($p < 0.05$) differences were observed between milk samples containing gums and the control. Carrageenan-maltodextrin showed most positive effect on the viability of *L. rhamnosus* strains. The addition of carrageenan, carrageenan-maltodextrin, pectin-carrageenan, and significantly ($p < 0.05$) enhanced levels of *β*-galactosidase activity of *L. rhamnosus* GG B101, *L. rhamnosus* GG B103 and *L. reuteri* DSM20016 strains with significant ($p < 0.05$) differences between control and treated strains. Guar-locust bean-carrageenan (GL) performed as the best *β*-galactosidase activity enhancer in *L. rhamnosus* GG B101 and *L. rhamnosus* B103 and as an inhibitor in *L. reuteri* DSM20016 and *L. reuteri* SD 2112. Guar-locust bean-carrageenan significantly ($p < 0.05$) inhibited *β*-galactosidase activity of *L. reuteri* SD 2112. *L. rhamnosus* GG B101 *and L. rhamnosus* B103 were the best strains to attain enhanced levels in growth, viability and beta-galactosidase activity. Our results showed that including both carrageenan-maltodextrin and guar-locust bean-carrageenan in milk could improve growth, viability and achieve an enhanced level of *β*-galactosidase activity in *L. rhamnosus* GG B101and *L. rhamnosus* GG B103. The information gleaned from this study could be used in the food industry to improve the quality of probiotic functional foods and thereby contribute to the alleviation of lactose malabsorption.

uture studies should explore the use of carrageenan, carrageenan-maltodextrin, pectin-carrageenan, and guar-locust bean-carrageenan to determine the viability and alpha/beta-galactosidase activity in yogurt.

In addition, acceptability test of yogurt containing carrageenan, carrageenan-maltodextrin, pectin-carrageenan, and guar-locust bean-carrageenan aimed at maximizing the use of these gums in yogurt for a more efficient lactose digestion should be done.

Impact carrageenan, carrageenan-maltodextrin, pectin-carrageenan, and guar-locust bean-carrageenan on acid whey production and texture in yogurt should be assessed. Concentration of gums directly affects acid whey production in yogurt and indirectly impact on the taste of yogurt. Effect of homogenization on the interaction between carrageenan, carrageenan-maltodextrin, pectin-carrageenan, and guar-locust bean-carrageenan and viability of *L. rhamnosus* GG B101 and *L. rhamnosus* B103 should be studied.

Other studies should be conducted in investigating the impact of carrageenan, carrageenan-maltodextrin, pectin-carrageenan, and guar-locust bean-carrageenan on protein expression.

A genetic engineering study should identify and clone the genes responsible for high beta-galactosidase activity of *L. rhamnosus* GG B101 *and L. rhamnosus* GG B103 in the presence carrageenan, carrageenan-maltodextrin, pectin-carrageenan, and guar-locust bean-carrageenan

Food engineering study should be conducted to identify potential use of *L. rhamnosus* strains and guar-locust bean-carrageenan in the formulation of functional symbiotic neutraceutical to alleviate lactose intolerance.

Clinical study to explore the symbiotic relationship between *L. rhamnosus* GG B101 and *L. rhamnosus* B103 and the guar-locust bean-carrageenan for enhanced β-galactosidase production for the treatment of Fabry's disease could be done.

Investigation into mechanism of interaction between gums and *L. rhamnosus* GG B101 and *L. rhamnosus* B103 should be conducted to determine how the probiotic interact with gums to increase β-galactosidase activity.

