Functional Properties of Aqueous Fruit Extracts Towards Probiotic and Pathogenic Bacteria

Sophorn Hap

A Thesis Submitted to
Auckland University of Technology
in Partial Fulfillment of the Requirements for the Degree of
Master of Applied Science (MAppSc)

2010

School of Applied Sciences

Supervisor: Dr. Noemi Gutierrez-Maddox

Table of Contents

List of Figures

List of Tables

Attestation of Authorship

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person (except where explicitly defined in the acknowledgements), nor material which to a substantial extent has been submitted for the award of any other degree or diploma of a university or other institution of higher learning."

Signed .....

Date .....

Acknowledgements

I would very much like to thank the following people to whom I owe a tremendous amount of gratitude, for all their cooperation, effort, support and guidance.

Firstly, I would like to thank the New Zealand International Aid and Development Agency, NZAID, for financial support throughout my studies. Without this support, I would have been unable to gain such valuable knowledge, experience and a Master's degree.

I am deeply indebted to my supervisor, Dr. Noemi Gutierrez-Maddox, whose advice, guidance and encouragement right from the beginning to the end enabled me to successfully complete the many experiments and especially the writing up of the thesis.

I would also like to express my sincere thanks to Dr. Stuart Young for statistical advice and Mr. Walter Wilson for his assistance with the microplate reader.

I am also thankful to Saeedeh, Dushi and the other AUT Applied Sciences Allied Staff for their assistance.

Finally, I am very grateful to my parents, brothers, sisters, nephews and nieces for their unstinting support and encouragement.

Abstract

Functional foods and ingredients provide health-promoting benefits to consumers in addition to their nourishing role. Exploitation of fruit ingredients that stimulate the growth of probiotic bacteria and suppress that of pathogenic bacteria could contribute to the success of health-promoting probiotic foods. This study investigated the functional properties of aqueous extracts of four New Zealand-grown fruits, namely, blueberry, strawberry, green kiwifruit, and feijoa, towards probiotic bacteria and food-borne pathogenic bacteria.

The fruit extracts were prepared by homogenizing dried fruit pulp in sodium phosphate buffer (pH 7.4), followed by centrifugation and filtration. Prior to assay, the fruit extracts were sterilized by a two-step membrane filtration using 0.45 μm and 0.22 μm membrane filters. Before mixing the fruit extract with the bacteria, the bacteria were allowed to adapt to the growth medium using two sub-cultures and the cell concentration was standardized at10^3 cells/mL.

Fruit extracts at 10%, 20%, and 30% were each used in a bioassay using a high-throughput method in a sterile 96-well microplate. A series of two-fold dilutions of the fruit extract was prepared resulting in an overall concentration range of 0.01 g/L to 30 g/L. The change in growth was calculated as Δ_{Growth} values after reading the optical density (620 nm) in a microplate reader. The fruit extracts were used in the assay as single extract and as combined extracts.

Results showed that the effect of the fruit extracts were dose- and species-dependent. At high concentrations, the four fruit extracts exerted growth-stimulating effect on the probiotic species of *Bifidobacterium* and *Lactobacilli*, except with *Bifidobacterium longum.* However, a biphasic effect was observed with strawberry, green kiwifruit and feijoa extracts, where at low concentrations, the extracts had growth-inhibiting effect on the probiotic bacteria while high concentrations had growth-stimulating effect.

In contrast, all fruit extracts were found to inhibit the pathogens used in this study which included both Gram-positive and Gram-negative bacteria. A biphasic effect was exerted on all the pathogens, except with feijoa extract towards *Listeria monocytogenes*. Feijoa extract exhibited significant inhibition towards *L. monocytogenes* even at low

concentrations. Based on functionality of a single extract, green kiwifruit seems to be the most effective extract. At high concentration, it exerted the greatest stimulatory effect on probiotic bacteria and inhibitory effect on pathogenic bacteria.

The combination of blueberry and feijoa extracts, similar to that of strawberry and kiwifruit extracts, exerted no beneficial stimulatory effect on the probiotic bacteria, except on *B. longum*. The growth of *B. longum* was enhanced in the presence of combined strawberry and green kiwifruit extracts. The two sets of combined extracts did not have any functional growth-inhibiting effect towards the pathogenic bacteria. The combined extracts of strawberry and feijoa were identified as undesirable due to their growth-stimulating effect on *Yersinia enterocolitica.*

These results are relevant for potential application of fruit extracts not only as functional ingredients in foods but also for cell propagation of probiotic bacteria using appropriate concentrations of the fruit extracts.

Chapter 1

Introduction

In answer to society's demand for a better lifestyle and increased longevity, a wide variety of food is being produced which contains ingredients that provide specific health benefits. These foods, known as functional foods, possess health-promoting and therapeutic properties in addition to their nourishing role. The production of functional foods and ingredients is one of the fastest growing areas in the food industry. The global market for the most common functional food, known as probiotics, increased from $14.9 billion in 2007 to an estimated $15.9 billion by the end of 2008. With a compound annual growth of 4.3%, it is estimated to reach $19.6 billion by 2013 (BCCResearch, 2008).

Probiotic foods contain live micro-organisms which have a beneficial effect on human health. Some probiotic foods have been developed to contain ingredients called prebiotics which are added to increase the number of beneficial bacteria in the host's intestines. The bacteria delivered to the host through probiotic food are meant to increase the level of beneficial micro-organisms to modulate the intestinal microflora. Prebiotics work by acting as additional nutrients for the probiotic bacteria, thus enabling them to thrive longer in the intestine. Fruit extracts are one of the main sources of prebiotic ingredients which have been shown to exhibit antimicrobial activity. Two ideal properties of probiotic food are: 1) the ability to provide and maintain viable bacteria at a minimum concentration of 10^6 colony forming unit (c.f.u) per milliliter (mL) of intestinal fluid and (Bourlioux, Koletzko, Guarner, & Braesco, 2003) 2) the inhibition of pathogens. The aim of the study is to investigate the functional properties of aqueous extracts of blueberry (*Vaccinium corymbosum*), strawberry (*Fragaria x ananassa*), green kiwifruit (*Actinidia deliciosa*), and feijoa (*Acca sellowiana* = *Feijoa sellowiana*), towards probiotic and pathogenic bacteria. This study will determine if the fruit extracts exert functional growth-enhancing effect on the probiotic bacteria and functional growth-inhibiting effect on the pathogenic bacteria. The effect on the bacteria will be evaluated by measuring the change in growth in the presence of single fruit extracts and in the presence of combined fruit extracts.

Results of this study could identify the fruit extract or combination of fruit extracts which could be added to probiotic microorganisms to improve the success of probiotic products. Further, the results could be applied to the development of novel products which are health-promoting and which contain pathogen-inhibiting compounds.

1.1 History of Probiotics

The beneficial properties of live microbial food supplements such as fermented milk have been documented over many centuries. Their use in the treatment of various illnesses is even mentioned in the Bible. Moreover, scientists such as Hippocrates and others considered fermented milk not only as a food product but as a medicine and prescribed it for disorders of the gastro-intestinal system (Oberman, 1985).

At the beginning of the 20th century, the Russian bacteriologist Elie Metchnikoff (Pasteur Institute, France) was the first to identify the beneficial effects of lactic acid bacteria present in fermented milk (Hughes & Hoover, 1995; O'Sullovan, Thorton, O'Sullivan, & Collins, 1992). He attributed the good health and longevity of Bulgarians to their large consumption of fermented milk known as yahourth. In 1908, he postulated a 'longevity-without-aging' theory. The principle of this theory was that lactic acid bacteria caused the displacement of toxin producing bacteria normally present in the intestine which could be responsible for longevity. Metchnikoff stated that lactic acid and other products produced by lactic acid bacteria in sour milk inhibited the growth and toxicity of anaerobic, spore-forming bacteria in the large intestine.

In 1899, Tissier (Pasteur Institute, France) isolated *Bifidobacteria* from the stools of breast-fed infants and found that they were a predominant component of the intestinal flora in humans (Ishibashi & Shimamura, 1993). Tissier recommended the administration of *Bifidobacteria* to infants suffering from diarrhoea, 'believing' that the *Bifidobacteria* would displace putrefactive bacteria responsible for most intestinal disorders, while re-establishing themselves as the dominant intestinal microorganisms (O'Sullovan et al., 1992). The first clinical trials of the effect of probiotics on constipation were conducted in the 1930s. In the 1950s, a probiotic product was licensed by the United States Department of Agriculture (USDA) as a drug for the treatment of scour (*Escherichia coli* infection) among pigs (Orrhage, Brismar, & Nord, 1994). During the last century, different micro-organisms were employed for their

ability to prevent and cure diseases, leading to the coining of the generic term, probiotics (Lidbeck, Overvik, Rafter, Nord, & Gustafsson, 1992).

Studies of lactic cultures in food continued throughout the 20th century and many reports have since yielded variable results around the benefits of the consumption of probiotic foods. Earlier research dealt with the use of fermented milk in the treatment of intestinal infections but recent studies have focused more on other aspects of potential health benefits that might be derived from these organisms, as well as strain selectivity to ensure survival of these bacteria in the gastrointestinal tract and the carrier food.

In 1994, the World Health Organization (WHO) deemed probiotics to be the next most important immune defense system when commonly prescribed antibiotics are rendered ineffective through antibiotic resistance (Kaila Kailasapathy & Chin, 2000; Levy, 2000). The use of probiotics where antibiotic resistance occurs is known as microbial interference therapy.

1.2 Probiotic Foods

Probiotics have been defined in several ways, depending on one's understanding of the mechanisms of action and their effect on human or animal well-being. The word probiotics is derived from Greek, meaning 'for life' and is traditionally used to describe the use of live microorganisms as food supplements that benefit the host through improvement of intestinal microbial balance (Fuller, 1989). Later, probiotics were redefined in terms of human nutrition, i. e. "Probiotics are live microbial food ingredients that have a beneficial effect on human health" (Salminen & Wright, 1998). This definition was further defined to "living microorganisms, which upon ingestion in certain numbers, exerts health benefits beyond inherent basic nutrition" (Guarner & Malagelada, 1998). However, Fuller (1992), defined probiotics as " mono- or mixed culture of live microorganisms which, when applied to animals or humans, beneficially affect the host by improving the properties of the indigenous microflora". In 2002, the joint Food and Agriculture Organization (FAO) and WHO redefined the term probiotics as "live microorganisms which when administered in adequate amounts confer health benefits on the host" (FAO/WHO, 2002).

The notion that food could serve as medicine was first conceived by the Greek philosopher and father of medicine Hippocrates, who wrote, "Let food be thy medicine and let medicine be thy food (Chow, 2002)." Probiotic foods, including dairy products, have been classically defined as 'foods containing live micro-organisms, which are believed to actively enhance health by improving the balance of microflora in the gut' (Gardiner, Heinemann, Bruce, Beuerman, & Reid, 2002). Adequate numbers of viable cells, namely the therapeutic minimum, need to be consumed regularly for the transfer of the 'probiotic' effect to consumers. Consumption should be more than 100 g per day of bio-yoghurt containing more than 10^6 cfu ml^{-1} (Arunachalam, 1999).

It is estimated that over 90 probiotic products containing *L. acidophilus* and *Bifidobacterium* spp., in the form of edible yogurt, buttermilk, frozen desserts and milk powder, are produced worldwide (Shah, 2000). Probiotic organisms themselves are also available in supplement forms such as powders, capsules and tablets (Shah, 2007). Probiotic foods are classified as functional foods and they are defined as ‘foods that contain some health-promoting component(s) beyond traditional nutrients’ (Shah, 2001). Functional foods are also known as designer foods, medicinal foods, nutraceuticals, therapeutic foods, superfoods, foodiceuticals, and medifoods (Shah, 2001). In general, the term refers to a food that has been modified in some way to become ‘functional’. One way in which foods can be modified is by the addition of probiotic microorganisms. Probiotic yoghurts, for instance, contain probiotic bacteria with health promoting components beyond traditional nutrients.

Traditionally, yoghurt is manufactured using *Streptococcus thermophilus* and *L. delbrueckii* ssp. bulgaricus as starter cultures. According to Metchnikoff, these organisms are claimed to offer some health benefits but they are not natural inhabitants of the intestine. Therefore, if yoghurt is to be considered as a probiotic product, *L. acidophilus*, *Bifidobacterium* and *L. casei* must be incorporated as dietary adjuncts. Yakult containing the *L. acidophilus* Shirota strain and fermented products containing *L. acidophilus*, *Bifidobacterium* spp., *L. casei* Shirota, *L. rhamnosus* GG, and *L. reuteri* have been developed in Europe (Shah, 2007). However, *L. acidophilus* and *Bifidobacterium* spp. are most commonly used as probiotics.

Different product types or supplements containing different viable micro-organisms with probiotic properties are commercially available either in lyophilised form or as

fermented food commodities. With the emphasis mainly on 'novel-type' fermented dairy products, a steadily increasing range of yogurt-like products is available on the European market.

1.3 Probiotic Microorganisms

Lactic acid bacteria (LAB) have been used in the preservation of food and in other areas of the food industry for many centuries. LAB are Gram-positive bacilli and cocci which metabolize carbohydrates fermentatively, producing lactic acid as the major end-product (homofermentative strain) or as a significant component in a mixture of end-products (heterofermentative strains) (Salminen & Wright, 1998).

In the dairy industry, probiotic bacteria belonging to the genera *Lactobacillus* and *Bifidobacterium* are the most widely used (Prasad, Gill, Smart, & Gopal, 1998); although some yeasts are also utilised (Donohue & Salminen, 1996). Species belonging to the genera *Lactococcus*, *Enterococcus*, *Saccharomyces* and *Propionibacterium* are also considered as probiotic bacteria. Some strains of *Streptococcus* and *Entercoccus* share the properties of LAB, though, *Streptococcus thermophilus* is the only species currently used in fermented dairy products (Donohue & Salminen, 1996). Most LAB are generally recognised as safe or having GRAS status for human consumption due to their ubiquity on the surface of the human body and in the gut, as well as their long history of safe usage in food products. However, members of the genera *Streptococcus* and *Enterococcus* contain many opportunistic pathogens and are not considered as having GRAS status for human consumption (Donohue & Salminen, 1996).

Probiotic microorganisms, together with their main metabolic products, used in fermented and unfermented milk products, including cheese, are shown in Table1. Information on their potential role in flavour production is provided, but the traditional lactic acid bacteria (i.e. starter cultures) are mainly responsible for much of the flavour and aroma (Tamime, 2005).

Table 1.1 Some selected characteristics of potential probiotic microorganisms used in dairy foods and their principal metabolic products (Tamime, 2005).

Starter organism	Metabolic product	Lactose fermentation
I. Lactic acid bacteria		
Pediococcus acidilactici	DL lactate	Homofermentative
Lactobacillus acidophilus, *gasseri*, *helveticus*, and *johnsonii*	DL lactate	Homofermentative
Lactobacillus casei, *reuteri*, *plantarum*, *rhamnosus*, and *fermentum*	DL lactate	Homofermentative
Bifidobacterium adolescentis, *animalis* subsp. *animalis*, *bifidum*, *breve*, *infantis*, *animalis* subsp. *lactis*, and *longum*	L(+) lactate, acetate	Homofermentative
Enterococcus faecium, and *faecalis*	L(+) lactate	Homofermentative
II. Yeasts		
Saccharomyces boulardii	? Ethanol, CO_2	

According to the previous definition, an impressive number of microbial species and genera are considered as probiotics (Table 1.2). However, only those strains classified as lactic acid bacteria are considered of importance in regard to food and nutrition.

Table 1.2 Microorganisms considered as probiotics (Holzapfel, Haberera, Snelb, Schillingera, & Veld, 1998).

Lactobacillus species	*Bifidobacterium* species	Other lactic acid bacteria	Non-lactic acid bacteria
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis*[1]	*Bacillus cereus* ('toyoi')[1,2]
L.casei	*B. animalis*	*Enterococcus faecium*	*Escherichia coli* ('Nissle 1917')
L. crispatus	*B. bifidum*	*Lactococcus lactis*[3]	*Propionibacterium freudenreichii*[2]
L. gallinarum[1]	*B. breve*	*Leuconostoc mesenteroides*[3]	*Saccharomyces boulardii*[2]
L. delbrueckii	*B. infantis*	*Pediococcus acidilactici*[3]	
L. gasseri	*B. lactis*[4]	*Sporolactobacillus inulinus*[1]	
L. johnsonii (*L. paracasei*)	*B. longum*	*Streptococcus thermophilus*	
L. plantarum			
L. reuteri			
L. rhamnosus			

[1] Main application for animals.
[2] Applied mainly as pharmaceutical preparations.
[3] Little is known about its probiotic properties.
[4] Probably synonymous with *B. animalis.*

1.4 Causes of Induced Changes in Gut Flora

The protective flora which establishes itself in the gut is usually stable but it can be influenced by some dietary and environmental factors. Excessive hygiene, antibiotic therapy, and stress are believed to be the most important factors that have a negative

influence on the imbalance of intestinal flora (Fuller, 1989; Lourens-Hattingh & Viljoen, 2001).

In the natural state, the new-born animal acquires its intestinal microflora mainly from its mother by direct or indirect routes. However, modern methods of animal rearing and husbandry often restrict access between mother and the new-born which prevents it from acquiring the full complement of characteristic microbes (Fuller, 1989). Currently, for example, chicken eggs are removed away from the hen and hatched in a clean incubator. Hence, there is no direct contact with the hen and the chicks must acquire any flora from the incubator environment. With mammals, the separation appears less complete but the tendency for limited acquisition of microflora still exists. The transfer of good bacteria from mother to child is minimized by the practice of excessive hygiene and the over-use of chemicals, sanitizing agents and disinfectants (Fuller, 1989).

The gastro-intestinal microflora can be severely affected by the administration of antibiotics as it causes an alteration in the gut ecosystem (Beaugerie, 2004). When an antibiotic is ingested, the overgrowth of species with a potential for pathogenicity is increased. This results in the disruption of the ecological balance of beneficial gut flora (Guarner & Malagelada, 2003).

The established protective indigenous microflora of the gastrointestinal tract can also be affected by stress (Tannock & Savage, 1974). Several animal studies have found that stress reduces the concentration of lactobacilli (a beneficial lactic acid bacteria in normal gut flora), resulting in an environment more favorable to pathogens (Bailey, Lubach, & Coe, 2004). The findings of Knowles, Nelson and Palombo (2008) confirmed that bacterial count significantly decreased during high-stress weeks compared to baseline weeks among human participants. Moreover, stress can produce drastic changes in gut physiology such as the increased production of bicarbonate, thereby, inhibiting the production of gut mucous and gastric acid. This will result in an environment less conductive to beneficial bacteria (Bailey & Coe, 1999). Protective flora is often reduced as a result of stress and the Western diet, which is low in fibre (Bengmark, 1998). Bengmark (1998) also noted that, on return to earth, astronauts had a significantly reduced ecoflora in which *L. plantarum* was totally eliminated in both the saliva and stools. He attributed these changes to the combination of stress and a fibre-deficient diet.

Probiotics are of potential value where the balance of gut microflora is adversely affected. The restoration of gut flora will enable the host animal to return to normal health.

1.5 Health Claims Associated with Probiotics

The human gastrointestinal (GI) tract is a highly specialized ecosystem which has evolved over time, both physiologically and microbiologically. The human body is inhabited by trillions of microbes and, under a microscope, there is a variety of microorganisms including probiotics. The latter can weigh approximately three pounds (Huffnagle & Wernick, 2007).

The human body consists of nearly ten trillion cells and it is host to one hundred trillion microbes. Nearly 80 percent of these microbes, including those important to the immune system, live in our intestines (Huffnagle & Wernick, 2007). In healthy people, the entire bacteria gut community forms a balanced microflora of 300-500 different species (Guarner & Malagelada, 2003). Although only 40 species predominate, they contribute significantly to the body's resistance to occasional infection (Tamime, 2005). However, changes in the composition of the gut microflora may disrupt that balance and leave the host more vulnerable to infection.

The consumption of probiotic products assists in maintaining good health, restoring body vigor, and in combating intestinal and other disorders (Mital & Garg, 1992). Clinical symptoms reportedly treated or having the potential to be treated with probiotics, include a decrease in the incidence and duration of diarrhoea, irritable bowel syndrome, inflammatory bowel disease, food allergies and atopic eczema. Probiotics also reduce lactose intolerance, hypertension, hepatic disease and enhance the immune system (Klaenhammer, 2000; Parvez, Malik, Kang, & Kim, 2006; Salminen & Wright, 1998). These conditions are believed to be associated with disturbances of the GI tract microflora, which cause various degrees of inflammation of the intestinal mucosa, leading to increased gut permeability (Salminen & Wright, 1998).

The alteration of gut microflora involved in many GI-related inflammatory diseases has been fully documented and is usually accompanied by imbalances in the intestinal microflora. When the healthy host-microbe interaction is disturbed, an immune response

can be induced by the resident bacteria (Salminen, Isolauri, & Onnela, 1995). Duchmann et al. (1995) have previously demonstrated that healthy individuals tolerate their own microflora but that patients with inflammatory bowel disease exhibit a disturbance in the tolerance of their microflora. Some of the properties and effects of probiotics for health maintenance and disease prevention will be discussed in the following sections.

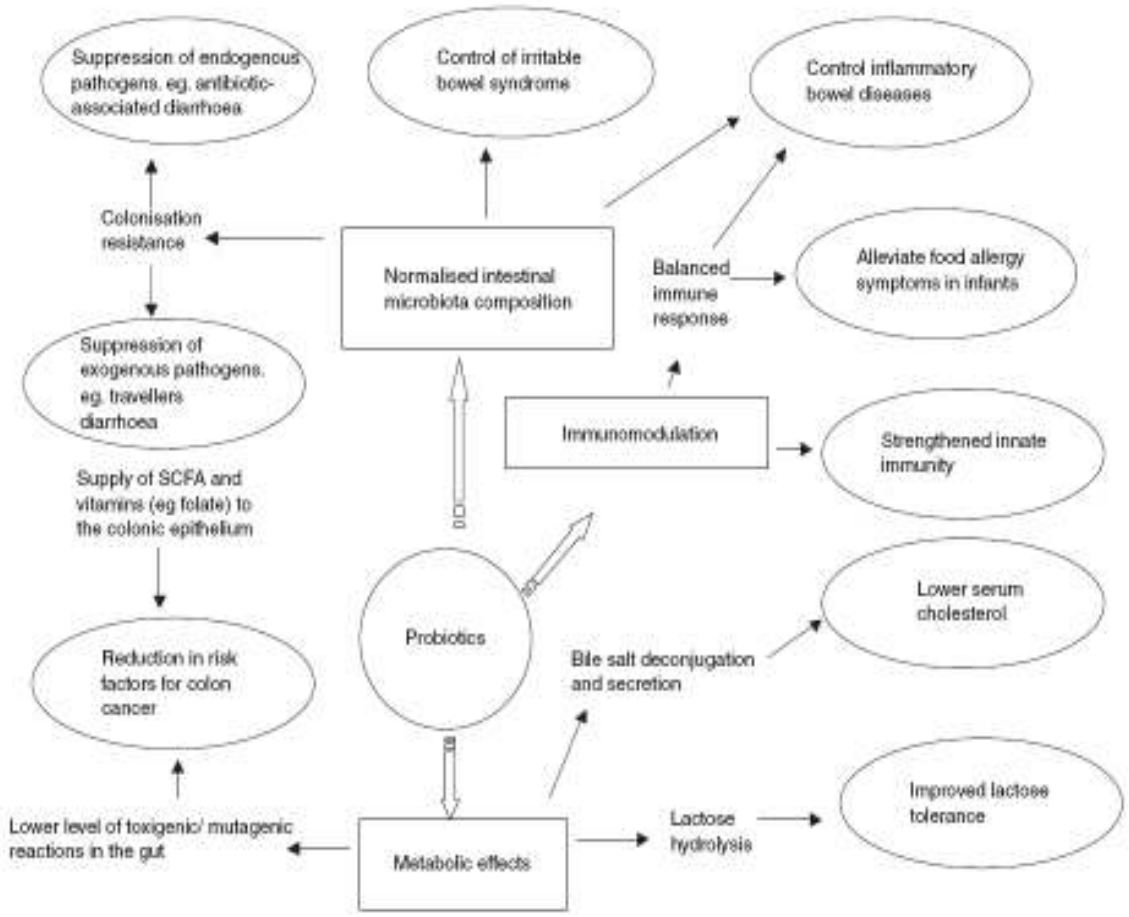

Figure 1.1 Various health benefits from probiotics consumption (Parvez et al., 2006).

1.5.1 Decreased Incidence and Duration of Diarrhoea

The most clearly reported and well-documented evidence of the health benefits of specific probiotics involves the treatment of viral diarrhoeal disorders in children. *Lactobacillus rhamnosus* GG has consistently reduced the duration of rotavirus-associated with diarrhoea in randomized, controlled trials (Guandalini et al., 2000; Raza et al., 1995). Furthermore, trials of other specific probiotic strains (including

Lactobacillus reuteri, *Bifidobacterium lactis* HN 019 and *Saccharomyces boulardii*) have shortened the duration and decreased the symptoms associated with acute viral diarrhoea (Shu, Qu, & Gill, 2001).

The effectiveness of regular supplementation with probiotics in the prevention of acute diarrhoea in children was shown in a placebo-controlled trial of undernourished Peruvian children (Oberhelman et al., 1999). *L. rhamnosus* GG and *S. boulardii* have also proved effective in reducing antibiotic-associated diarrhoea as well as in the treatment of *Clostridium difficile* diarrhoea (Arvola et al., 1999). Pelvic inflammation is a common adverse effect of diarrhoea and a randomized, controlled study by Salminen et al. (1988) showed a significant decrease in diarrhoea in patients with pelvic irradiation when receiving *L. acidophilus* NDCO 1748.

L. rhamnosus GG proved to be effective in the treatment of infant rotavirus diarrhoea in that it consistently reduced the duration of diarrhoea approximately by half in randomized controlled trials. It was also effective in the treatment of acute diarrhoea in Asian children (Pant et al., 1996). In another study, Kaila, Isolauri, Saxelin, Arvilommi, & Vesikari (1995) suggested that heat-inactivated *L. rhamnosus* GG was equally effective as living *L. rhamnisus* GG in reducing the duration of diarrhoea; however, the effect of the living probiotic was more pronounced on rotavirus specifically to immunoglobulin A response.

The mechanism by which *S. boulardii* exerts its action in preventing antibiotic-associated diarrhoea is not yet fully understood. Possible mechanisms which have been demonstrated in animals, include the production of a protease that inactivates the toxin A receptor, the production of increased levels of secretory IgA and IgA antitoxin A as well as competition for attachment sites (Pothoulakis et al., 1993). *S. boulardii* has also been shown to block *C. difficile* adherence to cells *in vitro* (Tasteyre, Barc, Karjalainen, Bourlioux, & Collignon, 2002).

1.5.2 Irritable Bowel Syndrome (IBS)

IBS is defined as a functional bowel disorder where abdominal pain is associated with a change in bowel habits whose symptoms are disordered defecation and distention (Camilleri, 2001). It has been suggested that symptoms of IBS could be (at least partly)

attributable to disturbed intestinal microflora (Bradley, Wyatt, Bayliss, & Hunter, 1987; King & Hunter, 1998). The increase in colonic gas production, commonly found in IBS subjects, has been explained by abnormal colonic fermentation (King & Hunter, 1998) and by excessive swallowing of air (Haderstorfer, Psycholgin, Whitehead, & Schuster, 2008).

Results obtained from studies investigating the role of intestinal microflora and colonic fermentation in IBS are contradictory and therefore, the question of whether a microbial imbalance exists in IBS subjects is open to debate. Despite this inconsistency, several studies have assessed the effect of bacterial treatment of IBS symptoms. Some reports suggest that lactobacilli can be useful in the therapy of functional intestinal disorders including cases of IBS. According to a study by Niedzielina, Kordeckia, & Birkenfeldb (2000) on the efficacy of *L. plantarum* 299V in patients with IBS, a marked improvement in the symptoms of IBS was noted. However, the researchers suggested that further studies on large cohorts of patients and a longer period of therapy in order to establish the place of *L. plantarum* in the treatment of IBS, were required.

Jonsonn et al. (1983) demonstrated that *L. plantarum* exerted a positive effect on several, well characterized symptoms of IBS and also provided a possible explanation of how this bacterium works. It is the unique ability of *L. plantarum* to catabolize arginine and generate nitric oxide. According to Wright, Rees, & Moncada (1992), nitrogen oxide may exert a positive effect on the motility of the large and small intestines, resulting in the improvement of the splanchnic circulation and immunological process.

In addition to a single bacterial strain treatment, the effect of a combination of bacterial strains on IBS was also tested. A liquid infusion of 18 bacteria into the caecum of IBS subjects improved the symptoms in 25 of 33 patients according to a preliminary report by Andrews & Borody (1993). In a study by Brigidi, Vitali, Swennen, Bazzocchi, & Matteuzzi (2001), a bacterial combination of VSL-3, containing four *Lactobacillus* strains, three *Bifidobacterium* strains and a *S. thermophilus* strain, was tested on 10 subjects with either IBS or functional diarrhoea (FD). In this preliminary study, no control group was included and both IBS and FD subjects reported a clear clinical improvement after the consumption of the probiotic mixture.

1.5.3 Inflammatory Bowel Disease (IBD)

IBD refers to disorders of an unknown cause that are characterized by chronic and recurrent intestinal infection. Ulcerative colitis (a relapsing inflammatory disorder of the colon), Crohn's disease (a chronic IBD occurring anywhere from the mouth to the anus), and pouchitis (a non-specific inflammation of the ileal reservoir) are generally included in IBD. The mechanisms responsible for the onset of IBD remain unknown, but it is assumed that IBD results from abnormal host responses towards some members of GI microflora or from a defective mucosal barrier (Marteau, Vrese, Cellier, & Schrezenmeir, 2001). In Crohn's disease and ulcerative colitis, an imbalance in the GI microflora has also been suggested (Dunne et al., 1999; Kennedy, Kirk, & Gardiner, 2000).

Although several promising animal studies have been performed to study the efficacy of probiotic bacteria in IBD (Schultz & Sartor, 2000), human studies have been relatively scarce. Malina, Suomalainen, Saxelin, & Isolauri (1996) assessed the immunostimulatory effect of *L. rhamnosus* GG (daily dose 2 x 10^{10} cfu for 10 days) on 14 children with Crohn's disease. *L. rhamnosus* GG was shown to stimulate the gut IgA immune response and the authors concluded that *L. rhamnosus* GG could have potential in promoting the gut's immunological barrier in this disease.

Furthermore, Plein and Hotz (1993) performed a pilot, double-blind, controlled study of the efficacy of *S. boulardii* on symptoms of Crohn's disease. Twenty patients with severe to moderate Crohn's disease were randomly assigned to receive either *S. boulardii* or a placebo for seven weeks together with the standard treatment. A significant reduction in the frequency of bowel movements and in disease activity was observed in the group receiving *S. boulardii* but not in the placebo group.

Venturi et al. (1999) proposed a different approach by studying the clinical efficacy of a probiotic mixture (preparation VSL-3) in ulcerative colitis subjects in remission. Twenty patients, intolerant or allergic to 5-aminosalicylic acid, consumed a VSL-3 preparation (bacterial daily dose 3 x 10^{12} cfu) for a year. The results suggested that VSL-3 might be a useful therapy in maintaining remission in ulcerative colitis patients.

1.5.4 Food Allergies and Atopic Disease

Atopy is defined by the individual predisposition to develop a group of inflammatory disorders in response to certain food or environment substances (Kirschbaum et al., 2003). Several studies have suggested a role for gut-colonizing bacteria in preventing and treating manifestations of food allergy and atopic disease, including atopic eczema, asthma, and other allergies. It has been proposed that children exhibiting allergies may have an aberrant gut microflora (Kalliomäki et al., 2001). The beneficial effects of probiotics on allergy development have primarily resulted from studies on infants and children (Kalliomaki et al., 2001; Pessi, Sütas, Hurme, & Isolauri, 2000). In a placebo-controlled study of high-risk infants, the supplementation of *L. rhamnosus* GG to both mothers and infants, significantly reduced the incidence of atopic disease by 2 years of age (Kalliomaki et al., 2001). In other studies, supplementation of hydrolyzed whey formula with *L. rhamnosus* GG and *B. lactis* Bb-12, significantly alleviated symptoms of atopic eczema in infants with milk hypersensitivity (Isolauri, Arvola, Sutas, Moilanen, & Salminen, 2000).

Another application of lactic acid bacteria would be in cases of allergic reactions in milk-fed infants due to casein, where lactobacilli degrade this protein to smaller peptides and amino acids. Sütas et al. (1996) demonstrated that hydrolysis of different casein proteins by *Lactobacillus casei* GG decreased the proliferation of mitogen-induced human lymphocytes compared to non-treated caseins. The effect of *Lactobacillus casei* GG supplement in infants with atopic eczema after elimination of cow's milk from the diet was investigated. The results indicated a significant reduction of the extent and intensity of infantile atopic dermatitis (Sutas, Hurme, & Isolauri, 1997).

Two hundred and thirty infants with suspected cow's milk allergy received *Lactobacillus* GG or placebo for a month in a randomized double-blind experiment, together with the elimination of cow's milk and skin treatment. Results suggested that the *Lactobacillus* GG group showed a greater decrease in infants suffering from IgE-associated atopic eczema/dermatitis syndrome (AEDS) when compared to the placebo group. This indicates that treatment with *Lactobacillus* GG may alleviate AEDS syndrome in IgE-sensitized infants (Viljanen et al., 2005). Furthermore, *Lactobacillus*

GG was reported to significantly alleviate symptoms of atopic eczema and cow's milk allergy in studies by Kirjavainen, Salminen, & Isolauri (2003).

Although the mechanism of action of probiotics in preventing allergy is only beginning to emerge, the effects are likely to be mediated by adhesion to intestinal mucus and mucosal surfaces (He et al., 2001; Ouwehand et al., 2001). The hypothesis proffered is that the prevention of early atopic disease in children may reduce the risk of developing food allergies and asthma later in life. Evidence that probiotics may reduce allergic symptoms in existing atopic disease is also accumulating for specific strains and their use in early infancy.

1.5.5 Reducing Lactose Intolerance

Lactose intolerance or more correctly lactose maldigestion occurs more frequently in adults (primary lactose maldigestion) and in people with bowel resection or enteritis (secondary lactose maldigestion). The inability to digest lactose adequately is due to the absence of β-galactosidase in the human intestine and can lead to varying degrees of abdominal discomfort (Kim & Gilliland, 1983).

It is well established that patients with lactose maldigestion experience better digestion and tolerance of lactose when contained in the form of yoghurt rather than in milk (Vrese et al., 2001). Yoghurt and other conventional starter cultures and probiotic micro-organisms in fermented milk play an important role in the improvement of lactose digestion and elimination of symptoms of intolerance in subjects prone to lactose maldigestion (Kim & Gilliland, 1983; Ouwehand, Salminen, & Isolauri, 2002). This could be due to the presence of β-galactosidase in the bacteria fermenting the milk. Upon ingestion, the bacteria are lyzed by bile in the small intestine, which releases the enzyme and degrades the lactose (Vesa, Marteau, & Korpela, 2000). Sonication of the *L. acidophilus* cells, prior to their addition, significantly alleviates lactose maldigestion, which would indicate that cell lysis promotes lactose digestion (McDonough, Wong, Hitchins, & Bodwell, 1985). This premise is supported by the fact that milk containing or fermented by *L. acidophilus*, which survives the intestinal transit intact, is not as effective as milk containing or fermented by *S. thermophilus*, as it is killed upon ingestion (Lin, Savaiano, & Harlander, 1991).

In clinical practice, the replacement of milk with yoghurt or fermented dairy products allows for better digestion and decreases diarrhoea and other symptoms of intolerance in patients (Arrigoni et al., 1994). The bacterial β-galactosidase activity of yoghurt is considered to be the main factor responsible for improving lactose digestion (Vrese et al., 2001). Yoghurt delays gastric emptying and intestinal transit causing a slower delivery of lactose to the intestine, thus optimizing the action of residual β-galactosidase in the small bowel and decreasing the osmotic load of lactose (Labayen et al., 2001). The beneficial effect is usually more commonly associated with products fermented with *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. However, some probiotics such as *L. rhamnosus* GG are not capable of fermenting lactose (Ouwehand et al., 2002).

1.5.6 Cholesterol Lowering Effect of Probiotics

According to Gilliland (1989), the consumption of fermented milk significantly reduces serum cholesterol in hypercholesterolemic individuals and significant reductions in plasma cholesterol levels are linked to a significant reduction in the risk of cardiovascular disease.

Certain *L. acidophilus* strains and some *Bifidobacterium* species are claimed to lower cholesterol levels within the intestines. Cholesterol coprecipitates with deconjugated bile salts as the pH declines as a consequence of lactic acid production by lactic acid bacteria (Marshall, 1996).

Although the ability of probiotics to reduce serum cholesterol is still a matter of debate, this ability may be linked to the activity of some *Lactobacilli* strains in deconjugating bile salts through the production of bile salt hydrolase (Marteau et al., 1995). However, faecal loss of bile acids could result in an increased requirement of cholesterol for *de novo* synthesis of bile salts, thereby reducing cholesterol levels. The oral administration of *L. johnsonii* and *L. reuterii* in pigs resulted in a decrease in serum cholesterol (Toit et al., 1998) and a similar reduction has been also demonstrated in rats with the oral administration of a probiotic mixture (Fukushima, Yamada, Endo, & Nakano, 1998). Likewise, the oral administration of *L. reuterii* in mice reduced serum cholesterol (Taranto, Medici, Pedigon, Holgado, & Vadez, 1998).

The use of a fermented product with the ability of *L. acidophilus* CRL 1014 and *E. faecium* reduced cholesterol by 54% and 55.4%, respectively when the cholesterol was added to the culture medium (Rossi, Giori, Holgado, & Valdez, 1994). Similar results were obtained by Fukushima & Nakano (1995), when rats, fed a diet rich in cholesterol, showed a reduction in the total cholesterol as well as an increase in the high density lipoprotein fraction. This was achieved by a daily intake of a probiotic product containing several species of *Lactobacillus*, *Streptococcus*, and yeast.

Another theory postulated was that *L. acidophilus* deconjugates bile acids into free acids, which are then excreted more rapidly from the intestinal tract than conjugated bile acids. As free bile salts are excreted from the body, the synthesis of new bile acids from cholesterol may reduce the total cholesterol concentration in the body (Gilliland & Speck, 1977).

1.5.7 Enhancing Immune Function

HIV-positive children with episodes of diarrhoea frequently experience malabsorption associated with possible bacterial overgrowth. *L. plantarum* 299V can be safely administered to immuno-compromised hosts, producing a positive effect on the immune response and potentially improving growth and development. The immune response may further be enhanced when one or more probiotics are consumed together and work synergistically, as when *Lactobacillus* is administered in conjunction with *Bifidobacteria* (Cunningham-Rundles et al., 2000).

The effect of probiotics on the immune response has been comprehensively reviewed. The majority of evidence from in vitro systems, animal models and humans suggests that probiotics can enhance both specific and nonspecific immune responses. These effects are believed to be mediated through activating macrophages, increasing levels of cytokines, increasing natural killer cell activity and/or increasing levels of immunoglobulin (Ouwehand et al., 2002).

Confirmation of enhanced immunity and increased resistance to infection has been demonstrated in both animals and humans. In an immunodeficient euthymic mouse model, *Lactobacillus* sp. and *Bifidobacteria* decreased disseminated systemic *Candida albicans* (Wagner, Warner, Roberts, Farmer, & Balish, 1997).

Healthy Japanese children consuming a probiotic formula containing *Bifidobacteria* showed increased faecal levels of total and anti-poliovirus IgA than prior to taking the formula (Fukushima, Kawata, Hara, Terada, & Mitsuoka, 1998). Co-colonization of rats with *Lactobacillus plantarum* and *Escherichia coli* resulted in higher circulating concentrations of total IgA and of *E. coli*-specific IgA and IgM than rats which were colonized with *E. coli* alone (Herias et al., 1999). Oral application of *Lactobacilli* led to macrophagous and lymphocytic stimulation and to the release of enzymes from murine peritoneal macrophages (Perdigon, Macias, Alvarez, Oliver, & Holgado, 1986).

1.5.8 Hypertension

Preliminary evidence indicates that probiotic bacteria or their fermented products may also play a role in blood pressure control, with animal and clinical studies documenting the antihypertensive effects of probiotic ingestion (Nakamura, Masuda, & Takano, 1996; Nakamura, Yamamoto, Sakai, & Takano, 1995). Elderly hypertensive patients, who consumed fermented milk with starter cultures such as *Lactobacllus helveticus* and *Saccharomyces cerevisiae* experienced reductions in systolic and diastolic blood pressure level (Hata et al., 1996). A similar decrease in systolic and diastolic blood pressure and the heart rate of hypertensive patients was also disclosed when powdered probiotic cell extracts were administered (Nakamura et al., 1995; Sawada et al., 1990). Considering the current epidemic of cardiovascular disease, regular consumption of probiotics may provide a modest prophylactic effect (Nakamura et al., 1995).

1.5.9 Anticarcinogenic Activity

Cancer is caused by the mutation or activation of abnormal genes which control cell growth and division but the presence of these abnormal cells do not always result in cancer as the normal cells can out-compete them. Moreover, the immune system recognizes and destroys most abnormal cells. It has been reported that probiotic cultures may confer a variety of important nutritional and therapeutic benefits on consumers, including anticarcinogenic and antimutagenic activity. Probiotics may suppress the growth of bacteria that convert procarcinogens into carcinogen, thereby reducing the amount of carcinogens in the intestine (Hata et al., 1996; Lee et al., 2004).

Several lactic acid bacteria have been shown to produce anticarcinogenic or antimutagenic activity and some of these activities are apparently due to compounds or substances produced by the organism during growth (Oda, Hasegawa, Komatsu, Kambe, & Tsuchiya, 1983). The antitumor action of probiotics is attributed to the inhibition of bacteria which converts procarcinogens to carcinogens, as well as the activation of the host's immune system and/or the reduction of the intestinal pH, thereby reducing microbial activity (Gilliland, 1989). Kailasapathy & Rybka (1997) reported several animal studies confirming that the intake of yogurt and fermented milk containing probiotic bacteria inhibited tumor formation and proliferation.

One hypothesis for the prevention or delay of tumor development by lactobacilli is that they may bind to mutagenic compounds in the intestine (Lidbeck et al., 1992), thereby decreasing the absorption of these mutagens. Intake of freeze-dried *L. casei* for 3 weeks reduced the urinary excretion of mutagens (Hayatsu & Hayatsu, 1993).

Dietary supplementation with a strain of *L. acidophilus* significantly suppressed the total number of colon cancer cells in rats in a dose-dependent manner (Santis, Famularo, & Simone, 2000). Another study indicated that *Lactobacillus* GG reduces the incidence and number of tumors in animals with artificially induced colon cancer (Goldin, Gualtieri, & Moore, 1996). *B. longum* can also inhibit the incidence of colon, liver, small intestinal and mammary tumors in rats (Orrhage, Brismar et al., 1994). Shahani, Friend, & Bailey (1983) emphasized that *L. acidophilus* super strain DDS1 produced the strongest antitumor activity.

1.5.10 Antimutagenic Activity

The antimutagenic effect of fermented milk has been shown to be effective against the range of mutagens and promutagens in various test systems based on microbial and mammalian cells. When these probiotic organisms bind mutagens to the cell surface, they are reported to reduce faecal enzymatic activities including β-glucuronidase, azoreductase, and nitroreductase, which are involved in the activation of mutagens (Goldin & Gorbach, 1984; Orrhage, Sillerstrom, Gustafsson, Nord, & Rafter, 1994).

Lankaputhra & Shah (1998) studied the antimutagenic activity of organic acids produced by probiotic bacteria against several mutagens and promutagens. The TA-100

mutant of *Salmonella typhimurium* (His^-) strain was used as a mutagenicity indicator organism and the Ames Salmonella test was employed in the mutagenicity test. In this study, butyric acid showed a broad spectrum antimutagenic activity against all mutagens or promutagens studied and live bacterial cells showed higher antimutagenicity than killed cells. The results revealed the importance of consuming live probiotic micro-organisms and of maintaining their viability in the intestine to provide efficient inhibition of mutagens.

1.5.11 Reduction of *Helicobacter pylori* Infection

Helicobacter pylori is a pathogenic bacterium that causes pelvic ulcers, type B and chronic gastritis. It is an opportunistic pathogen and patients are frequently asymptomatic (Armuzzi et al., 2001). The triple therapy antibiotic treatments can successfully eradicate *H. pylori*; however, sometimes the antibiotics not only cause side effects but also make the bacteria more antibiotic resistant. Probiotic organisms do not appear to eradicate *H. pylori* but they are able to reduce the bacterial load in patients infected with *H. pylori*. *Lactobacillus johnsonii* La1 and *Lactobacillus gasseri* OLL2716 have been found to reduce *H. pylori* colonization and inflammation whilst, *Lactobacillus casei* Shirota and *Lactobacillus acidophilus* are reported to inhibit growth (Cats et al., 2003; Felley et al., 2001).

Numerous studies have shown that various lactobacilli, or their metabolites, can inhibit or even kill *Helicobacter pylori in vitro* (Lorca, Wadstrom, Valdez, & Ljungh, 2001). Through catabolism, lactobacilli produce relatively large amounts of lactate which has been identified as an inhibitory and/or bactericidal factor (Aiba, Suzuki, Kabir, Takagi, & Koga, 1998; Midolo, Lambert, Hull, Luo, & Grayson, 1995). Other *in vitro* findings revealed that *Lactobacillus salivarius* WB 1004 not only inhibited adhesion of *H. pylori* to mouse and human gastric epithelial cells, but also reduced IL-8 secretion (Kabir et al., 1997). The study also showed that some (but not all) strains of *L. reuteri* share a surface glycolipid-binding protein with *H. pylori*, and thus will compete for binding sites in the host (Mukai et al., 2002).

1.6 Suppression of Bacterial Growth by Probiotics

The intestinal epithelium and the normal intestinal microflora represent a barrier to the movement of pathogenic bacteria, antigens and other noxious substances from the gut lumen. Normally, this barrier remains intact and provides normal intestinal function but, if either the epithelial cells or the normal microflora are disturbed, altered permeability facilitates the invasion of pathogens, foreign antigens and other harmful substances (Salminen, Isolauri, & Salminen, 1996).

Suppression of the growth of Gram-negative bacteria in the intestine can reduce the amount of endotoxin, which, in turn, attenuates endotoxin-associated organ damage. In such a scenario, the use of probiotic bacteria provides a viable therapy option. The most numerous probiotic bacteria colonising the small intestine are of the lactobacilli species; whereas in the colon, the majority is mainly bifidobacteria. Both have been shown to attenuate the growth of Gram-negative bacteria and the consumption of *Lactobacillus acidophilus* strain NP51 was found to reduce the number of *Escherichia coli* O157:H7 in fecal samples of cattle (Peterson et al., 2007; Younts-Dahl et al., 2005). *Lactobacillus* strains, LAP5 and LF33, obtained from swine and poultry, respectively, inhibited the growth of *E. coli* and *Salmonella typhimurium* in an *in vitro* culture system (Tsai et al., 2005).