8.1 List of Publications Related to the Subject of the Dissertation

Journal articles

1. Karlton-Senaye, B. D., Tahergorabi, R., Valerie, Giddings., & Ibrahim, A. Salam. (2014). *Effect of gums on the viability and β-galactosidase activity of Lactobacillus spp. in milk during refrigerated storage*. International Journal of Food Science and Technology. (Manuscript accepted).
2. Karlton-Senaye, B. D., Shahbazi A., & Ibrahim, S. A. (2013). *Impact of gums on the growth of probiotic microorganisms.* Springer. (book chapter- In press).
3. Karlton-Senaye, B. D., & Ibrahim, S. A. (2013). *Impact of gums on the growth of probiotics*. Agro FOOD Industry: Functionalfood and Nutraceuticals 24:4.

Poster presentations and conferences attended.

4. Karlton-Senaye, B. D., Tahergorabi, R., & Ibrahim, S. A. (2014). Impact of gums on the growth of *Lacto*B*acillus reuteri*. Poster accepted.114th General Meeting American Society for Microbiology. (May 17 - 20, 2014).
5. Karlton-Senaye, B. D., Tahergorabi, R., & Ibrahim, S. A. (2014). *Impact of gums on the growth of Lactobacillus reuteri.* Poster accepted. The 247th ACS National Meeting. (March 16-20, 2014).

6. Karlton-Senaye, B. D., Giddings, L. Valerie., Tahergorabi, Reza, Hayek, Saeed., Adjalood, L Suleiman., & Ibrahim, S.A. (2014). Impact of gums on the growth of *Lactobacillus reuteri*. Poster presentation at the North Carolina association of food consumer science teachers. (February 22, 2014).
7. Karlton-Senaye, B. D., Shahbazi, A., & Ibrahim, S.A. Impact of gums on the growth of *Lactobacillus reuteri*. Poster presentation at the 11th Annual Ronald E. McNair Research Symposium.(January, 29-30, 2013).
8. Karlton-Senaye, B. D., Shahbazi, A., & Ibrahim, S. A. Impact of gums on the growth of *Lactobacillus reuteri*. Poster presentation at North Carolina Association for Biomedical Research (NCABR).(July 13-16, 2013).
9. Karlton-Senaye, B. D., Shahbazi, A., & Ibrahim, S. A. Impact of gums on the growth of *Lactobacillus reuteri*. Poster presentation at Institute of Food Technologist. (July 13-16, 2013)
10. Karlton-Senaye, B. D., Shahbazi, A., & Ibrahim, S. A. Impact of gums on the growth of *Lactobacillus reuteri*. Poster presentation at the Southeastern Regional Meeting of the American Chemical Society. (November, 14–17, 2012).

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Appendix A

Table A.1

Average Population of Lactobacillus Strains in Modified Basal Media Containing Gums (0.5%)Incubated at 37°C for 12 Hours