According to Robinson & Samona (1992), acid produced by *Bifidobacteria* may well suppress the growth of pathogenic micro-organisms in the intestinal tract, since *Bifidobacteria* produce organic acids, namely, lactic and ethanoic (acetic) acid from glucose. The production of these acids in the intestine lowers the pH of the contents, thereby inhibiting the growth of undesirable bacteria as well as stimulating intestinal peristalsis and assisting in the physical removal of invading pathogens (Robinson & Samona, 1992). Probiotic microorganisms may prevent harmful bacterial colonization of a habitat by competing more effectively than an invading strain for adhesion sites. They also create an environment unfavorable to the growth of the invading harmful pathogens by producing antibacterial substances (Gurr, 1987).

Bifidobacteria can also prevent colonization of intestine by adventitious species through competition for both nutrients and space along the epithelial surfaces. In particular, *Bifidobacteria* participate in the formation of the ‘mucosa-associated flora’ in the colon,

where microorganisms adhere to the colon cells and embed in the mucin layer, thereby covering the epithelial layer. Thus, they act as a 'living barrier' to other micro-organisms (Robinson & Samona, 1992).

1.7 Market for Functional and Probiotic Foods

Fermented dairy products enriched with probiotic bacteria have developed into one of the most successful categories of functional foods. They have given rise to the creation of a completely new category of probiotic products, such as daily-dose drinks in small bottles. This category now had a market volume of more than 1000 million kg and was said to account for over Euro1.2 billion annually in retail sales in Europe (Saxelin, Tynkkynen, Mattila-Sandholm, & Vos, 2005). Worldwide, the market for fermented products, increased from $15.9 billion by the end of 2008. With a compound annual growth of 7%, it is estimated to reach $22.4 billion in 2013 (BCCResearch, 2009).

In 1997, functional food products accounted for 65% of the European functional food market, which was valued at US$ 889 million (Hilliam, 1998b). Leatherhead Food Research Association undertook a study of the market for functional food in the United Kingdom, France, Spain, Belgium, Netherlands, Denmark, Finland, and Sweden (Hilliam, 1998b). The results of the study indicated that the probiotic yoghurt market in these nine countries totalled more than 250 million kilograms in 1997 (Hilliam, 1998b). Of those countries, France represented the largest market, with sales of about 90 million kilograms, valued at US$ 219 million. Rapid growth of probiotic yoghurts in the German market was indicated during 1996-1997, where they increased by 150%; whereas the United Kingdom market only grew by 26% over the same period. On average, probiotic yoghurt accounted for about 10% of all yoghurts sold in the 9 countries studied. Denmark was observed to have the highest proportion of probiotic yoghurts (20%), followed by Germany and the United Kingdom (both at 13%) and then France (11%). At the lower end of the scale were The Netherlands and Belgium at 6%, followed by Finland and Sweden at 5% (Hilliam, 1998b). The market for functional foods in Europe could ultimately account for about 5% of total food expenditure, which, according to the prices at the time of the study, would equate to about US$ 30 billion.

However, the Japanese market is still dominated by original functional foods such as non-alcoholic drinks where dietary fibre and probiotics are significant functional

ingredients. Bikkle, considered to be the quintessential functional drink, was launched in 1993 by Suntory (Osaka, Japan) and contains bifidobacterial cultures, whey minerals, xylo-oligosaccharides, and dietary fibre. In its first year, total sales of 11 billion Japanese yen were achieved. Interestingly, the fermented milk drink Yakult which is classified as a functional food in Europe, is not regarded as such in Japan, as the presence of probiotics in isolation from other functional ingredients, does not carry functional food status. In addition to functional drinks, functional milk products and innovative products for children are found in a variety of foods and drinks such as ice cream, confectionery, biscuits, snacks, and calcium-fortified drinks. There have been several developments in the dairy products category, including that of yoghurt supplemented with oligosaccharides and calcium. It is expected that prebiotics and probiotics continue to be among the major functional food ingredients for the foreseeable future in Japan and estimation of the size of the functional food market, at the time of the study, was in the range of US$ 3-3.5 billion.

In the United States, vitamin- and mineral-enriched products continue to be among the more successful functional foods and it was predicted that the US market would experience the fastest growth rates in the future. Leatherhead Food Research Association 1996 Report indicated that the value of the global market for functional foods at US$ 6.6 billion in 1994, with Japan accounting for just under half. The market for the functional foods was forecasted to increase from US$ 2.5 billion to 3.3 billion by 2003 (Hilliam, 1998a).

1.8 Functional Ingredients

Functional ingredients are included in food products sold as dietary supplements, functional foods or nutraceuticals. This new generation of ingredients is specifically added to products to obtain an intended health-related effect on consumers. Furthermore, some dietary components can dramatically influence the factors for quality of life, such as the modulation of performance or risk reduction in acquiring a variety of diseases, through modification of one or more physiologic processes (Milner, 2000). Since this beneficial health effect is produced by a biological or physiological activity of the ingredient in the body, the term bioactive food ingredient or functional food ingredient can be used to encompass this class of ingredients (Meisel, 1997; Xu, 1998).

These functional ingredients are a diverse group of compounds and are designed to produce a positive effect on the health of consumers. The term functional is not intended to differentiate these ingredients from other ingredients historically consumed as part of the body supply of biologically active constituents. In fact, all foods should be considered as functional. Therefore, the term functional ingredient is intended to convey the function of these ingredients to produce a positive health outcome through physiological activity in the body (Kruger & Mann, 2003). Fruit and vegetables are sources of functional ingredients because they are rich in bioactive compounds such as polyphenols, carotenoids, flavonoids, and others (Day, Seymour, Pitts, Konczak, & Lundin, 2009). Functional ingredients are also found in animals, for example, peptides such as epidermal growth factor, opioid peptides, and lactoferrin from milk; arachidonic and docohexaenoic acids are also present in human milk (Ernst, 2002; Greeson, Sanford, & Monti, 2001).

In the human body, probiotic bacteria are not accidental passengers as they collectively perform functions essential to human health. The intestine-resided probiotic micro-organisms live in a challenging, competitive environment and they need support from their hosts. The latter simply deliver the ingredients or prebiotics to the probiotic bacteria in the gut. Food substrate is considered as one of the major factors in regulating the colonization of micro-organisms in the gastro-intestinal tract. Moreover, food assists to buffer the bacteria through the stomach and may contain other functional ingredients which could interact with probiotic microorganisms to alter their functionality. Colonic foods which promote the growth of useful bacteria are referred to as prebiotics and oligosaccharides such as lactulose, galacto-oligosaccharides, inulin, fructo-oligosaccharides and other food carbohydrates are well known examples of prebiotics. The potential for a synergistic effect when probiotics and prebiotics are combined together is realized, as prebiotics encourage the growth of probiotics. By increasing the amount of prebiotics in the diet, it is possible to increase and maintain healthy bacterial gut flora in the host (Sanders, 1998). Ingredients in certain food products may naturally contain prebiotics and these improve the functional efficacy of probiotics. A number of food components including non-specific substrates, plants and their extracts, and polyunsaturated fatty acids may also play an important role in probiotic efficacy (Bomba et al., 2006).

1.9 Health Benefits from Phytochemicals

The optimum diet recommended by most professional health organizations is a low-fat, low saturated fat, high-complex carbohydrate diet with a high intake of fruit, vegetables, whole-grain bread and rice. A diet rich in plant foods will provide sources of phytochemicals (non-nutritive substances in plants), which possess health protective effects. Phytochemicals are food components derived from naturally occurring ingredients (Bloch & Thomson, 1997). Fruit, vegetables, nuts and whole grains all contain an abundance of phenolic compounds, terpenoids, pigments and other natural antioxidants (including vitamins A, C, E). These have been associated with protection from and/or treatment of chronic diseases such as cardiovascular disease, cancer, diabetes, hypertension and other medical conditions (Bloch & Thomson, 1995). In addition to phytochemicals, fruit and vegetables are naturally low in fat, saturated fat, cholesterol, calories and sodium and are rich in potassium, fibre, folic acid, and vitamin C.

The Better Health Programme of 5 + a-day was developed as a tool to increase public awareness of the health benefits of fruit and vegetable consumption and promote adequate intake of known vitamins and minerals (Havas et al., 1994). Prevention is a more effective strategy than treatment of chronic diseases. Plant-based foods, such as fruit, vegetables, and whole grains, which contain significant amounts of bioactive phytochemicals, provide desirable health benefits beyond basic nutrition as well as reducing the risk of chronic diseases. The following are examples of the health benefits of phytochemicals in disease reduction and prevention.

1.9.1 Phytochemicals in Cancer Reduction

Fruit, vegetables, and whole grains contain a wide variety of antioxidant compounds (phytochemicals), such as phenolics and carotenoids and thus, help protect cellular systems from oxidative damage and could lower the risk of chronic diseases (Chu, Sun, Wu, & Liu, 2002; Sun, Chu, Wu, & Liu, 2002).

Of the 156 dietary studies which investigated the relationship between consumption of fruit and vegetables and the risk of cancer, 82% confirmed that fruit and vegetable consumption provided significant protection against cancer (Block, Patterson, & Subar,

1992). Subjects who ate a diet high in fruit and vegetables exhibited approximately half the risk of developing cancer and lower mortality rates (Ziegler, 1991). Fruit and vegetables proved most effective against cancers involving epithelial cells, which are found in carcinoma of the lung, esophagus, stomach, colon, and pancreas (Tavani & Vecchia, 1995). High intake of fruit and vegetables reduced the risk of many epithelial cancers, although the protection rate was generally of a lesser magnitude.

A study of 2,400 Greek women revealed that the risk of breast cancer was 46% lower in women with the highest intake vegetables (four to five servings a day), compared to women with the lowest vegetable intake (fewer than two servings a day). Furthermore, women with the highest intake of fruit (six servings a day), had a 35% lower risk of breast cancer, when compared to women with the lowest fruit intake (fewer than two servings a day) (Trichopoulou et al., 1995). Similar results were reported in a study by Liu (2004), where the risk of cancer was twice as high with a diet low in fruit and vegetables than that with a high intake. In 24 of 25 studies of lung cancer, a high fruit intake provided significant protection against carcinoma of the esophagus, oral cavity, and larynx. A high fruit and vegetable intake also afforded some protection against carcinoma of the pancreas and stomach in 26 out of 30 studies and against colorectal and bladder cancer in 23 out of 38 studies (Liu, 2004).

1.9.2 Phytochemicals in Cardiovascular Disease Prevention

Numerous studies have indicated a strong link between the dietary intake of phytochemicals and the reduction of the risk of heart disease. Dietary flavonoids intake was inversely associated with mortality from coronary artery disease to a significant extent, as well as inversely related to the incidence of myocardial infarction (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993). In a study, the intake of apples and onions, both rich in quercetin, was inversely correlated with total mortality and coronary mortality (Knekt, Jarvinen, Reunanen, & Maatela, 1996). In another study, the total intake of flavonoids (quercetin, myricetin, kaempferol, luteolin, and ficetin) was inversely correlated with the plasma total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations (Arai et al., 2000). The intake of quercetin alone was inversely related to total cholesterol and LDL plasma levels. According to Joshipura et al. (2001), the total fruit and vegetable intake were both individually associated with a decrease in the risk of coronary artery disease. The inverse association between total

consumption of fruit and vegetables and coronary artery disease was noted when dietary intake was greater than 4 servings a day.

In a study trial of patients with established coronary heart disease, who consumed a Mediterranean diet rich in fruit and vegetables and α-linolenic acid, showed a substantial reduction of the recurrence of coronary heart disease (Sánchez-Moreno, Jiménez-Escrig, & Saura-Calixto, 2000). Another intervention study of a diet rich in fruit and vegetables disclosed a significant decrease in blood pressure in 459 adults with borderline hypertension (Salonen et al., 2000).

The mechanisms through which fruit and vegetables protect against cardiovascular disease are potentially multiple. In general, the postulated beneficial constituents in fruit and vegetables include antioxidants, vitamins, folate, fibre, and minerals such as potassium. Rimm et al. (1998) reported that a higher intake of folate and vitamin B6 was significantly associated with a lower risk for coronary heart disease and fibre intake consistently so (Khaw & Barrett-Connor, 1987).

1.10 Antimicrobial or Bioactive Compounds in Plants

Plants have a natural defense mechanism against microbial infections. Antimicrobial peptides, lectins, phenolic compounds, terpenoids, essential oils and various other compounds are potentially involved in this phenomenon (Cowan, 1999). Raw and processed fruit, as well as waste products remaining after processing (peel, seeds, stems, and flesh) are good sources of these ingredients. In previous *in vitro* experiments, juices and extracts from berries, guava, and citrus fruit revealed antibacterial activities against Gram-negative and -positive bacteria (Cavanagh, Hipwell, & Wilkinson, 2003; Hoque, Inatsu, Juneja, & Kawamoto, 2007; Ryan, Wilkinson, & Cavanagh, 2001; Sagdic, Aksoy, & Ozkan, 2006; Vattem, Lin, Ghaedian, & Shetty, 2005).

Substances from plants have been shown to have anti-microbial activity. Some plant compounds have been shown to have an anti-adhesive effect on a wide range of bacteria (Wittschier et al., 2007). Alternatives to traditional antibiotics, antibacterial agents are being investigated due to the development of antibiotic resistance in infectious microorganisms. Consumption of fruit or application of fruit ingredients could provide

antibacterial agents to consumers. The effect of different fruit extracts on different pathogens will be discussed in the current study.

1.10.1 Bioactive Compounds in Berries

There is an increasing awareness of the positive health benefits of berries, which are rich sources in various bioactive compounds and which are thought to possess certain biological activities (Svarcova, Heinrich, & Valentova, 2007). They are rich in fibre, vitamins, minerals, folate and especially in phenolic compounds (Mullen et al., 2002; Tulipani et al., 2008; Vuorinen, Määttä, & Törrönen, 2000) and organic acids (Viljakainen, Visti, & Laakso, 2002). Phenolic compounds are secondary metabolites ubiquitous in all higher plants. Though the role of these compounds is not fully understood, they are believed to act as defence compounds against plant pathogens and are often induced as a response to various stress conditions (Puupponen-Pimia, Nohynek, Alakomi, & Oksman-Caldentey, 2005).

Phenolics occur in plant tissues as simple substituted phenols, mainly as glycosides, or as complex polymerised molecules with high molecular weights. Flavonoids, phenolic acids, lignans and complex phenolic polymers (polymeric tannins) are typical to berries (Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1999). Anthocyanins (anthocyanidin glycosides) are the predominating group of flavonoids present in berries. They are efficient absorbers of visible light, thus appearing as coloured substances, responsible for the characteristic orange/red/blue colours of berries, such as strawberries, raspberries, bilberries and red- and black currants. For example, 44% of phenolic compounds found in strawberries are anthocyanins.

Simpler phenolic acids, such as hydroxycinnamic acids and hydroxybenzoic acids, are also common in many berries (Herrmann & Nagel, 1989). Chlorogenic acid, which is an ester between caffeic and quinic acid, is another commonly occurring compound. Flavonoids and phenolic acids form the building blocks for polymeric tannins, which can be classified into hydrolysable and condensed tannins. Hydrolysable tannins are either gallotannins or ellagitannins and, when hydrolyzed gallotannins yield glucose and gallic acid. Ellagitannins contain one or more hydroxydiphenoyl residues which are linked to glucose as a diester together with gallic acid. Upon hydrolysis the hydrodiphenoyl residue undergoes lactonisation to produce ellagic acid and berries,

especially those of the family *Rosaceae*, genus *Rubus* (red raspberry, arctic bramble and cloudberry), are rich in ellagitannins (Häkkinen, Kärenlampi, Mykkänen, Heinonen, & Törrönen, 2000; Mullen et al., 2002). The latter and strawberries produce only ellagitannins based on a stable glucose conformation. In addition to pentagalloylglucose, they contain dimeric or polymeric ellagitannins with only a small amount of monomers. According to Viljakainen et al. (2002), the main acids of the wild-berry juices are invariably citric and malic acids, even though their concentrations vary widely from one to another. Furthermore, the juice of lingonberry, cranberry, cloudberry and black currant contain benzoic acid and lingonberry is especially high in benzoic acid concentration with a very low pH (pH 2.6–2.9). The pH of most berry juices is low (2.4–3.5) and therefore, advantageous in preventing microbial contaminations.

Table 1.3 Major classes of antimicrobial compounds from plants (Cowan, 1999).

Class	Subclass	Example (s)	Mechanism
Phenolics	Simple phenols	Catechol	Substrate deprivation
		Epicatechin	Membrane disruption
	Phenolic acids	Cinnamic acid	
	Quinones	Hypericin	Bind to adhesins, complex with cell wall, inactivate enzymes
	Flavonoids	Chrysin	Bind to adhesins
	Flavones		Complex with cell wall
		Abyssinone	Inactivate enzymes
			Inhibit HIV reverse transcriptase
	Flavonols	Totarol	?
	Tannins	Ellagitannin	Bind to proteins
			Bind to adhesins
			Enzyme inhibition
			Substrate deprivation
			Complex with cell wall
			Membrane disruption
			Metal ion complexation
	Coumarins	Warfarin	Interaction with eucaryotic DNA (antiviral activity)
Terpenoids, essential oils		Capsaicin	Membrane disruption
Alkaloids			
		Berberine	Intercalate into cell wall and/or DNA
		Piperine	
Lectins and polypeptides			
		Mannose-specific agglutinin	Block viral fusion or adsorption
		Fabatin	Form disulfide bridges
Polyacetylenes		8S-Heptadeca-2(*Z*),9(*Z*)-diene- 4,6-diyne-1,8-diol	?

1.10.2 Bioactive Compounds in Green Kiwifruit

Green kiwifruit (*Actinidia deliciosa*) is unusual in many aspects when compared to other fruit, in relation to flavor, color, aroma, shape and nutritional content (Ahmet,

Sezai, & Nihat, 2007). Kiwifruit extract contains nutritional components and desirable bioactive compounds, which include polyphenols, ascorbic acid and water-soluble polysaccharides (pectic polysaccharides) (Sun-Waterhouse et al., 2009). It also possesses high levels of antioxidants (Szeto, Tomlinson, & Benzie, 2002), vitamin C (Ahmet et al., 2007), folic, citric, glutamic acids (Cassano, Donato, Conidi, & Drioli, 2008), and dietary fibre (Chan, Leung, Tong, & Wong, 2007).

According to Dawes & Keene's study (1999) on the identification of the phenolic compounds in kiwifruit juice, the main highly acidic phenolic compounds in kiwifruit juice were coumaric and caffeic acid derivatives, as well as chlorogenic, protocatechuic acid, and a derivative of 3, 4-dihydroxybenzoic acid. The weaker acidic phenolic compounds in clarified juice include low levels of catechin and epicatechin, procyanidin dimers B3, B2, or B4, and oligomers. Glycosides of quercetin and kaempferol were identified to consist of both the monoglycosides (quercetin and kaempferol 3-rhamnoside, quercetin 3-glucoside) and the diglycoside (rutinoside).

1.10.3 Bioactive Compounds in Feijoa

The feijoa plant (*Feijoa sellowiana*) (synonym, *Acca sellowiana*), is a monotypic species of the family Myrtaceae and it is also commonly known as the pineapple guava or guavasteen, since it is related to the guava genus, *Psidium guajava* L (Weston, 2010). The feijoa is a very aromatic fruit and contains high levels of phenolic components and the natural substance flavone has been identified as the principle phenol (Weston, 2010). The tannins in the feijoa were identified as proanthocyanidin tannins, whose phenolic composition were largely responsible for antioxidant activity (Foo & Porter, 1981). The feijoa is also known to contain high amounts of vitamin P (P)-active polyphenols, such as catechin, leucoanthocyanins, flavonoids, proanthocyanins, and naphthoquinones (Nakashima, 2001).

Large amounts of ascorbic acid, carotenoids, xanthophylls, flavonoids and minerals have also been reported to be present in the feijoa (Basile, Conte, Rigano, Senatore, & Sorbo, 2010; Romero-Rodriguez, Vazquez-Orderiz, Lopez-Hernandez, & Simal-Lozano, 1994). The skin and pulp of the feijoa produce a highly aromatic, volatile oil which contains many volatile compounds such as terpenes, tannins, quinones, steroidal saponins, flavonoids as well as methyl- and ethyl-benzoat (Binder & Flath, 1989).

1.11 Effect of Fruit Bioactive Compounds on Probiotic Bacteria

Phenolic compounds are the main constituents present in berries (Mullen et al., 2002; Vuorinen et al., 2000) claimed to be attributed to the increase in growth of *Lactobacilli* and *Bifidobacteria*. Another compound of berries which were assumed to contribute to the increase in growth of *Lactobacillus hilgardii* was gallic acid and flavonoid (catechin). These compounds not only activated the growth but also increase the bacterial population as this bacterium was able to metabolize these compounds (Alberto, Farı´as, & Nadra, 2001). Inulin and fructo-oligosaccharides of berries were also another cause of significant increase in bifidobacterial population (Gibson, Beatty, Wang, & Cummings, 1995).

Typical compounds such as sugars and small proteins and other food-specific phytochemical classes including phenolics and organic acids present in aqueous extracts of berries were also reported to increase the growth of probiotic microorganisms. The growth promoting activity of these blueberry and strawberry functional ingredients on some specific probiotic bacteria, namely *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Bacillus lactis* was reported by Sutherland et al. (2009).

Anthocyanins which are pigments present in berries were also reported to play role in the stimulation of growth of probiotic bacteria. Anthocyanins pigments such as pelargodin 3-monoglucoside, cyanidin 3-monoglucoside and delphinidin 3-monoglucoside were identified by Pratt, Powers, & Somaatmadja (1960) and these anthocyanins from strawberries actively influenced the growth of *Lactobacillus acidophilus*. This finding was consistent with the finding of Werlein et al. (2005) who detected an influence of strawberry anthocyanins on the growth rate of *L. acidophilus*. The conclusion was based on the fact that both *Lactobacilli* and *Bifidobacteria* possess the enzyme beta-glucosidase which can convert delphinidin and malvidin glycosides, the two anthocyanin most commonly found in foods, into other compounds with different bioavailability and bioactivity (Ávila et al., 2009).

Carbohydrates present in berries such as pectins and pectic-oligosaccharides are another compounds reported to attribute to the increase in growth of certain probiotic bacteria. When functional ingredients reach in the colon, it should usually be selectively fermented by probiotic bacterial genera, such as *Bifidobacterium* and *Lactobacillus* (Gibson & Roberfroid, 1995). The evidence that the carbohydrates stimulated the

growth of probiotic bacteria was shown by (Olano-Martin, Gibson, & Rastall, 2002) who studied on the comparison of the *in vitro* bifidogenic properties of pectins and pectic-oligosaccharides. They indicated that pectic olisaccharides have a bifidogenic effect, and selected bifidobacteria showed high growth rates on these substrates. The finding was supported by Manderson et al. (2005) who have also demonstrated that pectic oligosaccharides from orange peel showed prebiotic properties increasing the bifidobacterial numbers.

Phenolics, cumaric acid, caffeic acid, gallic acid and catechin are compounds present in kiwifruit which are believed to have growth promoting effect on probiotic bacteria. The growth rate stimulation by gallic acid and catechin and the increase in cell density could be related to their ability to metabolize phenolic compounds (Alberto et al., 2001). Reguant et al. (2000) who studied the influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine, reported that catechin and quercetin are beneficial for *Oenococcus oeni* activity. The conclusion was based on their consideration that phenolic compounds serve as oxygen scavenger and reduce the redox potential of wine. This is true with the fact that lactic acid bacteria grow better in the oxygen-free culture. Another evidence was proven by Barthelmebs, Divies, & Cavin (2000) who reported that *Lactobacillus plantarum* displays substrate-inducible decarboxylase activities on *p*-coumaric, caffeic, and ferulic acids.