Gums	*L. rhamnosus GG* B103	*L. rhamnosus GG* B101	*L. delbrueckii ssp.bulgaricus* SD35	*L. delbrueckii ssp.bulgaricus* SD33	*L. acidophilus* SD 16	*L. acidophilus* EF7	*L. reuteri* DSM20016	*L. reuteri* CF2-2F
Control	7.24 ± 0.01^{ac}	7.14 ± 0.06^{a}	7.95 ± 0.04^{c}	6.87 ± 0.04^{a}	7.56 ± 0.06^{cd}	5.92 ± 0.03^{a}	7.72 ± 0.03^{ab}	8.10 ± 0.14^{ab}
Dextrose	7.49 ± 0.14^{bcd}	8.47 ± 0.06^{e}	8.35 ± 0.06^{d}	7.60 ± 0.11^{a}	8.27 ± 0.04^{bc}	5.99 ± 0.09^{a}	8.45 ± 0.06^{c}	8.44 ± 0.33^{b}
Pectin-Dextrose	7.65 ± 0.07^{cd}	8.30 ± 0.28^{ce}	7.93 ± 0.04^{c}	7.48 ± 0.08^{a}	8.26 ± 0.04^{cd}	5.82 ± 0.03^{a}	8.38 ± 0.06^{c}	8.25 ± 0.30^{ab}
Pectin	7.30 ± 0.28^{ab}	8.03 ± 0.03^{bc}	7.66 ± 0.04^{ab}	7.59 ± 0.08^{a}	8.31 ± 0.01^{cd}	5.88 ± 0.06^{a}	7.92 ± 0.04^{ac}	8.03 ± 0.03^{ab}
Carrageenan	7.80 ± 0.14^{d}	8.20 ± 0.06^{be}	7.54 ± 0.03^{a}	7.59 ± 0.09^{a}	8.18 ± 0.04^{cd}	6.46 ± 0.06^{a}	8.25 ± 0.06^{bc}	8.22 ± 0.03^{ab}
Carageenan-Maltodextrin	7.78 ± 0.04^{d}	8.23 ± 0.04^{be}	8.02 ± 0.03c	7.56 ± 0.04^{a}	7.65 ± 0.04^{a}	6.43 ± 0.03^{a}	8.30 ± 0.23^{bc}	8.24 ± 0.03^{ab}
Locust Bean	6.86 ± 0.04^{a}	8.33 ± 0.03^{ce}	7.51 ± 0.04^{a}	7.33 ± 0.74^{a}	8.24 ± 0.03^{d}	5.95 ± 0.06^{a}	7.92 ± 0.023^{ac}	8.32 ± 0.028^{ab}
Guar	7.65 ± 0.04^{cd}	8.45 ± 0.04^{de}	8.05 ± 0.04^{c}	7.09 ± 0.04^{a}	8.13 ± 0.03^{bc}	5.93 ± 0.04^{a}	8.06 ± 0.06^{ac}	8.37 ± 0.04^{b}
Pectin-Carrageenan	7.56 ± 0.06^{bcd}	8.25 ± 0.07^{be}	7.87 ± 0.01^{bc}	7.13 ± 0.01^{a}	8.19 ± 0.06^{cd}	6.00 ± 0.89^{a}	7.53 ± 0.03^{a}	8.34 ± 0.06^{ab}
Inulin	7.49 ± 0.04^{bcd}	8.09 ± 0.06^{bcd}	8.29 ± 0.11^{d}	6.97 ± 0.01^{a}	8.35 ± 0.04^{d}	5.78 ± 0.01^{a}	8.11 ± 0.01^{ac}	7.87 ± 0.03^{ab}
Guar-locust bean-Carrageenan	7.46 ± 0.04^{bcd}	8.23 ± 0.04^{be}	7.95 ± 0.01^{c}	7.29 ± 0.06^{a}	7.99 ± 0.06^{b}	6.04 ± 0.04^{a}	7.60 ± 0.42^{a}	7.71 ± 0.014^{a}
Xanthan	7.72 ± 0.04^{d}	8.17 ± 0.06^{be}	8.04 ± 0.09^{c}	7.65 ± 0.03^{a}	7.56 ± 0.06^{a}	5.82 ± 0.03^{a}	7.61 ± 0.04^{a}	8.34 ± 0.28^{ab}

Note. Results expressed as means ± standard deviation ($n = 2$). Means in the same column with different letters are significantly ($p<0.05$) different.

Appendix B

Table B.1

Profile of pH values of basal media containing different gums (0.5%) Incubated at 37°C for 12 Hours