1.12 Effect of Fruit Bioactive Compounds on Pathogenic Bacteria

Berry extracts inhibited the growth of Gram-negative bacterial species but not Gram-positive *Lactobacillus* species (Puupponen-Pimia¨ et al., 2002). These variations may reflect differences in cell surface structures between Gram-negative and Gram-positive bacteria. In particular, the outer membrane of Gram-negative bacteria functions as a preventive barrier against hydrophobic compounds (Helander et al., 1998).

Ellagitannins is one of the bioactive compounds in berries which was confirmed about their widest bactericidal activity against pathogenic bacteria (Rauha et al., 2000). Ellagitannins could be one of the components in strawberries, cloudberries and raspberry causing the inhibition against *Salmonella*, *E. coli* CM871 and especially *Typhimurium* (R. Puupponen-Pimia et al., 2001) since ellagic acid was the main phenolic compound in the hydrolyzed berry extracts of the genera *Robus* and *Fragaria*

(strawberry) (Häkkinen et al., 2000). Ellagic acid is a product of hydrolysis from ellagitannins, which, together with gallotannins, form the predominant group of tannins in these berries (Macheix, Fleuriet, & Billot, 1990). However, strawberry extract contained only small amounts of ellagitannins and this may explain the moderate antimicrobial effects against *Salmonella* bacteria (Puupponen-Pimia¨ et al., 2005).

Catechin exhibited its antimicrobial properties against *Achromobacter* sp. (Scalbert, 1991). Kaempferol was found to induce a significant decrease in the number of *Helicobacter pylori* in gerbil's stomach after oral treatment (Kataoka et al., 2001).

Polyphenols were shown to inhibit the growth and adhesion of gut pathogens. Organic acids also played an important role in the growth inhibition of some bacteria (Parkar, Stevenson, & Skinner, 2008). *Salmonella enteritidis* was inhibited by up to 0.2% of citric acid (Ruzickova, 1996).

Pathogenic bacteria strains, both Gram-positive and Gram-negative, were selectively inhibited by bioactive berry compounds (Puupponen-Pimia¨ et al., 2005). In another report by the same authors on the action of berry phenolics against human intestinal pathogens, they found that phenolic berry extracts inhibited the growth of *Salmonella*, *Escherichia*, *Staphylococcus*, *Helicobacter*, *Bacillus*, *Clostridium* and *Campylobacter* species but not *Listeria* species and that *Salmonella*, *Staphylococcus*, *Helicobacter* and *Bacillus* strains were the most sensitive bacteria for the berry extracts. Flavonoids are other berry compounds that exhibit various physiological activities including anti-inflammatory, antiallergic, anti-carcinogenic, anti-hypertensive, anti-artthritic and anti-microbial activities (Middleton, Kandaswami, & Theoharides, 2000).

Kiwifruit extract showed high antimicrobial activities against both Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) and Gram-negative (*Salmonella typhymurium* and *Escherichia coli*) pathogenic bacteria, with the water extracts exhibiting the greatest antimicrobial activity (Molan, Kruger, De, & Drummond, 2007).

Flavone is one of the active compounds present in *Feijoa sellowiana* fruit and it showed a high antimicrobial activity against bacterial strains. *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Proteus vulgaris* showed a very high sensitivity to flavone (Basile et al., 2010). Fungus *Rhyzotonia solani* was the most sensitive strain to the action of flavone. Pro-anthocyanidins is another compound of feijoa which may

attribute to the inhibitory effect against pathogens in this study as it is suggested by Puupponen-Pimia¨ et al. (2002) that the inhibitory effects of berry extracts may not be due to simple phenolics but to more complex polymers such as pro-anthocyanidins, ellagitannins and tannins.

Chapter 2

Materials and Methods

2.1 Materials

2.1.1 Fruits

Four different fruits were used in this study: Blueberry (*Vaccinium corymbosum*), strawberry (*Fragaria x ananassa*), green kiwifruit (*Actinidia deliciosa*), and feijoa (*Acca sellowiana*) were purchased from an Auckland supermarket.

2.1.2 Media and Chemicals

Lactobacilli MRS (de Man, Rogosa and Sharp) agar and broth (Difco Laboratories Inc, Detroit MI) were purchased from Fort Richards Ltd, Auckland. Tryptic Soy Broth (Becton, Dickinson and Co.), mono-Sodium hydrogen orthophosphate anhydrous (NaH_2PO4) and di-Sodium hydrogen orthophosphate anhydrous (Na_2HPO_4) (BDH AnalaR Chemicals Ltd, Poole, England) were obtained from Auckland University of Technology (AUT) Applied Sciences laboratory.

2.1.3 Other Materials

Sterile multiple-well plates (96-well), round bottom with lid (Newton, NC 28658, USA) were purchased from Global Science, Auckland. GasPak^{TM} EZ Gas generating sachets and GasPak^{TM} EZ incubation chambers (Becton, Dickinson, USA) were purchased from Fort Richards Ltd. Auckland. Sterile Millipore filter papers, (Millex 0.45 μm, 0.22 μm) were purchased from Thermo Fisher Scientific (Biolab Limited), Auckland. Whatman No. 41 filter paper was obtained from W & R Balston Limited, England.

High throughput growth results were obtained using a Multiskan FC (version 2.5) microplate photometer (Thermo Scientific) purchased from Medica Pacifica Ltd, Auckland. Sorval RC 5C Superspeed Centrifuge was obtained from Bio-Strategy

Distribution, Auckland. Harvest Maid Food Dehydrator (Model No: FD-1000) was obtained from Hydraflow Industries Limited, New Zealand. Multichannel pipettor was purchased from Total Lab Systems Ltd, Auckland. Speed Stick Mixer (Model, ZIP 114) was purchased from Mitre 10 Mega, New Zealand.

2.1.4 Bacterial Strains

Three strains of *Bifidobacterium* and one strain of *Lactobacillus casei* were isolated from Yoplait, Naturalea and Biofarm yoghurts as described in **Section 2.2.4**. *Lactobacillus reuteri* and *Bifidobacterium longum* were a gift from Massey University. *Lactobacillus acidophilus* and *Lactobacillus plantarum* were purchased from Fonterra Research Centre, Palmerston North.

The seven species of pathogenic bacteria were obtained from the culture bank of AUT Applied Sciences Laboratory: *Samonella enteritidis*, *Escherichia coli* 0157:H7, *Salmonella typhimurium*, *Yersinia enterocolitica*, *Bacillus cereus*, *Vibrio parahaemolyticus* and *Listeria monocytogenes*.

2.2 Methods

2.2.1 Fruit Dehydration

Except for blueberry, the fruit were washed and cut into thin slices and were arranged in a single layer on food dehydrator trays for drying. The temperature of the dehydrator was set at 50 ^{o}C and drying time was dependent on the water content of each fruit. Blueberry was dried for 52 hours, strawberry for 18 hours, green kiwifruit for 22 hours, and feijoa for 24 hours. The weight of the fruit slices was taken before and after dehydration. The weight of dried fruit was used to calculate the amount of buffer required to prepare the fruit extract.

2.2.2 Buffer Preparation

The study required 25 mM sodium phosphate buffer, pH 7.4 for fruit extraction. Equal volumes of 25 mM monobasic sodium phosphate (NaH_2PO_4) and 25 mM dibasic

sodium phosphate (Na_2HPO_4) solutions were initially mixed together. The pH was adjusted to 7.4 using NaH_2PO_4 solution.

2.2.3 Fruit Extraction

Fruit extracts were prepared according to a modified method described by Hammad, Nemer, & Kawar (2000); Rosendale et al., (2008) and Sun-Waterhouse, Farr, Wibisono & Saleh (2008). The dehydrated fruit slices were mixed with 25 mM sodium phosphate buffer, pH 7.4 at a proportion of 100 mg per ml and homogenized using a speed stick mixer. The crude extract was then centrifuged at 8500 rpm for 15 minutes at 4 ^{o}C to remove solids. The supernatant was then filtered through Whatman (No. 41) filter paper under vacuum. Finally, the extract was dispensed into 100 ml bottles and kept in a freezer at -7 ^{o}C for storage.

The fruit extracts were sterilized using a two-step membrane filtration method. Frozen fruit extracts were thawed at room temperature and passed through sterile 0.45 μm membrane filters. The filtrate was then further passed through a 20 ml syringe filter fitted with 0.22 μm membrane filter. The sterile extracts were then partitioned into sterile test tubes and frozen at -85 ^{0}C until required. Each extract sample was used only once to prevent any freeze/thaw-induced denaturation of active compounds (Rosendale et al., 2008).

2.2.4 Isolation of Probiotic Bacteria

Three brands of New Zealand yoghurts (Yoplait, Naturalea, and Biofarm) were used as sources of some of the probiotic microorganisms used in this study. *Lactobacilli* and *Bifidobacteria* were isolated using a five-phase streak plating method on MRS agar. The streak plates were incubated anaerobically in GasPak^{TM} EZ incubation chambers with anaerobe pouches at 37 ^{o}C for four days.

Colour and size of colonies were used as indicators in this preliminary isolation. The isolated colonies were picked off using a sterile inoculating loop and streak-plated again into fresh MRS agar. The morphology and purity of the cultures were assessed microscopically by Gram staining. The Gram staining method was carried out according

to the procedure described by Harrigan (1998), with a slight modification. In addition to Gram reaction, Gram staining allowed determination of cell morphology and arrangement. By comparing cell morphology of the presumed probiotic strains to those in the studies by Jones & Collins (1986) and Kandler & Weiss (1986), different species of probiotic bacteria named on the labels of the four yoghurts were confirmed.

2.2.5 Microbial Growth Conditions and Maintenance

The probiotic bacteria used were grown anaerobically in MRS agar plates at 37 ^{o}C for 48 hours. The cultures were maintained by sub-transferring onto fresh MRS agar plates every fortnight and stored in a 4 ^{o}C refrigerator until required. The pathogenic bacteria tested were grown in Columbia Sheep Blood (CSB) Agar overnight at 37 ^{o}C. *Salmonella enteritidis*, *Escherichia coli* 0157:H7, *Salmonella typhimurium*, and *Yersinia enterocolitica* were maintained by sub-culturing onto fresh Blood Agar every fortnight. *Bacillus cereus*, *Vibrio parahaemolyticus* and *Listeria monocytogenes* were transferred onto fresh CSB agar once a week.

2.2.6 Inoculum Preparation

Inocula for probiotic bacteria and pathogenic bacteria were obtained from fresh cultures in MRS agar and Columbia Sheep Blood agar, respectively. Inocula for the test were prepared by taking a colony of pathogenic bacteria and a few colonies for probiotic bacteria from an agar plate culture. The colony was picked off with a sterile inoculating loop and then transferred into a test tube containing either 10 ml of MRS broth for probiotic bacteria or Tryptic Soy Broth (TSB) for the pathogens. The MRS broth inoculated with probiotic bacteria was incubated anaerobically for 48 hours at 37 ^{o}C. The TSB inoculated with pathogenic bacteria was incubated aerobically overnight. A second subculture was performed by pipetting 10 μl from the previous broth which was then incubated anaerobically for another 48 hours for probiotic bacteria, and aerobically for 24 hours for pathogenic bacteria.

Two subcultures were performed with both probiotic and pathogenic microorganisms to ensure that organisms were fully adapted to the new broth media prior to use for any microbial experiment. Bacterial inocula for the microbial assays were standardized after

estimating the culture density with a haemocytometer and adjusted with sterile broth to obtain a concentration of 10^3 cells ml^{-1} (Rosendale et al., 2008).

2.2.7 Testing the Effect of Fruit Extract on the Growth of Bacteria

A high throughput bio-assay using a 96-well microplate to determine optical density (OD) at 620 nm was used throughout this work. This assay was performed to test the effects of fruit extracts on both probiotic and pathogenic bacterial strains. The effects were determined by comparing the change in the growth of cultures in the presence of the extract or extracts relative to the growth of unsupplemented control cultures. The assay was performed using the method of Rosendale et al. (2008) which used serial two-fold dilutions of fruit extract mixed with bacterial medium to assess the response of a particular bacterium.

The experiments were carried out both as single extract and combined extract assays.

2.2.7.1 Single Extract Assay

Three different concentrations of each fruit extract were used in the assay, namely, 10%, 20% and 30% (v/v). A sterile 96-well microplate was used for each assay. Each microplate contained one type of fruit extract at a specific concentration being assayed on only one species of bacteria. Further dilutions of fruit extracts (10%, 20%, 30%) were prepared by conducting a two-fold dilution series across a 96-well microplate. Therefore after using this procedure, the lowest concentration tested in this study was 0.01 $g.l^{-1}$ of fruit extract and the highest concentration was 30 $g.l^{-1}$.

Wells from column number 2 to colunm number 12 were filled with 50 µl of sterile bacterial growth medium. The first column of the 96-well plate was loaded with 90 µl of growth medium and 10 µl of a fruit extract (say 10%).

Mixing was carried out by using a multi-channel pipettor, moving from left to right, with the first column containing the highest concentration. This resulted in eleven dilutions, each replicated in eight wells in the one plate and each dilution was half the concentration of the previous one. Eight control wells were included which contained

growth medium without any fruit extract. These control wells were in the last column of the 96-well microplate.

The wells were inoculated with an equal volume (50 µl) of bacterial inoculum at a standardized inoculation density. The zero growth values (the blank), T_o, were determined by measuring the optical density (OD) of the plate immediately prior to incubation at a wavelength of 620 nm using a Thermo Multiskan FC (Thermo Fisher Scientific) 96-well plate reader. The microplates were covered with lids to prevent cross contamination, and then incubated anaerobically for probiotic bacteria and aerobically for pathogenic microorganisms at 37 ^{o}C for 16 hours. After incubation, the OD was measured to determine the growth of the cultures at late log phase-early stationary phase growth of the microorganisms, T_{16}.

The pH of the culture medium, before and after incubation with bacteria, was measured using a PHM201 Portable pH meter (Radiometer Analytical S.A.S, France). The measurement was taken both in the presence of the maximum extract concentration used and in the control cultures. These measurements were used to exclude either changes in pH or buffering of the media, as reason for any observed increases or decreases in bacterial growth.

The assay was done twice for each experiment using a fruit extract.

2.2.7.2 Combined Extracts Assay

For this assay, two fruit extracts were combined and mixed well to test their effects on the growth of the same microorganisms used in the single assay. Blueberry extract was combined with feijoa extract and strawberry extract with green kiwifruit extract. The combined extracts assays were carried out exactly as described for single extract assays, except that the extracts were mixed but the total volume was kept the same. This assay was performed twice for each combination.

2.2.8 Growth Analysis

2.2.8.1 Single Extract Assay

To compare the effect of fruit extracts on a variety of microorganisms, the change of (delta) growth, (Δ_{Growth}), used by Rosendale, et al (2008) was adopted. The Δ_{Growth} represents a standardized value denoting any change in the growth of bacteria in the presence of fruit extract(s), relative to the growth of bacteria in the unsupplemented control. The Δ_{Growth} value was calculated by converting the OD to a percentage of the control OD and then subtracting 100. Graphs of Δ_{Growth} were generated and the line of best fit was applied to all curves.

$$\Delta_{Growth} = \left[\frac{(Extract\ OD - Extract\ blank\ OD) x 100}{(Control\ OD - Control\ blank\ OD)} \right] - 100$$

* Δ_{Growth} represents the magnitude of change in the growth of bacteria relative to the bacteria in the control sample which had not been supplemented with fruit extract. If the calculated value of Δ_{Growth} is positive, that means the growth is stimulated by the fruit extract. If the Δ_{Growth} value is negative, that means the growth is inhibited by the fruit extract.

* Extract OD is the optical density of a bacterium in culture supplemented with a fruit extract measured at 16 hrs, that is, after incubation.

* Extract blank OD is the optical density of a bacterium in culture supplemented with a fruit extract measured at zero hr, that is, before incubation.

* Control OD is the optical density of medium, without fruit extract, with a bacterium measured at 16 hrs.

* Control blank OD is the optical density of medium, without fruit extract, with a bacterium measured at zero hr.

The zero hour reading was subtracted from the reading at 16 hours end-point to eliminate all changes in optical density not due to growth. Hence, potential variations in

microplate density, media colour or any other unknown factors could be accounted for. Moreover, non-inoculated extract controls were routinely included to confirm the sterility of the extracts.

The results were entered into a database and subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) for Windows Statistical Package Version 16.0. A one-way analysis of variance (ANOVA) was used to compare growth across concentrations. To identify significant differences in bacterial growth between fruit extract and control, the Tukey test was applied in this study. Probability levels of < 0.05 or values of $p < 0.05$ at the 95% confidence interval were considered significant.

2.2.8.2 Combined Extracts Assay

The combined extracts experiments were carried out similar to that of single extract assay which was described in Section 2.2.8.1. However, the volume for the assay was not varied. This means that each extract in a combination used only half of the volume of the highest dose applied in single extract experiment.

The Δ_{Growth} values were calculated and the dose-response profiles were plotted in a graph. An additional analysis used by Rosendale et al. (2008) was adopted in this study to be able to compare the efficacy of the combined extract with that of single extract. The effect could be described as good, poor, desirable and undesirable after comparisons using the following two methods:

Comparison 1: The Δ_{Growth} values of the combined extracts assays were significantly ($p<0.05$) higher than the sum of Δ_{Growth} values of single extract assays (equation 1).

Does $\Delta_{Growth\ AB}$ exceed ($\Delta_{Growth\ A} + \Delta_{Growth\ B}$)? (Equation 1)

Comparison 2: The Δ_{Growth} values of the combined extracts assays were significantly higher than the highest Δ_{Growth} values from either one of the single extract assays (Equation 2).

Does $\Delta_{Growth\ AB}$ exceed either $\Delta_{Growth\ A}$ or $\Delta_{Growth\ B}$? (Equation 2)

According to the two assigned methods, any results from the combined extracts experiments that fulfilled comparison 1 were termed "good" and those that did not fulfill comparison 1 were termed "poor". Any results of the combined assay that fulfilled both Comparison 1 and Comparison 2 were termed "desirable" and those that failed to meet the two comparisons were termed "undesirable".

Chapter 3

Results

3.1 Single Extract Assay

Optical density readings from each species supplemented with varying concentrations of fruit extract were collected. To determine the dose-response profile of the bacteria to a fruit extract, the ANOVA test was performed by using the optical density values from each concentration of fruit extract. The Tukey test was used to determine the concentrations that produced significant effects; that is whether growth-stimulating or growth-repressing effects. Using a two-fold serial dilution of the fruit extracts at concentrations of 10%, 20%, 30% (v/v), a concentration range from 0.01 $g.l^{-1}$ to 30 $g.l^{-1}$ was obtained. These resulted in up to 48 replicates for each concentration.

To demonstrate any difference in the dose response of several bacteria to any fruit extract, the Δ_{Growth} values (Chapter **2**, Section **2.2.8**) were calculated and plotted against the concentrations of the fruit extract. All the Δ_{Growth} data obtained from the experiments are summarized in tables in Appendix 1. The Δ_{Growth} values indicate the extent of growth relative to the unsupplemented control cultures. Values of Δ_{Growth} above zero indicate a stimulation of growth of the bacteria in the presence of a fruit extract. Any Δ_{Growth} values below zero meant that the fruit extract had a growth-repressing effect.

3.1.1 Effect of Blueberry Extract on Growth of Probiotic Bacteria and Pathogens

3.1.1.1 Probiotic Bacteria

The growth of all species of probiotic bacteria tested was significantly stimulated by increasing concentrations of blueberry extract, except for *Bifidobacterium longum*. The Δ_{Growth} profiles of *Bifidobacterium* species and *Lactobacillus* species in the presence of blueberry extract are shown in **Fig. 3.1** and **Fig. 3.2**, respectively. The greatest stimulatory effect of the extract was observed with the species of *Bifidobacterium* and *Lactobacillus* which were both isolated from Yoplait yoghurt. However, the

stimulatory effect of blueberry extract on Yoplait *Lactobacillus casei* was about three times higher than that of Yoplait *Bifidobacterium*. The highest ΔGrowth value obtained from Yoplait *L. casei* was 180%, while that from Yoplait *Bifidobacterium* was 60%. The Tukey test showed that the minimum stimulatory concentration of blueberry extract on Yoplait *L.casei* was 1.25 $g.l^{-1}$ ($p<0.05$). The minimum stimulatory effect of the extract obtained with Yoplait *Bifidobacterium* was 0.63 $g.l^{-1}$ ($p<0.05$).

Interestingly, at $p<0.05$, enhancement of growth of Naturalea *Bifidobacterium* isolate required the same minimum concentration of blueberry extract (0.63 $g.l^{-1}$) as with Yoplait *Bifidobacterium*. The minimum concentration of the extract which was found to stimulate the growth of Biofarm *Bifidobacterium* was 1.88 $g.l^{-1}$ ($p<0.05$).

The Δ_{Growth} values (**Fig. 3.1**) show that the degree of stimulatory effect of blueberry extract on isolates of *Bifidobacterium* (Naturalea) and of *Bifidobacterium* (Biofarm) was lower than that on *Bifidobacterium* (Yoplait).

In contrast to the effect of blueberry extract on the above isolates, the presence of the extract elicited a repressing effect on the growth of *B. longum* despite an apparent increase in the Δ_{Growth} value obtained at 10 $g.l^{-1}$. This apparent change was not statistically significant ($p>0.05$).

All identified species of *Lactobacillus*, namely, *L. reuteri*, *L. acidophilus*, and *L. plantarum*, tested with blueberry extract showed an increase in growth in the presence of the extract. The growth of *L. reuteri* was only significantly enhanced in the presence of blueberry extract of at least 0.63 $g.l^{-1}$. Again, this was the same minimum stimulatory concentration obtained with both Yoplait *Bifidobacterium* and Naturalea *Bifidifobacterium.*

L. acidophilus also reached significantly higher level of growth in the presence of blueberry extract of at least 0.12 $g.l^{-1}$ ($p<0.05$). The Δ_{Growth} values (**Fig. 3.2**) show that blueberry extract exhibited the least growth enhancing effect on *L. plantarum*, the growth of which only increased with a minimum concentration of 5 $g.l^{-1}$.

Blueberry extract at a concentration of 10 $g.l^{-1}$ (Tukey test, $p<0.05$) had the strongest significant stimulatory effect on the growth of the above three identified species of *Lactobacillus*.