Treatments	*L. rhamnosus GG* B103	*L. rhamnosus* GG B101	*L. delbrueckii ssp.bulgaricus* SD35	*L. delbrueckii spp.bulgaricus* SD33	*L. acidophilus* SD 16	*L. acidophilus* EF7	*L. reuteri* DSM20016	*L. reuteri* CF2-2F
Control	6.47	5.90	5.53	6.72	6.09	6.15	5.47	5.49
Dextrose	5.67	5.70	5.50	5.29	5.43	5.63	4.90	4.85
Pectin-Dextrose	5.99	5.32	5.87	5.77	6.00	6.35	5.35	5.32
Pectin	5.86	5.30	5.97	5.92	6.26	6.23	5.56	5.58
Carrageenan	6.26	5.03	6.34	6.60	6.55	5.34	6.21	6.16
Carageenan-Maltodextrin	6.37	5.73	6.37	6.59	6.49	5.65	6.25	6.19
Locust Bean	6.36	6.33	6.35	6.61	6.02	6.46	5.52	5.50
Guar	6.32	5.70	6.33	6.47	6.06	6.02	5.51	5.51
Pectin-Carrageenan	6.21	5.80	6.23	6.44	6.06	6.31	5.46	5.46
Inulin	6.27	5.92	6.18	6.37	5.96	6.48	5.36	5.45
Guar-Locust bean-Carrageenan	6.39	6.23	6.34	6.60	6.03	5.72	5.65	5.50
Xanthan	6.14	5.58	6.21	6.41	5.90	5.60	5.53	5.45

Appendix C

Table C.1

Average Population of Lactobacillus Strains in Modified Basal Media Containing Different Gums (0.25%) Incubated at 37°C for 12 Hours

Treatments	*L.rhamnosus GG* B103	*L. rhamnosus GG* B101	*L. delbrueckii ssp.bulgaricus* SD35	*L. delbrueckii ssp.bulgaricus* SD33	*L. acidophilus* SD 16	*L. acidophilus* EF7	*L. reuteri* DSM20016	*L. reuteri* CF2-2F
Control	7.24	7.14	7.96	6.87	6.90	5.92	7.72	8.1
Dextrose	7.39	7.03	8.35	8.20	7.56	6.35	8.16	8.4
Pectin-Dextrose	7.29	7.09	7.93	8.03	8.08	6.27	7.04	8.34
Pectin	7.42	7.38	7.66	7.61	8.01	6.33	7.88	8.05
Carrageenan	7.12	7.23	7.54	7.92	7.95	6.52	8.27	7.93
Carageenan-Maltodextrin	7.48	7.52	8.02	7.89	7.03	6.47	8.21	8.09
Locust Bean	6.79	7.52	7.51	7.65	7.08	5.87	8.41	8.05
Guar	7.37	7.60	8.05	8.11	8.02	5.95	8.25	8.21
Pectin-Carrageenan	7.11	6.95	7.87	8.05	8.07	5.91	7.78	7.93
Inulin	7.41	7.58	8.28	8.15	7.68	5.86	7.68	8.03
Guar-Locust bean-Carrageenan	7.18	7.24	7.95	7.95	8.16	5.98	7.40	7.90
Xanthan	7.69	7.54	7.99	7.91	7.53	5.89	8.08	8.4

Appendix D

Table D.1

Profile of pH Values of Basal Media Containing Different Gums (0.25%) Incubated at 37°C for 12 Hours

Treatments	*L. rhamnosus* GG B103	*L. rhamnosus GG* B101	*L. delbrueckii ssp.bulgaricus* SD35	*L. delbrueckii spp.bulgaricus* SD33	*L. acidophilus* SD 16	*L. acidophilus* EF7	*L. reuteri* DSM20016	*L. reuteri* CF2-2F
Control	7.24	6.47	5.53	6.72	6.09	6.95	5.47	5.49
Dextrose	7.39	6.35	5.50	5.73	6.06	6.60	5.27	5.23
Pectin-Dextrose	7.29	5.99	5.87	6.10	6.28	7.03	5.67	5.55
Pectin	7.42	5.86	5.97	6.17	6.59	7.02	5.71	5.67
Carrageenan	7.12	6.26	6.34	6.71	6.63	6.20	6.17	6.10
Carageenan-Maltodextrin	7.48	6.37	6.37	6.71	6.58	5.85	6.35	6.14
Locust Bean	6.79	6.36	6.35	6.53	6.06	7.26	5.51	5.51
Guar	7.37	6.32	6.33	6.55	6.10	7.17	5.52	5.50
Pectin-Carrageenan	7.11	6.21	6.23	6.56	6.04	6.86	5.51	5.51
Inulin	7.41	6.27	6.18	6.45	6.10	7.21	5.46	5.48
Guar-locust bean-Carrageenan	7.18	6.39	6.34	6.65	6.09	7.10	5.36	5.54
Xanthan	7.69	6.14	6.21	6.50	6.07	7.22	5.53	5.51