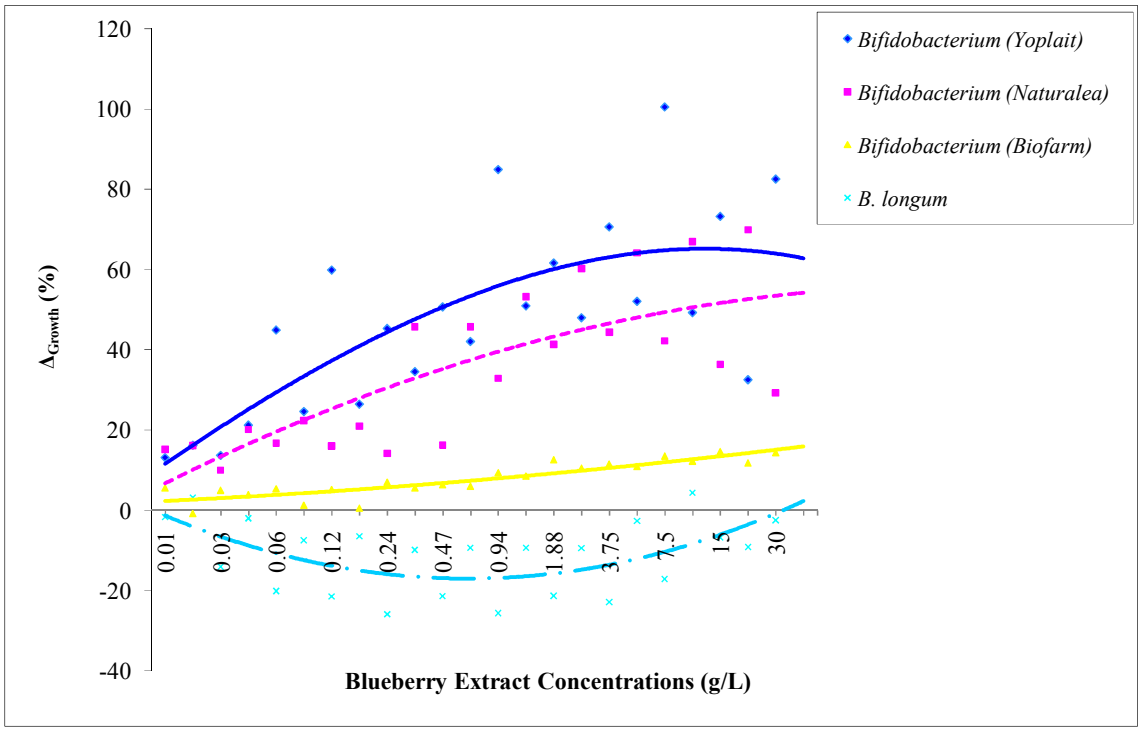

Figure 3.1 Effect of blueberry extract on growth of *Bifidobacteria* species.

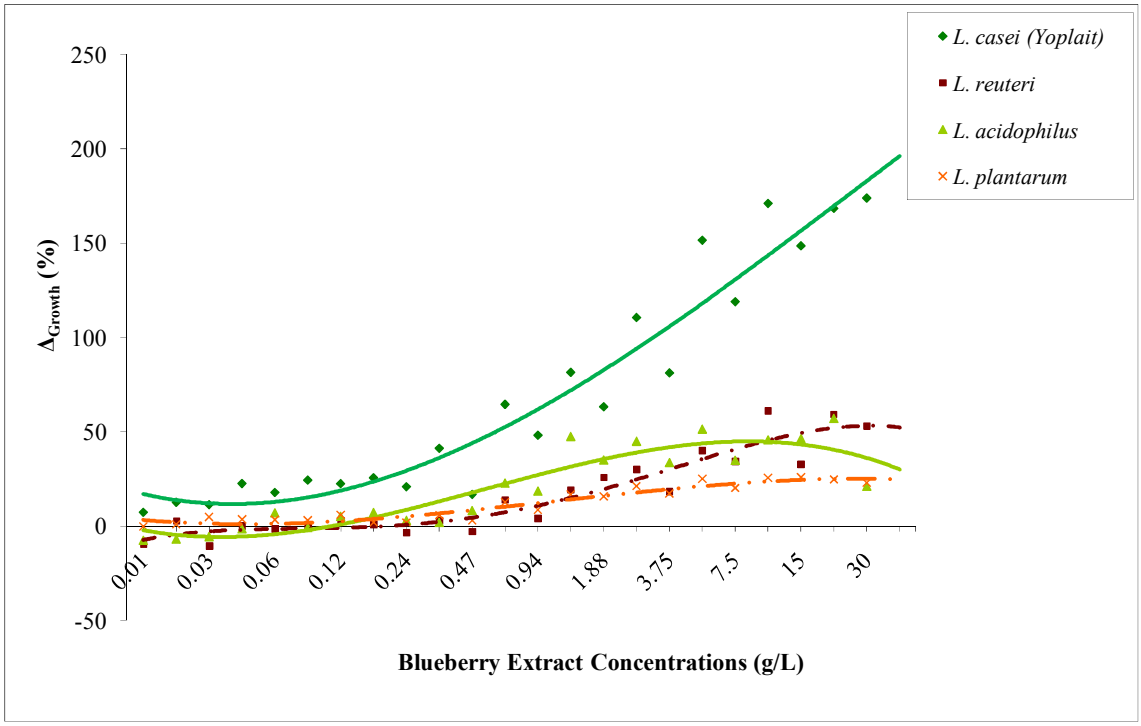

Figure 3.2 Effect of blueberry extract on growth of *Lactobacilli* species.

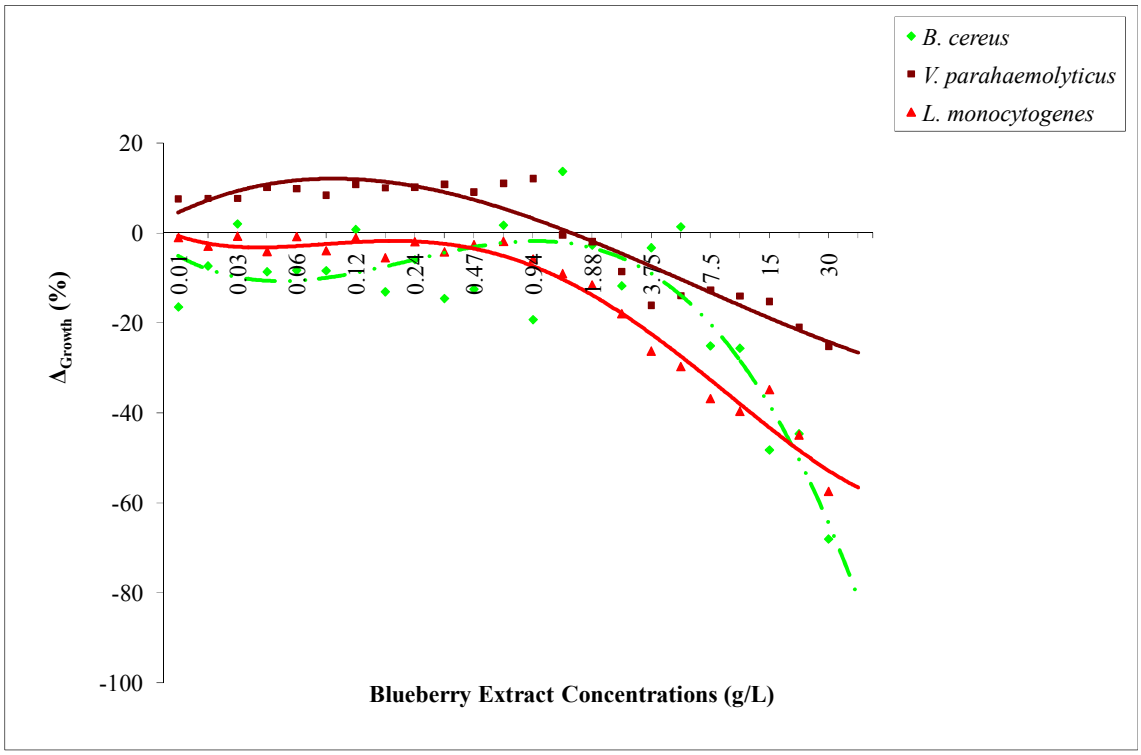

Figure 3.3 Effect of blueberry extract on the growth of pathogenic bacteria.

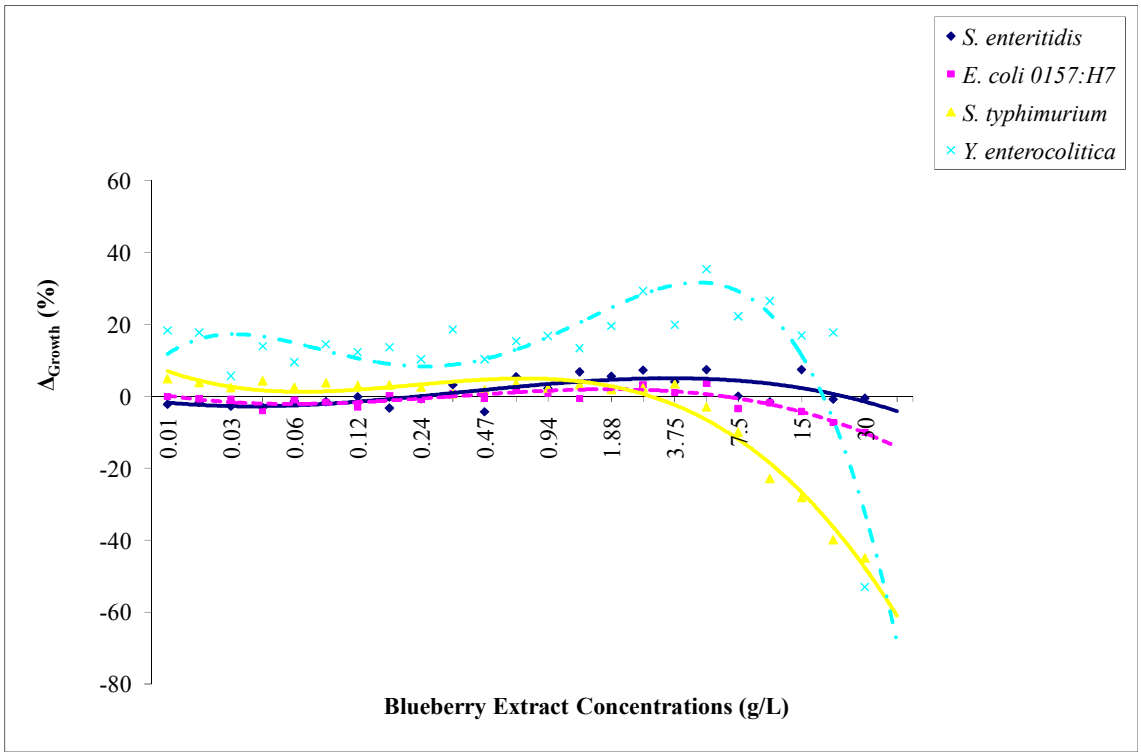

Figure 3.4 Effect of blueberry extract on the growth of pathogenic bacteria.

3.1.1.2 Pathogenic Bacteria

The single extract assay, as done with the probiotic bacteria, was performed using a few species of pathogenic bacteria which included Gram-positive and Gram-negative species. The dose-response profiles in the presence of blueberry extract are shown in **Fig. 3.3** and **Fig. 3.4**.

The highest concentration of blueberry extract at 30 $g.l^{-1}$ significantly inhibited the growth of all the pathogens tested ($p<0.05$). However, the growth of *Y. enterocolitica* (**Fig. 3.4**) was stimulated by all concentrations of blueberry extract lower than 30 $g.l^{-1}$ until its growth was significantly inhibited at this concentration ($p<0.05$). This effect is referred to as "biphasic" in this study. Blueberry also showed a biphasic effect on *V. parahaemolyticus* which was more distinct (phases were almost equally divided) than that on *Y. enterocolitica*. Concentrations lower than 1.4 $g.l^{-1}$ had a stimulatory effect while concentrations higher than 1.4 $g.l^{-1}$ had an inhibitory effect ($p<0.05$) on the growth of *V. parahaemolyticus*.

Blueberry extract exerted its strongest inhibitory effect on *B. cereus*, the growth of which was suppressed at even the lowest concentration of the extract. Significant growth inhibition occurred at a concentration range of 5 $g.l^{-1}$ to 30 $g.l^{-1}$ ($p<0.05$). *L. monocytogenes* was also strongly inhibited by all concentrations of blueberry extract although at a lesser degree than with *B. cereus*.

S. enteritidis and *E. coli* 0157:H7 were significantly inhibited ($p<0.05$) by blueberry extract only at the highest concentration of 30 $g.l^{-1}$.

3.1.2 Effect of Strawberry Extract on Growth of Probiotic Bacteria and Pathogens

3.1.2.1 Probiotic Bacteria

The growth of all species of probiotic microorganisms tested was markedly stimulated by strawberry extract, except for *Bifidobacterium longum*. The Δ_{Growth} profiles of *Bifidobacterium* species and *Lactobacillus* species in response to the presence of strawberry extract are shown in **Fig. 3.5** and **Fig. 3.6**, respectively. There was an increase in Δ_{Growth} values with increasing concentrations of strawberry extract. Values

of Δ_{Growth} greater than 100% which were obtained from *Bifidobacterium* (Yoplait) and *Lactobacillus casei* (Yoplait) indicated that the greatest stimulatory effects of strawberry extract were on these two isolates. The growth of *Lactobacillus reuteri* and *Lactobacillus acidophilus* were similarly enhanced by the fruit extract. However, the effect on these organisms was only about one-third that on *Bifidobacterium* (Yoplait).

According to Tukey test, the significant lowest and highest stimulatory concentrations of strawberry extract on *Bifidobacterium* (Yoplait) was 0.63 $g.l^{-1}$ and 30 $g.l^{-1}$, respectively ($p<0.05$).

With *L. casei* (Yoplait), Δ_{Growth} value increased significantly with a minimum of 1.25 $g.l^{-1}$ but at concentration of strawberry extract higher than 15 $g.l^{-1}$ the effect seemed to be inhibitory. No experiment was done to confirm this; hence, observations were derived from the line of best fit for this organism.

The minimum concentration ($p<0.05$) of strawberry extract which stimulated the growth of *Bifidobacteriu*m (Naturalea), *Bifidobacterium* (Biofarm), and *L. reuteri* was 10 $g.l^{-1}$. Increase in the growth of *L. acidophilus* and *L. plantarum* required a minimum concentration of the extract at 2.5 $g.l^{-1}$ and 20 $g.l^{-1}$ ($p<0.05$), respectively.

3.1.2.2 Pathogenic Bacteria

The dose response profiles of the pathogenic bacteria in the presence of strawberry extract are shown in **Fig. 3.7** and **Fig. 3.8**.

Results show that strawberry extract stimulated the growth of most pathogens to varying extent before any marked inhibition occurred with the highest concentration used in this study. Except for *Yersinia enterocolitica*, the growth of all pathogens tested with strawberry extract were inhibited by its highest concentration of 30 $g.l^{-1}$.

An inhibition of growth could be arranged in order of inhibitory strength of extract as follows: *Salmonella typhimurium* , *Salmonella enteritidis* , *Bacillus cereus* , *Listeria monocytogenes, Escherichia coli* 0157:H7. While *Vibrio parahaemolyticus* was inhibited at the highest concentration of strawberry extract (30 $g.l^{-1}$), its growth was markedly enhanced at concentrations lower than 30 $g.l^{-1}$.

Significant growth in *S. typhimurium* did not occur with strawberry extract concentration below 5 $g.l^{-1}$. Strawberry extract at 2.5 $g.l^{-1}$ enhanced the growth of *S. enteritidis* before a significant suppression of growth was obtained at 15 $g.l^{-1}$ ($p<0.05$). It was observed that the extract did not only have a lesser inhibitory effect against *S. enteritidis* but also a narrow range of effective concentration than that on *S. typhimurium*.

The growth of *E. coli* 0157:H7 was similarly enhanced, although to a lower extent, by the strawberry extract. The growth of this bacterium was significantly reduced ($p<0.05$) with strawberry concentration of at least 10 $g.l^{-1}$. *B. cereus* and *L. monocytogenes* showed a significant decrease in growth ($p<0.05$) in the presence of strawberry extract at 2.5 $g.l^{-1}$ and 5 $g.l^{-1}$, respectively.

The growth-reducing effect of strawberry extract on *V. parahaemolyticus* was observed only at the highest concentration (30 $g.l^{-1}$). Concentrations lower than 30 $g.l^{-1}$ were stimulatory to the growth of the bacterium. However, the stimulatory effect of the extract was significantly decreased at 3.75 $g.l^{-1}$ ($p<0.05$).

Contrary to its inhibitory effect, strawberry extract had a significant stimulatory effect ($p<0.05$) on the growth of *Y. enterocolitica* at concentrations higher than 0.24 $g.l^{-1}$. An increase in the concentration of the extract markedly stimulated the growth of the bacteria until a concentration of 20 $g.l^{-1}$, where a significant decrease in growth stimulation was obtained ($p<0.05$). Further, if the curve were extrapolated, strawberry could potentially inhibit *Y. enterocolitica* at a concentration higher than 45 $g.l^{-1}$.

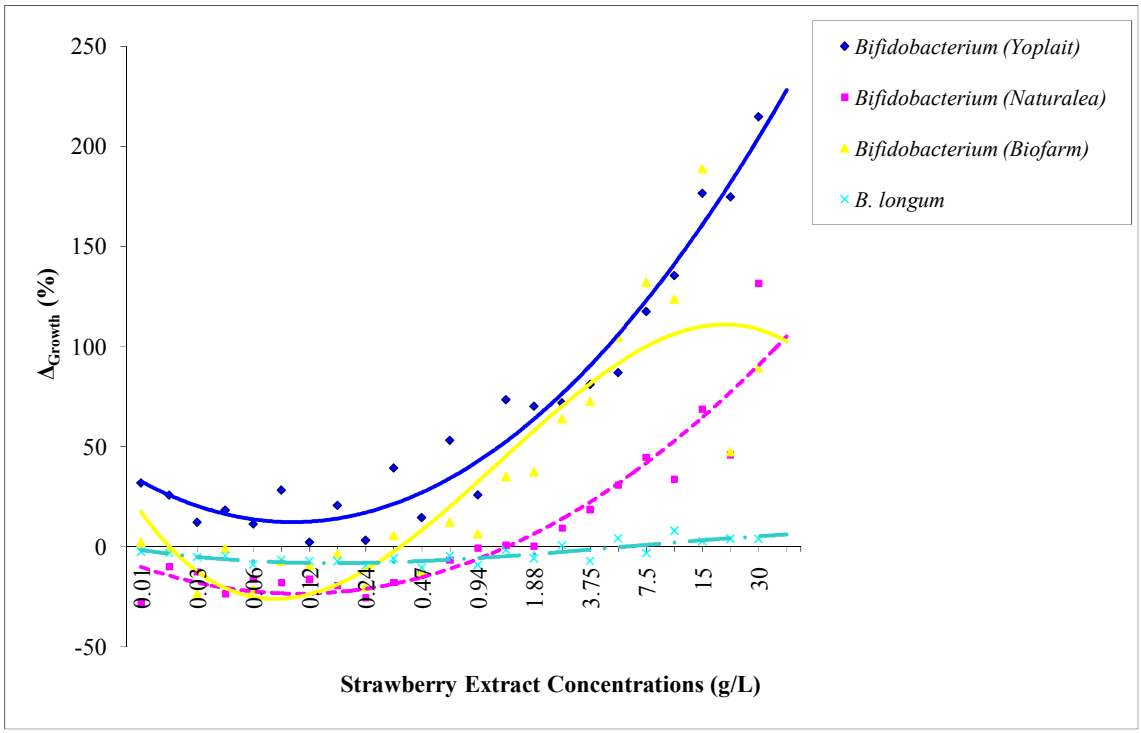

Figure 3.5 Effect of strawberry extract on growth of *Bifidobacteria* species.

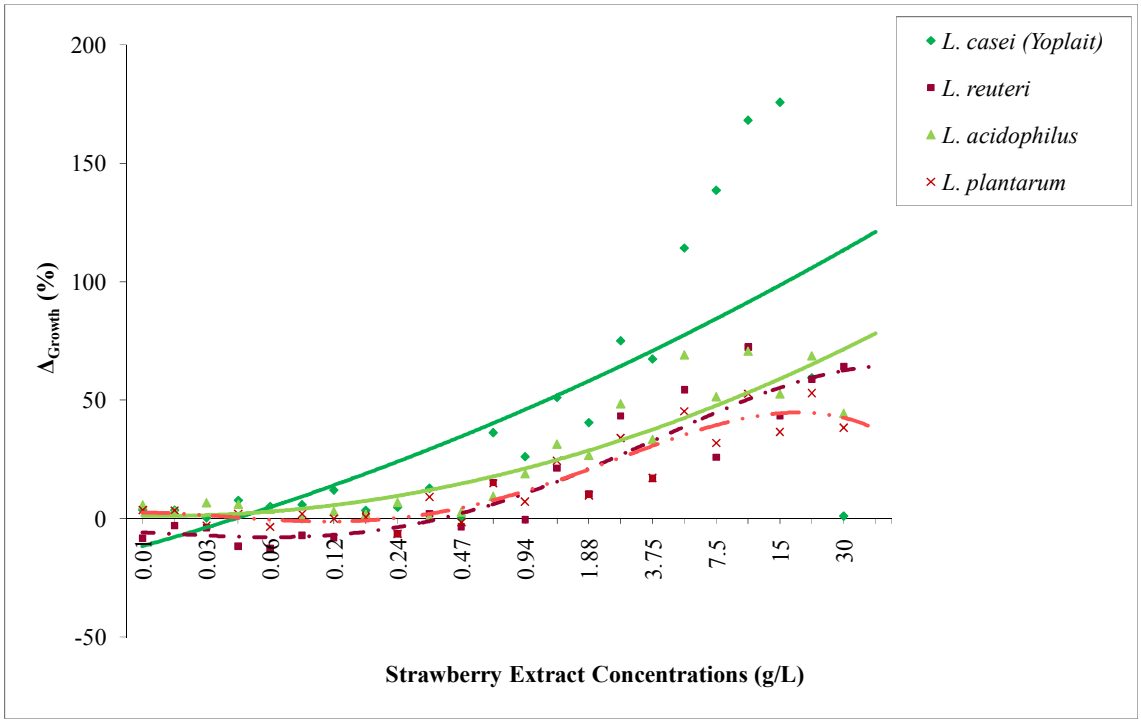

Figure 3.6 Effect of strawberry extract on growth of *Lactobacilli* species.

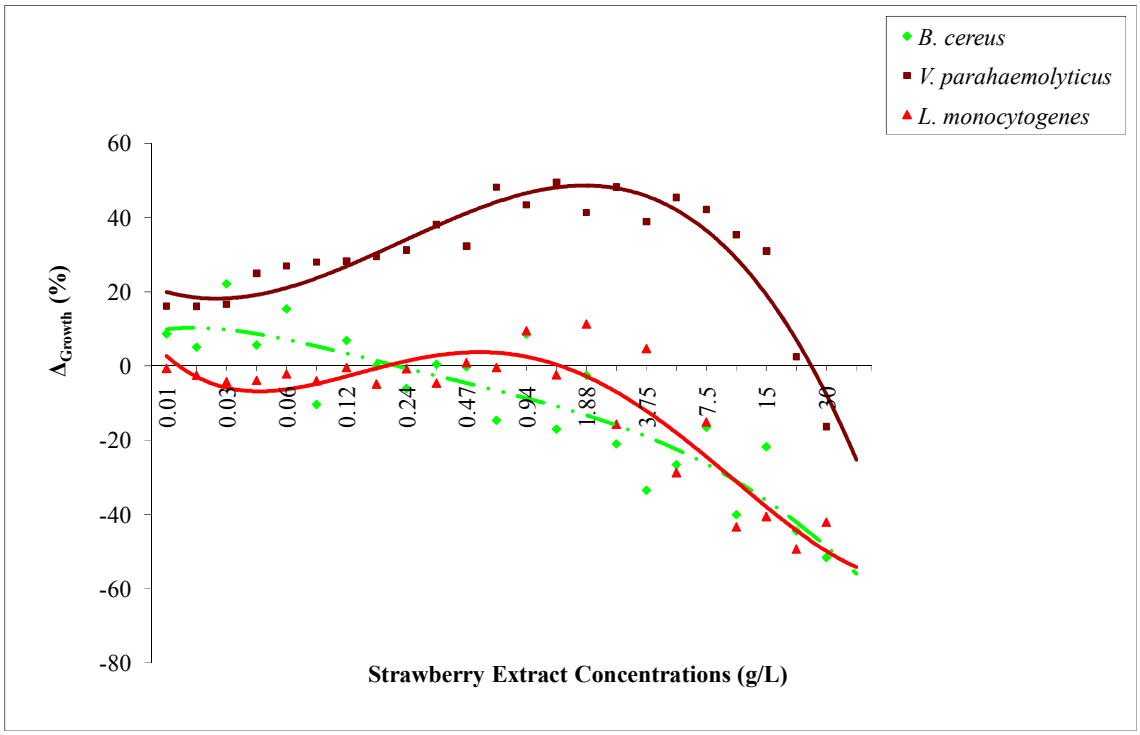

Figure 3.7 Effect of strawberry extract on growth of pathogenic bacteria.