Appendix E

Table E.1

Average Population of Lactobacillus Strains in Modified Basal Media Containing Different Gums (0.5%) Incubated at 37°C for 6 Hours

Treatments	*L. rhamnosus GG* B103	*L. rhamnosus GG* B101	*L. delbrueckii ssp.bulgaricus* SD35	*L. delbrueckii ssp.bulgaricus* SD33	*L. acidophilus* SD 16	*L. acidophilus* EF7	*L. reuteri* DSM20016	*L. reuteri* CF2-2F
Control	5.01	5.03	6.52	6.26	6.21	6.06	5.78	5.86
Dextrose	5.68	5.59	6.36	6.34	6.21	6.10	6.13	4.70
Pectin-Dextrose	5.51	6.00	6.16	7.26	6.63	6.46	6.09	5.32
Pectin	5.56	5.85	6.45	7.16	6.40	6.32	5.73	5.33
Carrageenan	4.83	5.68	6.24	6.12	5.51	6.77	5.76	5.38
Carageenan-Maltodextrin	4.00	5.85	6.34	7.04	5.88	6.71	5.73	5.41
Locust Bean	5.06	5.81	6.39	6.24	6.43	6.19	6.26	5.70
Guar	5.85	6.08	6.03	6.19	6.46	6.16	6.22	5.85
Pectin-Carrageenan	4.76	5.97	6.42	6.45	6.75	6.10	6.07	5.62
Inulin	5.06	5.76	6.43	6.23	6.64	6.13	6.12	5.70
Guar-Locust bean-Carrageenan	5.67	5.80	6.89	6.33	6.46	6.23	6.37	5.94
Xanthan	5.74	6.20	6.41	6.38	6.38	5.99	6.14	4.70

Appendix F

Table F.1

Average population of Lactobacillus strains in Basal Media Containing Different Gums (0.25%) Incubated at 37°C for 6 Hours

Treatments	*L. rhamnosus GG* B103	*L. rhamnosus GG* B101	*L. delbrueckii ssp.bulgaricus* SD35	*L. delbrueckii ssp.bulgaricus* SD33	*L. acidophilus* SD 16	*L. acidophilus* EF7	*L. reuteri* DSM20016	*L. reuteri* CF2-2F
Control	5.01	5.03	6.36	6.7	6.21	6.06	5.78	5.86
Dextrose	5.51	5.38	6.35	6.66	6.20	6.48	5.59	5.23
Pectin-Dextrose	5.63	6.01	6.34	6.32	6.12	6.74	6.06	5.20
Pectin	5.62	5.48	6.4	6.18	6.10	6.61	6.11	5.54
Carrageenan	5.52	5.68	6.26	6.72	5.32	6.73	5.73	5.38
Carageenan-Maltodextrin	5.71	5.72	6.31	6.71	6.30	6.68	5.52	5.30
Locust Bean	5.76	5.62	6.06	6.08	6.09	6.18	6.15	5.60
Guar	5.80	5.77	6.37	6.61	6.28	6.17	6.01	5.53
Pectin-Carrageenan	5.45	5.75	6.41	6.57	6.16	6.14	5.98	5.49
Inulin	4.96	5.43	6.33	6.61	6.26	6.15	5.92	5.34
Guar-Locust bean-Carrageenan	4.94	5.76	6.33	6.70	6.33	6.20	6.09	6.03
Xanthan	5.46	5.75	6.31	6.55	6.62	6.13	6.38	5.23