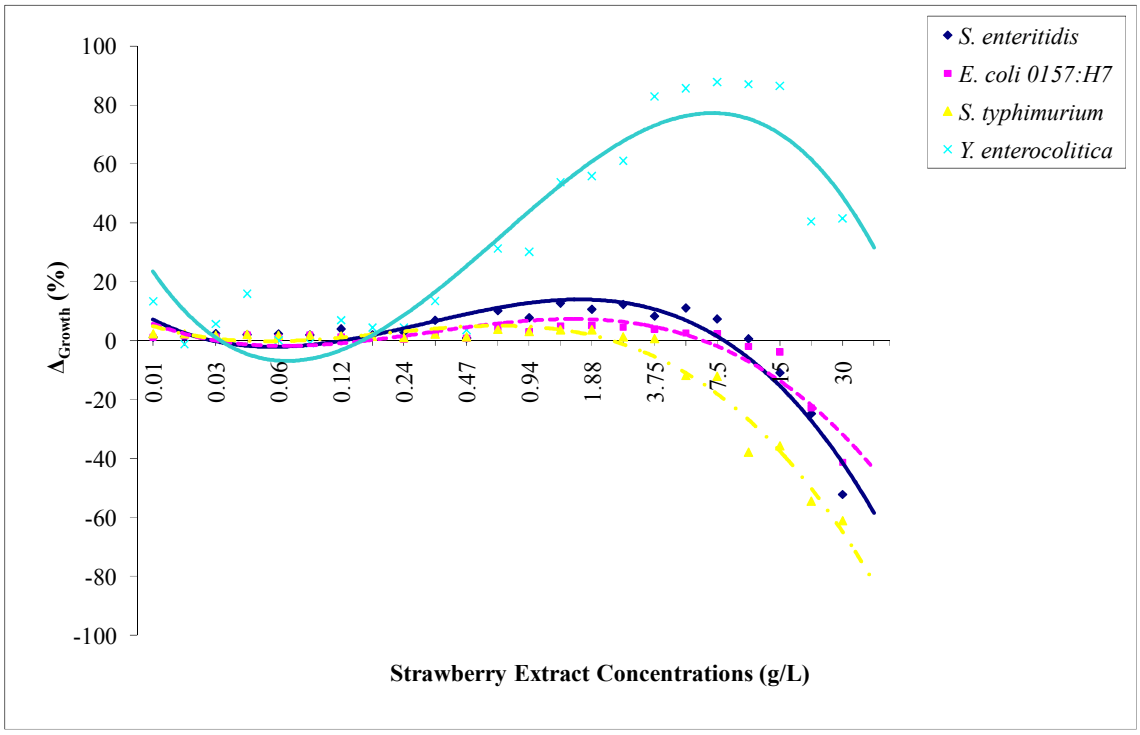

Figure 3.8 Effect of strawberry extract on growth of pathogenic bacteria.

3.1.3 Effect of Green Kiwifruit Extract on Growth of Probiotic Bacteria and Pathogens

3.1.3.1 Probiotic Bacteria

Green kiwifruit extract was able to enhance the growth of all the probiotic bacteria studied but the effect of the extract was dose- and strain-dependent. **Fig. 3.9** and **Fig. 3.10** show the effect of green kiwifruit extract on the growth of *Bifidobacterium* and *Lactobacilli* species, respectively.

At $p<0.05$, lower concentrations of kiwifruit extract (i.e. less than 1.0 $g.l^{-1}$) exerted a growth-repressing effect, while higher concentrations (i.e. 1.0 $g.l^{-1}$) stimulated the growth of all *Lactobacillus* species used in this study.

Lactobacillus acidophilus should have been the most positively affected by green kiwifruit extract but its growth was highly inhibited by the highest concentration used (30 $g.l^{-1}$). Since no further experiment were carried out to determine the effect of concentrations higher than 30 $g.l^{-1}$, the line of best fit was drawn for *L. acidophilus* which showed growth stimulation for this species being less than that in *L. reuteri*.

Similar inhibitory effect at lower concentrations and stimulatory effect at higher concentrations of green kiwifruit extract were observed with *Bifidobacterium* (Yoplait) and *B. longum*. The effect of green kiwifruit extract on *Bifidobacterium* (Naturalea) and *Bifidobacterium* (Biofarm) was stimulatory until 30 $g.l^{-1}$ and 15 $g.l^{-1}$, respectively which then became inhibitory to the bacteria. The line of best fit and lack of further trials did not make the inhibition of *Bifidobacteria* (Naturalea) apparent.

A concentration of green kiwifruit extract 3.75 $g.l^{-1}$ and lower than 30 $g.l^{-1}$ produced a growth-enhancing effect on all species of *Bifidobacteria* and *Lactobacilli*, except for *B. longum*. With *B. longum*, green kiwifruit extract was found to be inhibitory at all concentrations ($p<0.05$).

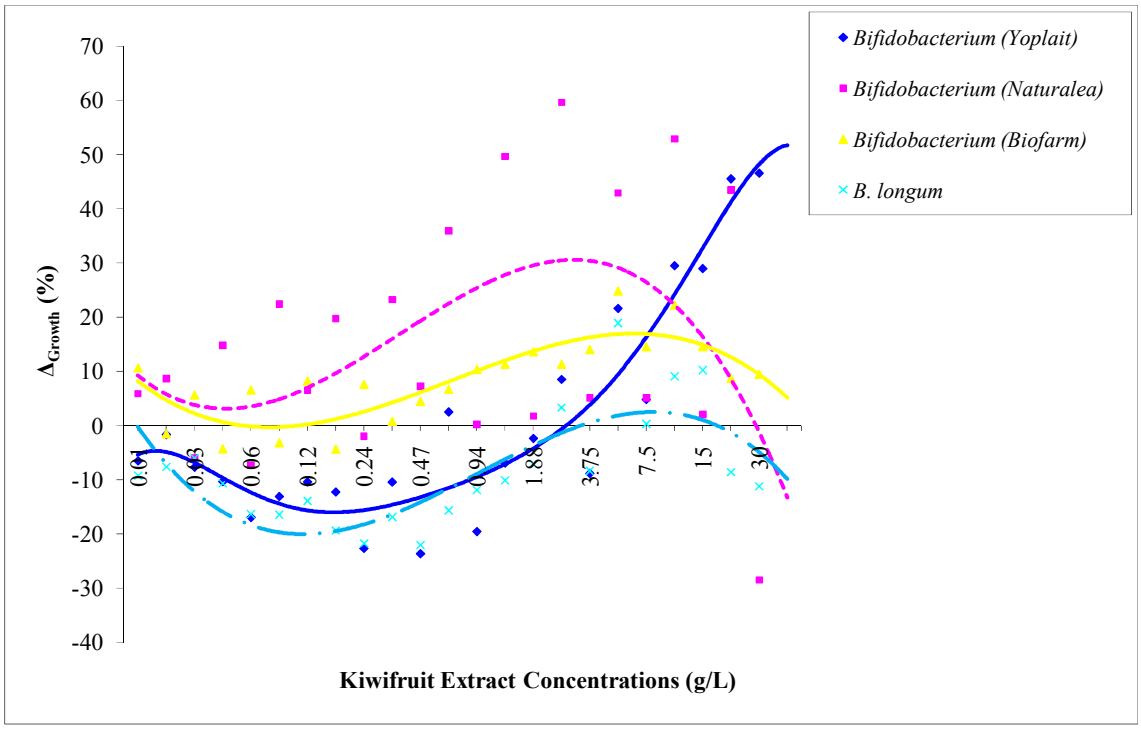

Figure 3.9 Effect of green kiwifruit extract on growth of *Bifidobacteria* species.

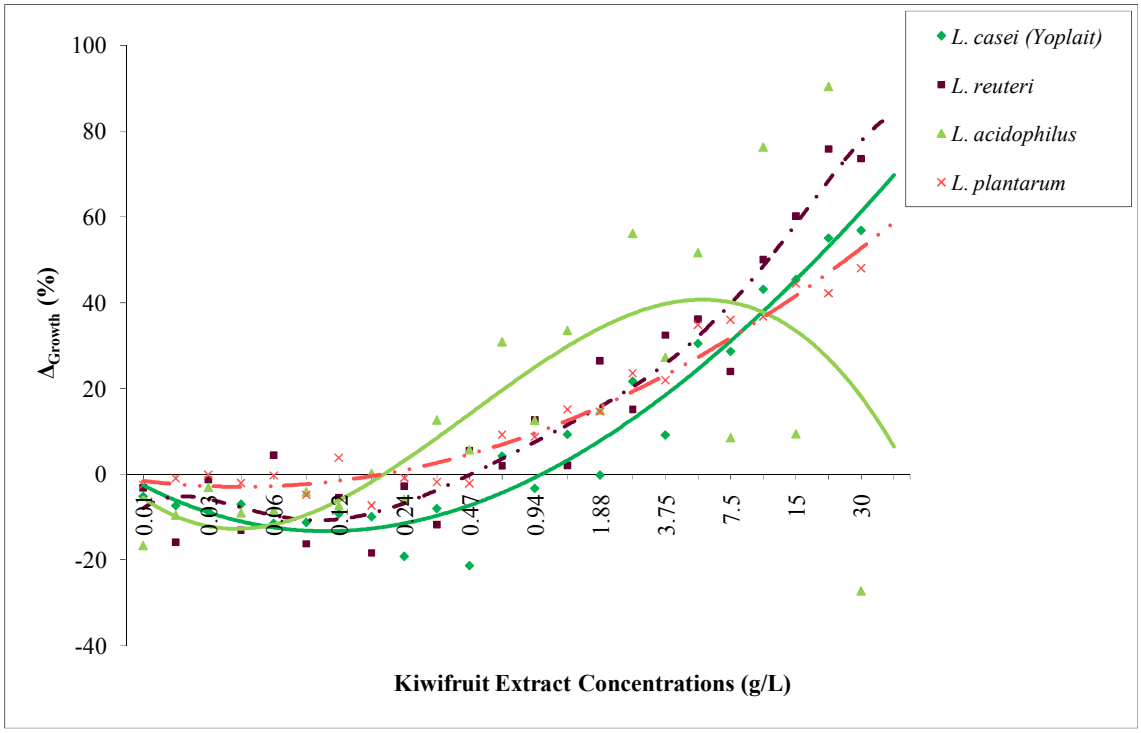

Figure 3.10 Effect of green kiwifruit extract on growth of *Lactobacilli* species.

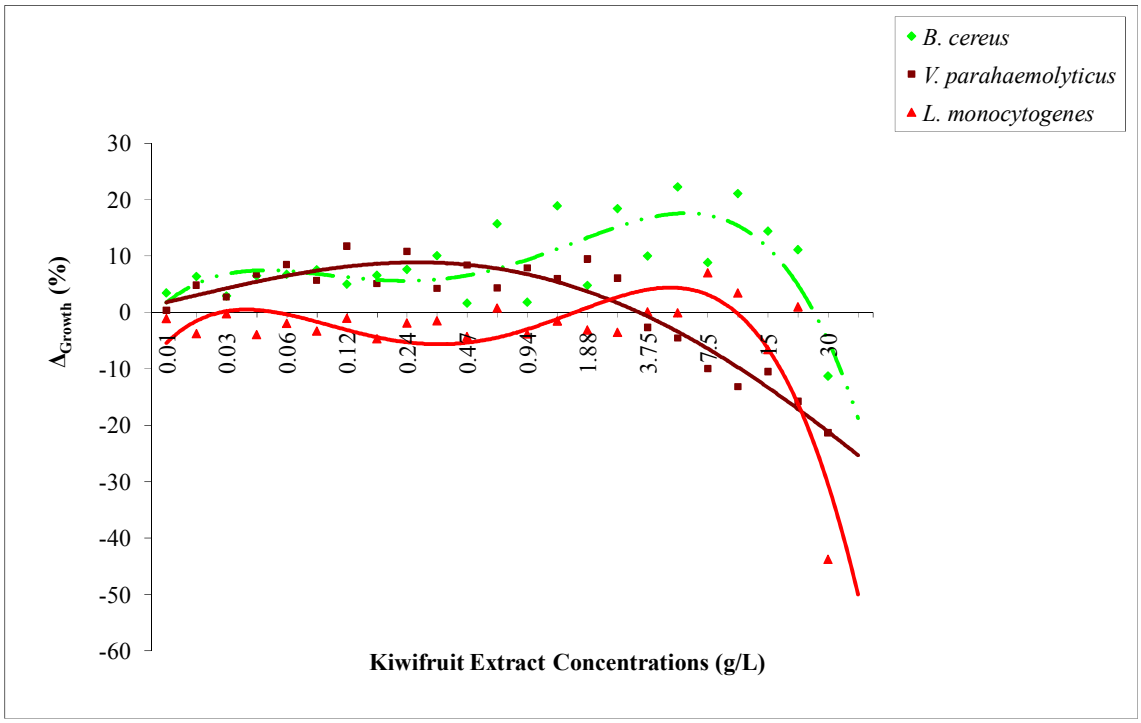

Figure 3.11 Effect of green kiwifruit extract on growth of pathogenic bacteria.

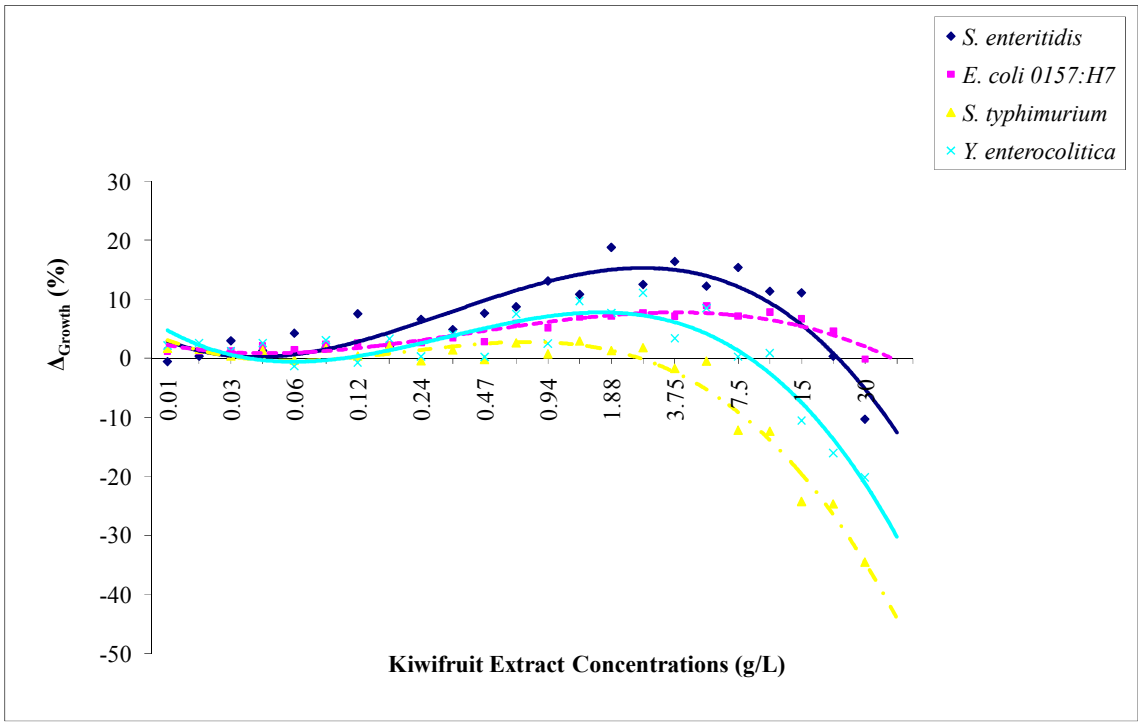

Figure 3.12 Effect of green kiwifruit extract on growth of pathogenic bacteria.

3.1.3.2 Pathogenic Bacteria

The Δ_{Growth} values for the pathogenic bacteria grown with varying concentrations of green kiwifruit extract are shown in **Fig. 3.11** and **Fig. 3.12**.

The ANOVA results (p<0.05) indicated that relatively low concentrations of green kiwifruit extract exerted growth stimulatory effect, while high concentrations had growth-inhibitory effect on all the pathogenic species used in this assay. In contrast to the dual effect of the highest concentration (30 $g.l^{-1}$) on probiotic bacteria, green kiwifruit extract at this concentration exerted only growth-inhibiting effect on the pathogens.

Green kiwifruit extract caused significant growth inhibition of pathogenic bacteria such as *S. typhimurium* and *V. parahaemolyticus* at a concentration as low as 3.75 $g.l^{-1}$ (p<0.05) which is ten-fold lower than the concentration inhibitory to the probiotic bacteria.

Although inhibition of *L. monocytogenes* occurred at higher concentrations, a very low Δ_{Growth} value indicated that green kiwifruit extract at 30 $g.l^{-1}$ exerted its strongest inhibitory effect on this bacterium. The growth of *B. cereus*, *E. coli* 0157:H7 and *S. enteritidis* were suppressed only at the highest concentration of the extract.

3.1.4 Effect of Feijoa Extract on Growth of Probiotic Bacteria and Pathogens

3.1.4.1 Probiotic Bacteria

Results of the growth assays using feijoa extract are shown in **Fig. 3.13** and **Fig. 3.14**. The effect of feijoa extract was found to be dose-and species-dependent. At certain concentrations, feijoa extract exerted growth-stimulating effect on all the species of probiotic bacteria used in this study. This meant that a biphasic effect was exerted by feijoa extract.

Concentrations lower than 2.5 $g.l^{-1}$ were inhibitory to *L. casei* (Yoplait), while concentrations below 1.0 $g.l^{-1}$ were inhibitory to *Bifidobacterium* (Naturalea) and

Bifidobacterium (Biofarm) (p<0.05). Concentrations higher than these values had growth-stimulating effects on the bacteria.

With *L. acidophilus* and *Bifidobacterium* (Yoplait), the increase in stimulation with concentration reached a maximum stimulatory concentration, after which the effect of the extract became inhibitory. Hence, feijoa extract at concentration higher than 5.0 $g.l^{-1}$ (p<0.05) were inhibitory to *L. acidophilus* and *Bifidobacterium* (Yoplait). The highest concentration (30 $g.l^{-1}$) added to *L. acidophilus* had a significant strong inhibitory effect (p<0.05).

Significant increases (p<0.05) in the growth of *L. reuteri* and *L. plantarum* were obtained in the presence of the highest concentration of feijoa extract (30 $g.l^{-1}$). The greatest stimulatory effect of feijoa extract was obtained with *L. reuteri*.

B. longum was inhibited by all concentrations of feijoa extract, with significant inhibition effected by the extract at 30 $g.l^{-1}$.

3.1.4.2 Pathogenic Bacteria

The dose response profiles of the pathogens in the presence of feijoa extract are shown in **Fig. 3.15** and **Fig. 3.16**.

The stimulatory and inhibitory effects of feijoa extract on *V. parahaemolyticus* were most pronounced and markedly significant (p<0.05). Feijoa extract also exerted double-edged effects on the pathogenic bacteria used in this investigation, except on *L. monocytogenes* and *B. cereus*.

Significant increases (p<0.05) in the growth of species of *V. parahaemolyticus*, *S. enteritidis*, *E. coli* 0157:H7 and *Y. enterocolitica* used in this study were obtained in the presence of feijoa extract from 1.0 $g.l^{-1}$ to 5.5 $g.l^{-1}$. Concentrations higher than 15 $g.l^{-1}$ were found to be inhibitory to these pathogenic bacteria (p<0.05).

Feijoa extract appeared to be growth-repressing at all concentrations with *L. monocytogenes* and *B. cereus*. Significant ($p < 0.05$) greatest reductions in growth

occurred in the presence of the highest concentration of extract (30 $g.l^{-1}$). A weak stimulation of growth was observed with *B. cereus* at 0.01 $g.l^{-1}$.

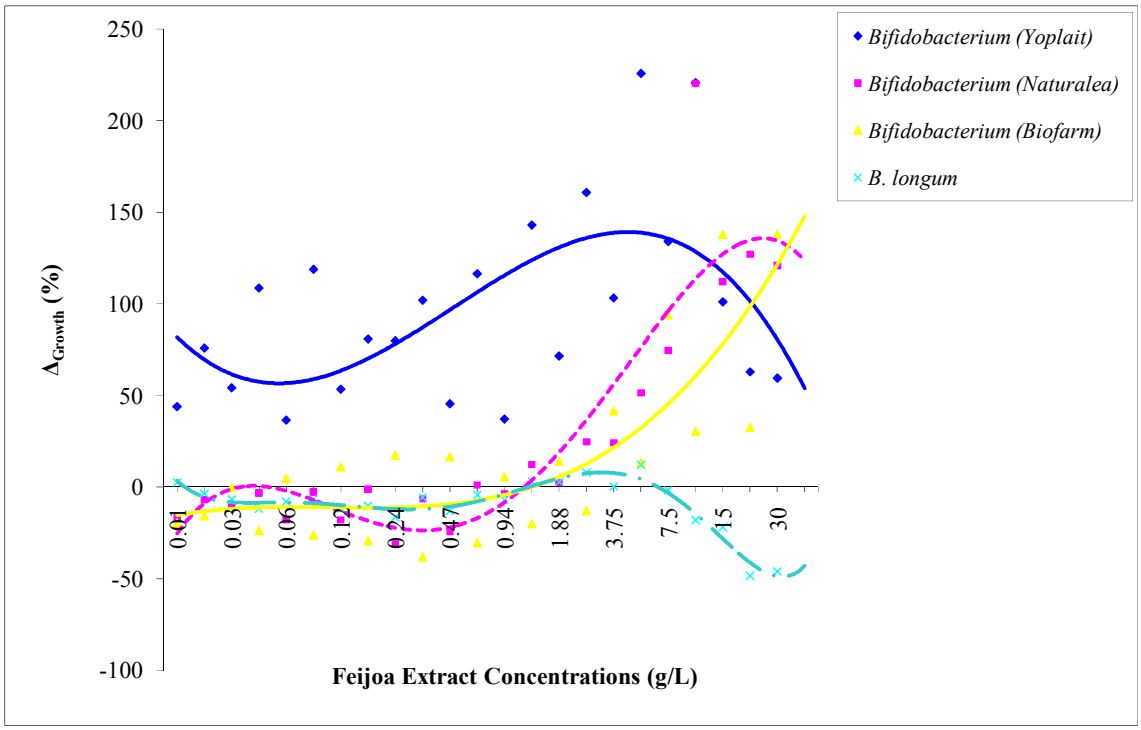

Figure 3.13 Effect of feijoa extract on growth of *Bifidobacteria* species.

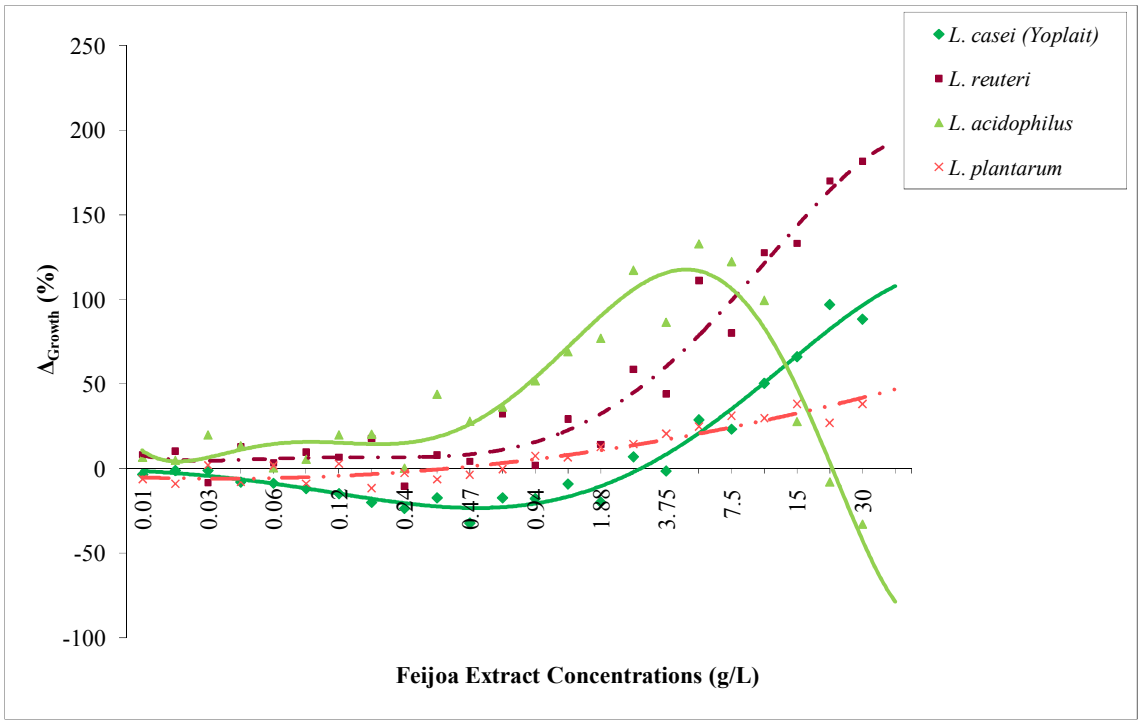

Figure 3.14 Effect of feijoa extract on growth of *Lactobacilli* species.

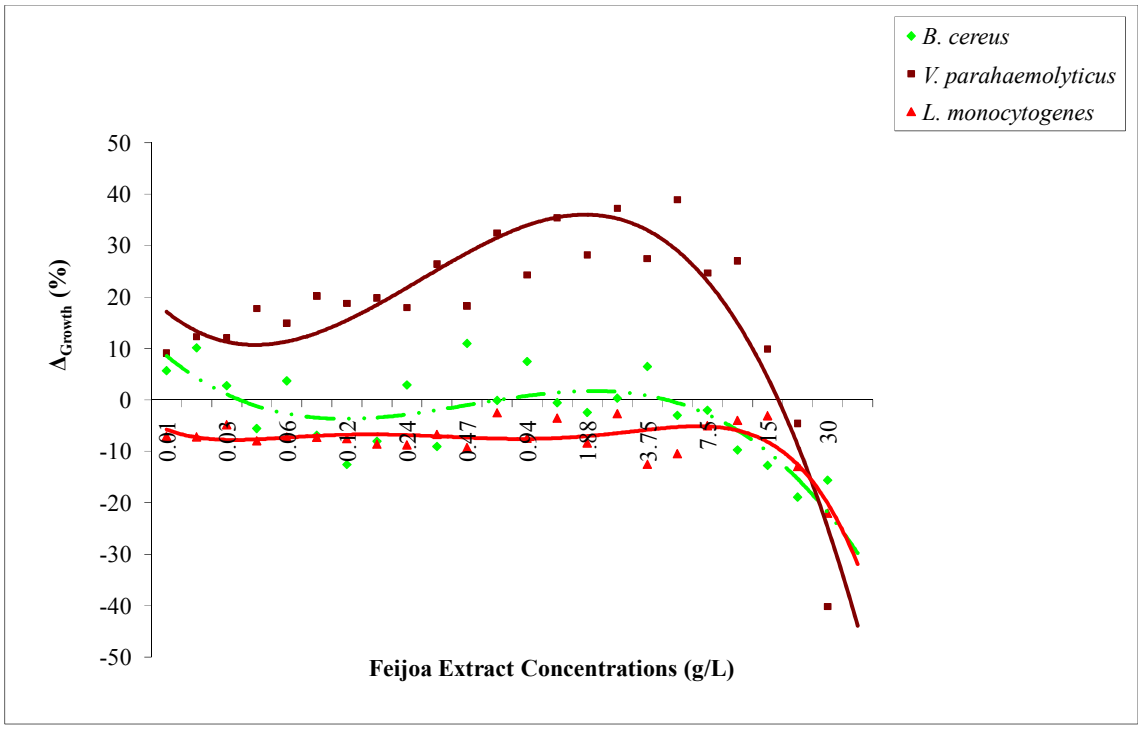

Figure 3.15 Effect of feijoa extract on growth of pathogenic bacteria.

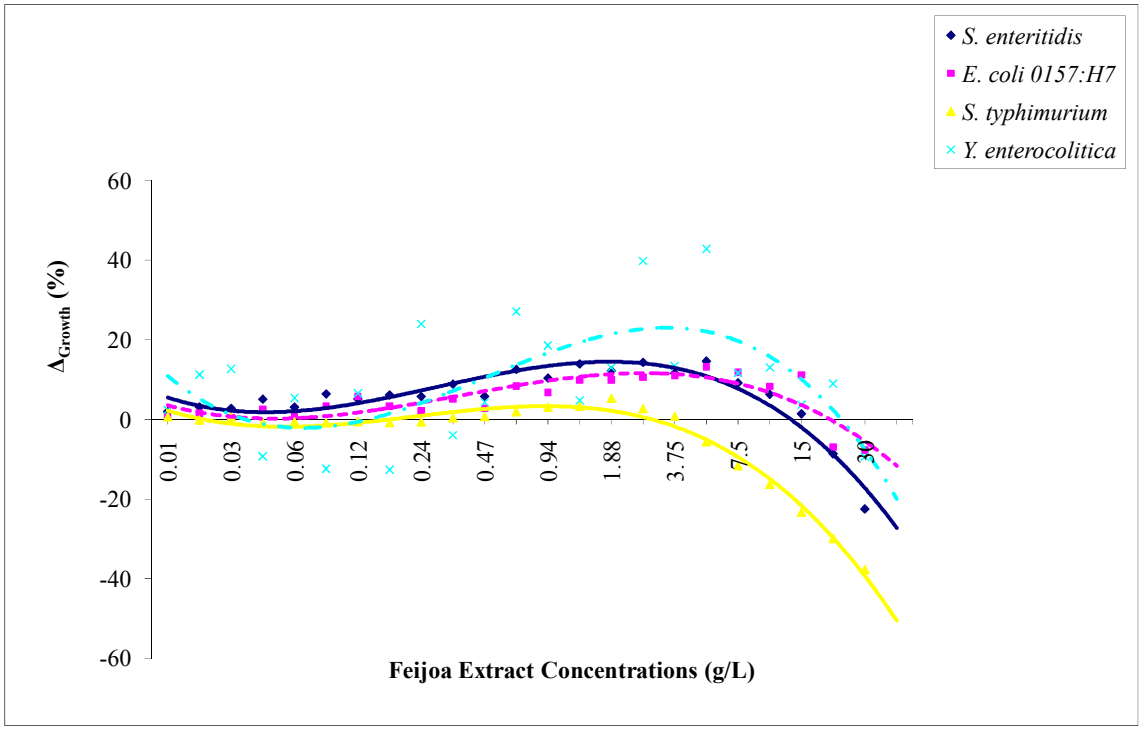

Figure 3.16 Effect of feijoa extract on growth of pathogenic bacteria.

3.2 Combined Extract Assay

A combination of two fruit extracts were used in growth assays to determine any stimulatory or inhibitory interactions between the fruit extracts which could enhance stimulation or inhibition effects on microorganisms.

Two fruit extracts in equal proportions were added into bacterial cultures as done with single extract assay but keeping the assay volume the same. Each method resulted in each fruit extract present at half of the concentration of the highest dose when tested singly.

The Δ_{Growth} values were calculated and the dose-response profiles were plotted in a graph. An additional analysis used by Rosendale et al. (2008) was adopted in this study to compare the efficacy of the combined extracts with that of a single extract. The effect could be described as good, poor, desirable and undesirable after comparisons using the following two methods:

Comparison 1:

Does $\Delta_{Growth\ AB}$ exceed ($\Delta_{Growth\ A} + \Delta_{Growth\ B}$)?

Comparison 2:

Does $\Delta_{Growth\ AB}$ exceed either $\Delta_{Growth\ A}$ or $\Delta_{Growth\ B}$?

According to the two assigned methods, any results from the combined extracts experiments that fulfilled Comparison 1 were termed "good" and those that did not fulfil Comparison 1 were termed "poor". Any results of the combined assay that fulfilled both Comparison 1 and Comparison 2 were termed "desirable" and those that failed to meet the two comparisons were termed "undesirable".

Due to budget and time constraints, only two sets of combinations were tested. A dark-coloured fruit extract was mixed with a pale-coloured fruit extract. Blueberry was mixed with feijoa extract; strawberry was mixed with green kiwifruit extract.

3.2.1 Effect of Combined Blueberry and Feijoa Extracts on Growth of Probiotic Bacteria and Pathogens

3.2.1.1 Probiotic Bacteria

The dose-response profile shows an apparent biphasic effect of blueberry and feijoa extracts on *Bifidobacterium* (Yoplait) (**Fig. 3.17**) *B. longum* (**Fig. 3.17**), *L. acidophilus* (**Fig. 3.18**) and *L. reuteri* (**Fig. 3.18**). However, although the growth of all probiotic bacteria seemed to have been stimulated by relatively high concentrations of combined blueberry and feijoa extracts as seen on the graph, the analytical comparisons revealed that this combination resulted in growth of all probiotic bacteria that is lesser than expected from the contributing fruit extracts. Hence, the combined blueberry and feijoa extracts actively suppressed the growth of all probiotic bacteria.

3.2.1.2 Pathogenic Bacteria

The apparent effects of the combined blueberry and feijoa extracts on pathogens are shown in **Fig. 3.19** and **Fig. 3.20**.

The graphs show that the highest concentration of the combined extracts was inhibitory to all pathogens, except *S. enteritidis*. But further analysis using the two comparisons described previously revealed that the growth of pathogenic bacteria was enhanced by this combination which is marked as "c" in **Table 3.1**. The combination of blueberry and feijoa extracts was identified as significantly undesirable for the inhibition of *E. coli* 0157:H7, *Y. enterocolitica*, *V. parahaemolyticus* and *L. monocytogenes*.

The growth of these pathogenic bacteria was stimulated by the combined blueberry and feijoa extracts to the extent that was even greater than the sum of the individual extracts. Further, the combined blueberry and feijoa extracts increased growth of the pathogens greater than the more stimulatory individual extract did.

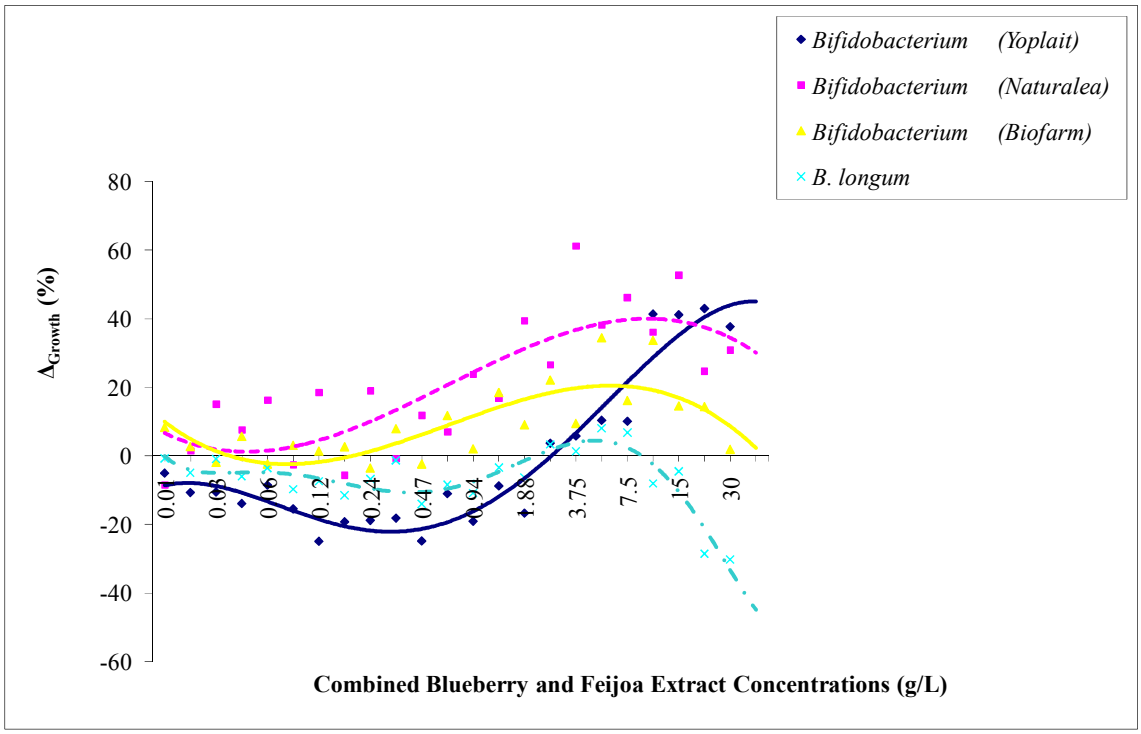

Figure 3.17 Effect of combined blueberry and feijoa extracts on growth of *Bifidobacteria* species.

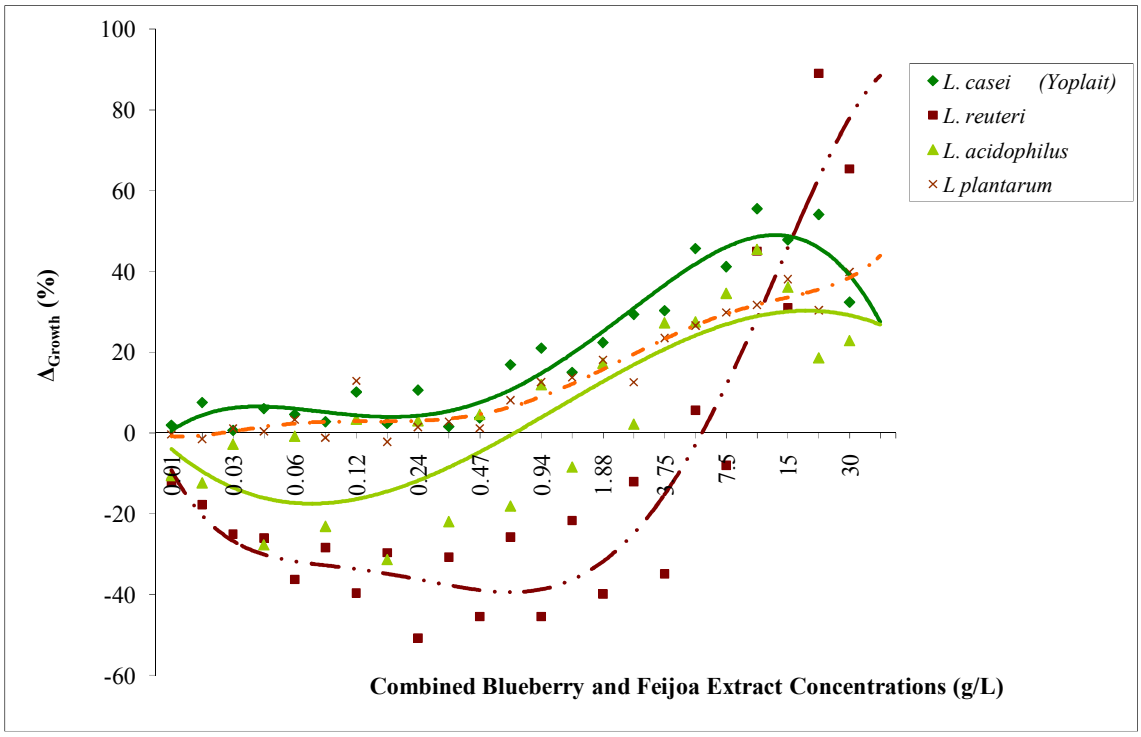

Figure 3.18 Effect of combined blueberry and feijoa extracts on growth of *Lactobacilli* species.

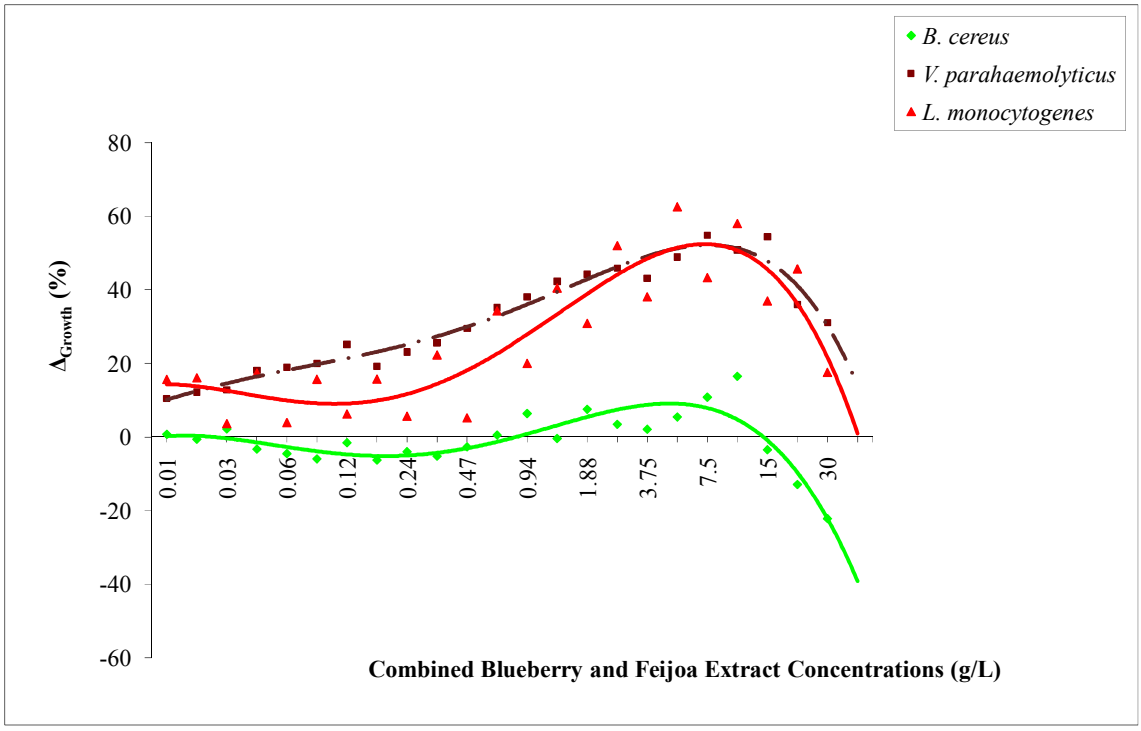

Figure 3.19 Effect of combined blueberry and feijoa extracts on growth of pathogenic bacteria.

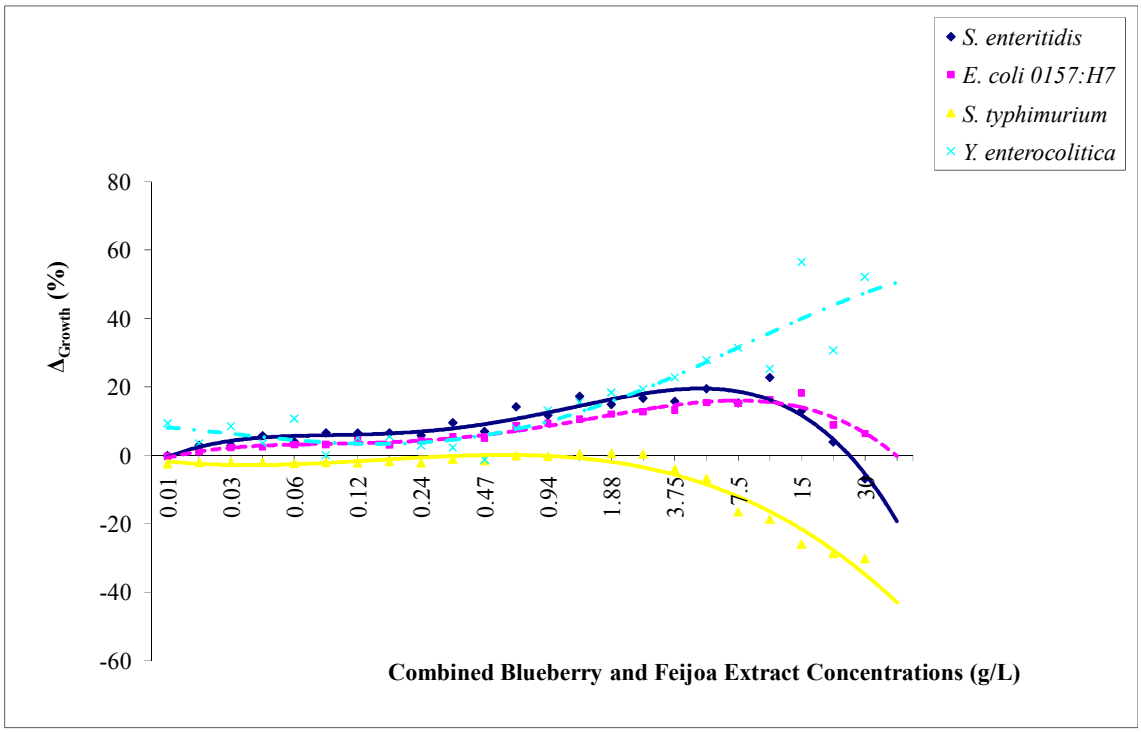

Figure 3.20 Effect of combined blueberry and feijoa extracts on growth of pathogenic bacteria.

Table 3.1: Comparison between highest Δ_{Growth} values of bacteria in single extracts and combined blueberry-feijoa extracts assays.

Microorganisms	Single extract assay		Combined extracts assay
	$\Delta_{Growth\ Blueberry}$	$\Delta_{Growth\ Feijoa}$	$\Delta_{Growth\ Blueberry + Feijoa}$
Bifidobacterium (Yoplait)	100	225	43^{d}
Bifidobacterium (Naturalea)	69	220	61^{d}
Bifidobacterium (Biofarm)	15	137	34^{d}
Bifidobacterium longum	4	12	8^{d}
Lactobacillus casei (Yoplait)	173	97	55^{d}
Lactobacillus reuteri	61	181	89^{d}
Lactobacillus acidophilus	57	132	45^{d}
Lactobacillus plantarum	26	38	39^{d}
Salmonella enteritidis	-1	-22	-7^{c}
Escherichia coli 0157:H7	-10	-7	$+6^{c,b}$
Salmonella typhimurium	-45	-37	-30^{c}
Yersinia enterocolitica	-53	-9	$+5^{c,b}$
Bacillus cereus	-68	-18	$-22^{c,b}$
Vibrio parahaemolyticus	-25	-40	$+31^{c,b}$
Listeria monocytogens	-57	-22	$+17^{c,b}$

Notes:

Δ_{Growth} , %

a = Desirable combined effect = fulfilled both comparisons 1 and 2. There was increased probiotic growth or decreased pathogenic growth due to both: 1) Δ_{Growth} from combined extracts exceeds the sum of Δ_{Growth} from individual extracts, and 2) Δ_{Growth} from combined extracts exceeds the extreme Δ_{Growth} from an individual extract.

b = Undesirable combined effect = did not meet both comparisons 1 and 2. There was decrease of probiotic growth or increase of pathogenic growth due to both: 1) Δ_{Growth} from combined extracts exceeds the sum of Δ_{Growth} from individual extracts, and Δ_{Growth} 2) from combined extracts exceeds the extreme Δ_{Growth} from an individual extract.

c = Growth exceeds the sum of the individual extracts.

d = Growth less than the sum of the individual extracts.

3.2.2 Effect of Combined Strawberry and Green Kiwifruit Extracts on Growth of Probiotic Bacteria and Pathogens

3.2.2.1 Probiotic Bacteria

The apparent effect of combined strawberry and green kiwifruit extracts on the probiotic microorganisms was similar to that of combined blueberry and feijoa extracts. There was an apparent direct relationship between the concentration of the combined extracts and stimulation of growth of all the probiotic bacteria except that of *B. longum* (**Fig. 3.21** and **Fig. 3.22**).

An apparent biphasic effect is shown in the graphs for *Bifidobacterium* (Yoplait), *B. longum* (**Fig. 3.21**) and *L. reuteri* (**Fig. 3.22**).

Closer analysis using comparison 1 and comparison 2 showed the actual effect of the combined extracts of strawberry and green kiwifruit (**Table 3.2**). Except for *B. longum*, this particular combination produced a "d" effect on all the probiotic bacteria which meant that there was no stimulation of growth in the presence of combined strawberry and green kiwifruit extracts ($p<0.05$).

The only probiotic bacterium in which the growth was significantly enhanced ($p<0.05$) by the combined strawberry and green kiwifruit extracts was *B. longum*. This combination had a significantly desirable "a" effect for producing more growth in this probiotic species.

3.2.2.2 Pathogenic Bacteria

Fig. 3.23 and **Fig. 3.24** show the apparent effect of the combined strawberry and green kiwifruit extracts on the pathogenic bacteria. The graphs suggest that the highest concentration of the combined extracts was inhibitory to all the pathogens tested. However, the analytical comparisons revealed different effects. Results of comparisons 1 and 2 revealed that in the presence of the combined strawberry and green kiwifruit extracts, all the pathogens produced more growth than the mathematical sum of the individual extracts called "c" effect.

For the analytical comparisons, the combined strawberry and green kiwifruit extracts produced a significantly strong stimulation for the growth of *Y. enterocolitica* ($p<0.05$) which exceeded both the mathematical effect of the two extracts and the effect of the more stimulatory extract. Hence, the combined strawberry and green kiwifruit extracts are undesirable for inhibition of *Y. enterocolitica*.

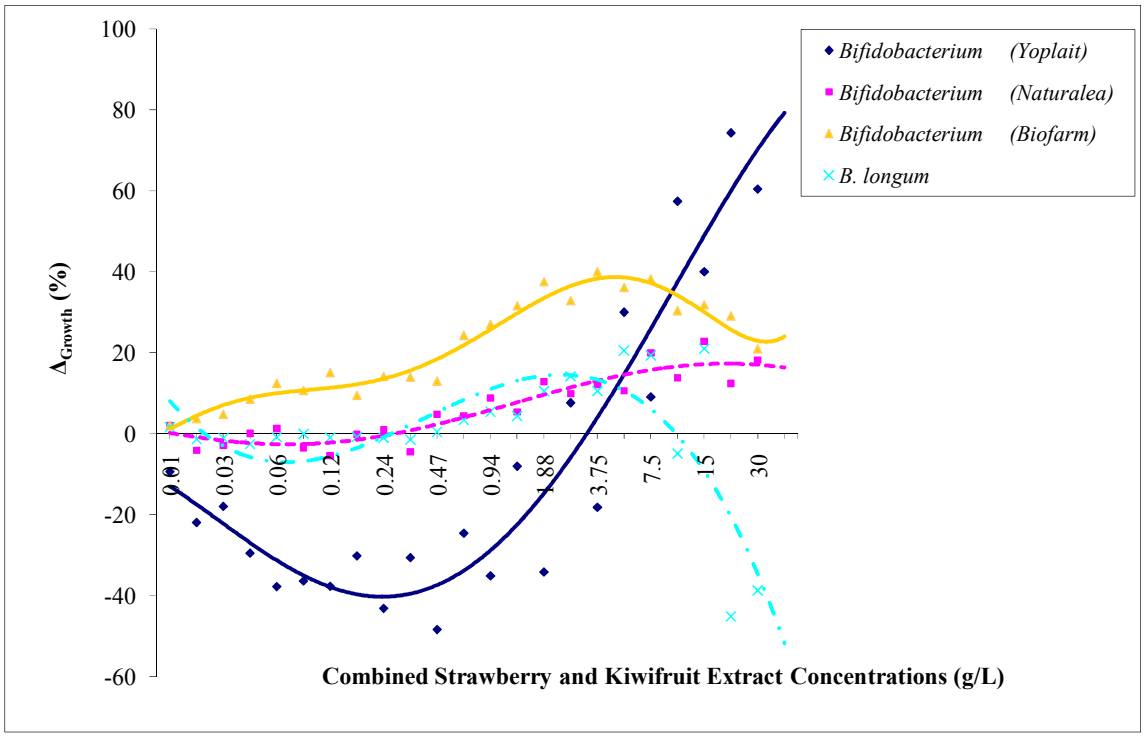

Figure 3.21 Effect of combined strawberry and green kiwifruit extracts on growth of *Bifidobacteria* species.

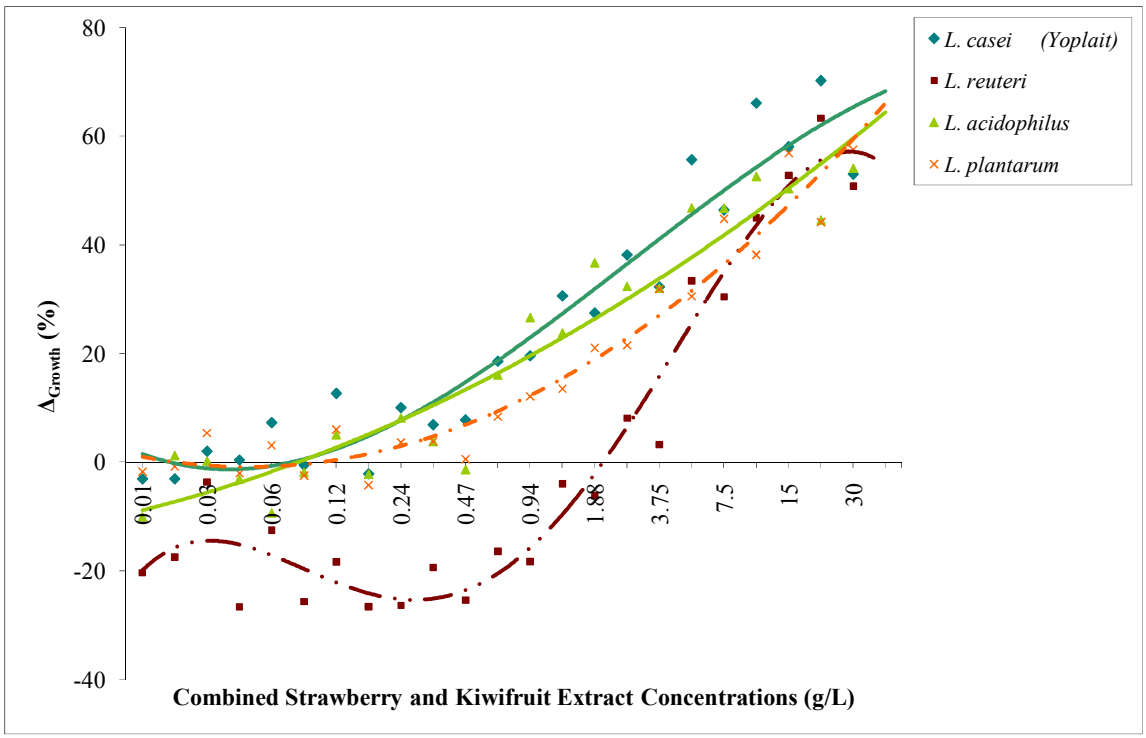

Figure 3.22 Effect of combined strawberry and green kiwifruit extracts on growth of *Lactobacilli* species.

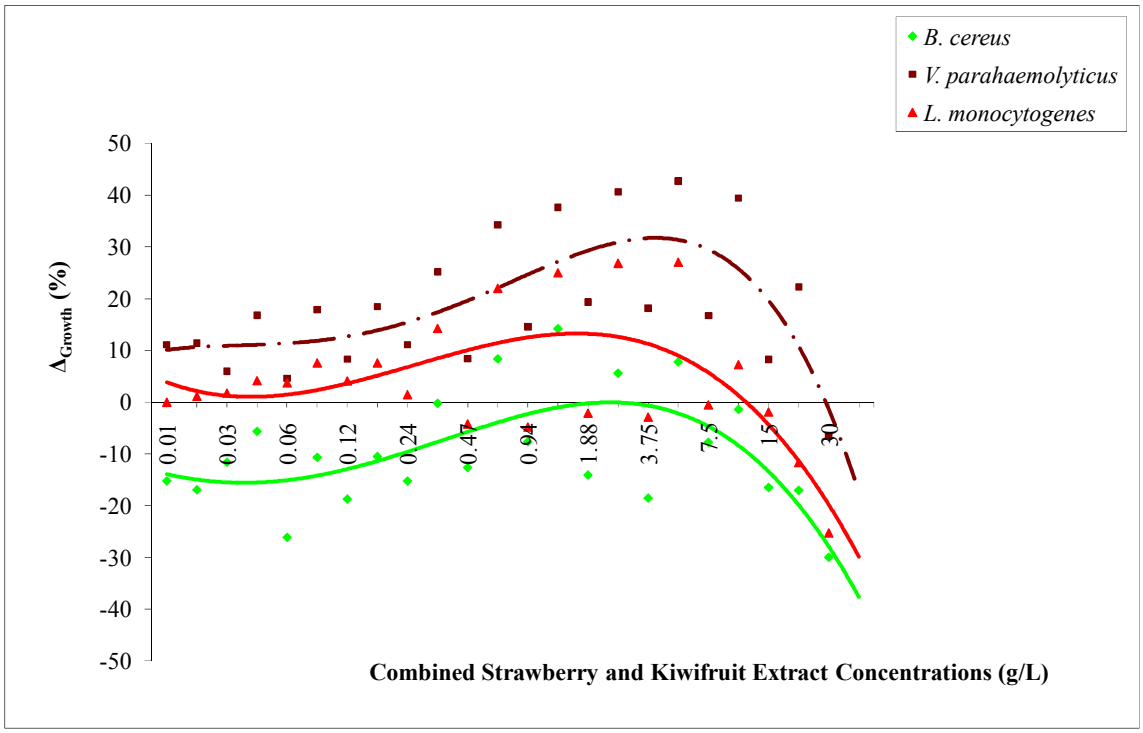

Figure 3.23 Effect of combined strawberry and green kiwifruit extracts on growth of pathogenic bacteria.

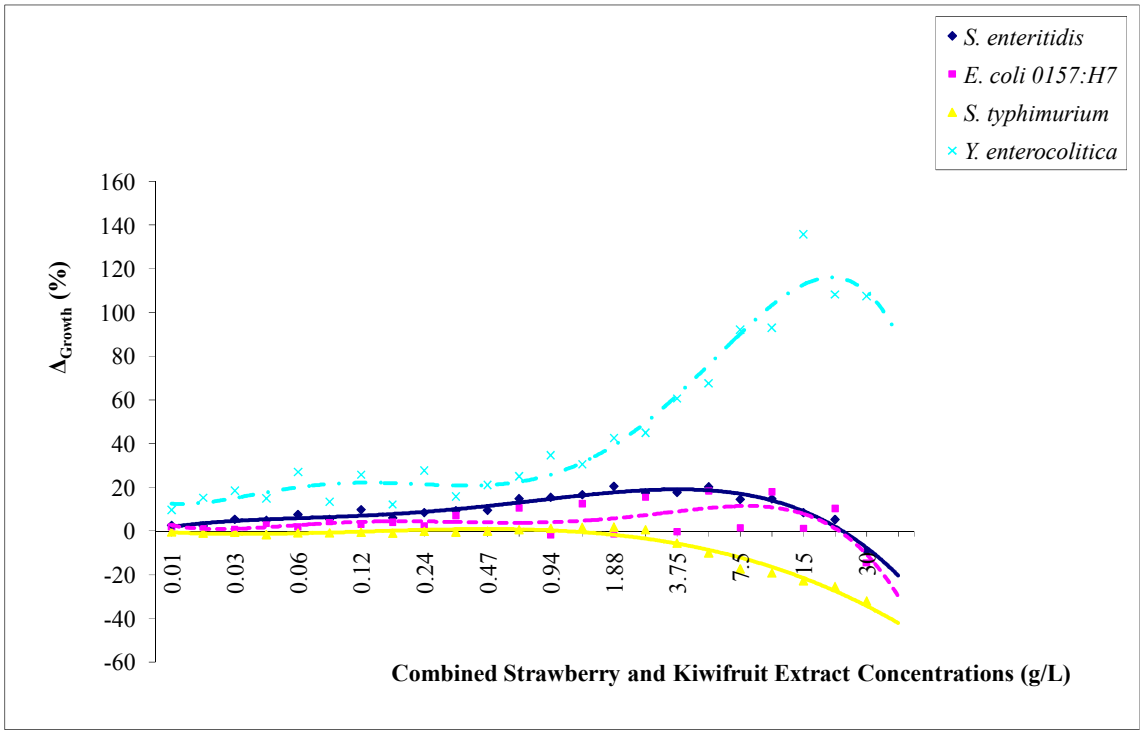

Figure 3.24 Effect of combined strawberry and green kiwifruit extracts on growth of pathogenic bacteria.

Table 3.2: Comparison between highest Δ_{Growth} values of bacteria in single extracts and combined strawberry-green kiwifruit extracts.

Microorganisms	Single extract assay		Combined extracts assay
	$\Delta_{Growth\ Strawberry}$	$\Delta_{Growth\ Kiwifruit}$	$\Delta_{Growth\ Strawberry + Kiwifruit}$
Bifidobacterium (Yoplait)	214	46	74[d]
Bifidobacterium (Naturalea)	131	52	22[d]
Bifidobacterium (Biofarm)	188	24	39[d]
Bifidobacterium longum	7.7	10	20[c,a]
Lactobacillus casei (Yoplait)	175	56	70[d]
Lactobacillus reuteri	72	75	63[d]
Lactobacillus acidophilus	70	90	54[d]
Lactobacillus plantarum	53	48	57[d]
Salmonella enteritidis	-52	-10	-9[c]
Escherichia coli 0157:H7	-41	-0	-14[c]
Salmonella typhimurium	-50	-34	-32[c]
Yersinia enterocolitica	+41	-20	+107[b]
Bacillus cereus	-51	-11	-30[c]
Vibrio parahaemolyticus	-16	-21	-6[c]
Listeria monocytogens	-49	-43	-25[c]

Notes:

Δ_{Growth} , %

a = Desirable combined effect = fulfilled both comparisons 1 and 2. There was increased probiotic growth or decreased pathogenic growth due to both: 1) Δ_{Growth} from combined extracts exceeds the sum of Δ_{Growth} from individual extracts, and 2) Δ_{Growth} from combined extracts exceeds the extreme Δ_{Growth} from an individual extract.

b = Undesirable combined effect = did not meet both comparisons 1 and 2. There was decrease of probiotic growth or increase of pathogenic growth due to both: 1) Δ_{Growth} from combined extracts exceeds the sum of Δ_{Growth} from individual extracts, and Δ_{Growth} 2) from combined extracts exceeds the extreme Δ_{Growth} from an individual extract.

c = Growth exceeds the sum of the individual extracts.

d = Growth less than the sum of the individual extracts.

Chapter 4

Discussion

The functional properties of four fruit extracts were investigated for the growth enhancement of probiotic bacteria and growth inhibition of pathogenic bacteria. The results demonstrated the ability of fruit extracts to have direct growth enhancing and growth inhibiting effects on probiotic and pathogenic bacteria, respectively. In general, the four fruit extracts exhibited both growth promoting and growth inhibiting activities to some extent in all microorganisms studies. Results also showed that different bacterial species exhibited different sensitivities to fruit extract.

4.1 Single Extract Effect

4.1.1 Effects of Blueberry Extract and Strawberry Extract on Growth of Probiotic Bacteria

Blueberry extract and strawberry extract at 30 $g.l^{-1}$ was observed, in this study, to exert a significant enhancement of growth in all strains of probiotic bacteria, except for *B. longum*. However, strawberry extract had a biphasic effect on *Bifidobacterium* (Biofarm) and *Bifidobacterium* (Naturalea) isolates and concentrations lower than about 2 $g.l^{-1}$ inhibited growth of these bacteria. Strawberry extract produced better stimulation of probiotic bacteria isolates growth than blueberry. *Bifidobacterium* (Yoplait) indicated the highest increase in growth in the presence of blueberry extract.

The growth-enhancing effect of blueberry extract observed in this study could be attributed to a variety of active compounds present in berries. A similar increase in the growth of lactobacilli and bifidobacteria was observed in other studies (Mullen et al., 2002; Rosendale et al., 2008; Vuorinen et al., 2000). The exact mechanism by which the active compounds increase the growth of probiotic bacteria is not completely understood but the active compounds could either serve as an additional energy source or exert an anti-oxidizing effect. The growth increase observed in lactobacillus and bifidobacterium was attributed to phenolic compounds which are the main constituents in blueberries and strawberries (Mullen et al., 2002; Vuorinen et al., 2000). Molan, Lila,

Mawson, & De (2009) suggest that the phenolic compounds in blueberry extract were utilized by lactobacillus and bifidobacterium for growth.

Other compounds of berries assumed to contribute to the increase in growth of *Lactobacillus hilgardii* are gallic acid and flavonoid (catechin). These compounds not only activated the growth but also increased the bacterial population as this bacterium is able to metabolize these compounds (Alberto et al., 2001). Inulin and fructo-oligosaccharides of berries are other sources which significantly increase the population of bifidobacteria (Gibson et al., 1995).

Typical compounds such as sugars and small proteins and other food-specific phytochemical classes, including phenolics and organic acids present in aqueous extracts of berries have also been reported to positively affect the growth of probiotic microorganisms. Moreover, the growth promoting activity of the functional ingredients of blueberry and strawberry on *Lactobacillus rhamnosus*, *Lactobacillus reuteri* and *Bacillus lactis* has been reported by Sutherland et al. (2009).

Anthocyanins which are pigments present in berries are also claimed to play a role in the stimulation of growth of probiotic bacteria. Anthocyanin pigments such as pelargodin 3-monoglucoside, cyanidin 3-monoglucoside and delphinidin 3-monoglucoside were identified by Pratt et al. (1960) and, when present in strawberries, they actively influenced the growth of *Lactobacillus acidophilus* (Pratt et al., 1960). This result was consistent with that of Werlein, Ku¨temeyer, Schatton, Hubbermann, & Schwarz (2005) who detected an influence of strawberry anthocyanins on the growth rate of *L. acidophilus*. The conclusion was based on the fact that both lactobacilli and bifidobacteria possess enzyme β-glucosidase which can convert the two most common anthocyanins, delphinidin and malvidin glycosides, into other compounds with different bioavailability and bioactivity (Ávila et al., 2009).

Berry fruit extracts can also function as prebiotics, as the complex polysaccharide pectins and pectic-oligosaccharides present, have also been suggested to increase the growth of certain probiotic bacteria. Gibson & Roberfroid (1995) proposed the selective utilization of these rather complex polysaccharides as an energy reserve in the colon for bifidobacterium and lactobacillus. Evidence that carbohydrates stimulated the growth of probiotic bacteria was supported by (Olano-Martin et al., 2002), who studied the

comparison of the *in vitro* bifidogenic properties of pectins and pectic-oligosaccharides. They indicated that pectic olisaccharides have a bifidogenic effect and selected bifidobacteria showed high growth rates on these substrates. This finding was supported by Manderson et al. (2005), who also demonstrated that pectic oligosaccharides from orange peel showed prebiotic properties which increased the bifidobacterial numbers.

The growth enhancing effect of berry extract could also be explained by the antioxidant effect of the extract (Ka¨hko¨nen, Hopia, & Heinonen, 2001; Schotsmans, Molan, & MacKay, 2007) which could modulate the oxidative stress in the medium generated by metabolic activities. Consequently, a more beneficial environment for the growth and multiplication of these bacteria is provided. They observed that when blueberry extract was added to the broth, a substrate for metabolism became available for the bacteria leading to growth enhancement in relation to the controls grown without the addition of the blueberry extract. Hence, they concluded that the growth of bacteria may be exerted by one or more of the above mentioned mechanisms.

An attempt to explain the biphasic effect of berries is taken up later in this section. Similar studies reported that the incorporation of aqueous extracts of strawberry and blueberry at a concentration of about 30 g/L into probiotic preparations has the potential to modify the intestinal microbial profile and thereby increase the number of beneficial bacteria.

4.1.2 Effect of Green Kiwifruit Extract on Growth of Probiotic Bacteria

With the exception of *B. longum*, the dose effect of green kiwifruit extract on the probiotic bacteria was observed to be strain-dependent and biphasic on two dimensions. Significant stimulation of growth by green kiwifruit extract, present on its own, was obtained with all probiotic bacteria at concentrations higher than 3.75 g/L. However, most strains of lactobacilli, at lower concentrations, demonstrated that green kiwifruit extract was inhibitory whilst at higher concentrations the extract was stimulatory. The same effect was observed on *Bifidobacterium* (Yoplait). The biphasic effect on *Bifidobacterium* (Naturalea), *Bifidobacterium* (Biofarm) and *Lactobacillus acidophilus* was at a different dimension. Higher concentrations of green kiwifruit extract on these probiotic bacteria were significantly inhibitory rather than stimulatory.

Only a few studies exist on the effect of green kiwifruit extract on probiotic bacteria. The growth stimulatory property obtained in this study is supported by Molan et al. (2007) who showed the impact of the kiwifruit extract on the growth of three strains of lactic acid bacteria (*Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium breve*). Kiwifruit extract did not significantly affect the growth of these bacteria at low concentrations (0.5-2.5 $mg.ml^{-1}$). However, the addition of 5 $mg.ml^{-1}$ resulted in a significant increase in the number of the above three species of lactic acid bacteria.

The inhibitory concentrations of green kiwifruit extract on probiotic bacteria had not previously been reported, hence, the results of this study are a significant finding. However, the mechanism responsible for the inhibitory effect still remains to be elucidated.

The increase in the growth of probiotic bacteria could be explained by the effect of compounds in green kiwifruit, similar to those present in other fruit, which have been reported to have growth stimulating properties.

The oligosaccharides present in green kiwifruit extract could serve as prebiotic compounds and thus nourish the population of probiotic microorganisms (Bengmark, 1998). The stimulatory property of green kiwifruit extract could also be explained from a study on Zyactinase which is a freeze-dried extract used in kiwifruit digestion relief product (Weir, Peng, Bian, Matharu, & Shu, 2008). Zyactinase contains a protease complex, fibre, pectins and fructo-oligosaccharides. Weir et al. (2008) showed that Zyactinase significantly increased the growth of probiotic bacteria *Lactobacillus reuteri*, *Lactobacillus acidophilus*, *Pediococcus acidilactici*, and *Lactobacillus plantarium*. Furthermore, Molan et al. (2007) obtained a significant increase in the number of lactic acid bacteria (*Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, and *Bifidobacterium breve*) in the presence of 5 $mg.ml^{-1}$ of green kiwifruit extract.

Another factor, to which growth stimulation of probiotic bacteria could be attributed to, is the antioxidant activity present in kiwifruit since it has a high antioxidant content (Szeto et al., 2002). As suggested for berries, the antioxidant activity in green kiwifruit could increase the growth of bacteria by modulating the oxidative stress in the medium generated by the metabolic activity of the bacteria (Ka¨hko¨nen et al., 2001).

Aqueous extract of green kiwifruit contains sugar, some proteins, phytochemicals such as phenolics and organic acids. These compounds showed a significant growth enhancing effect on *L. reuteri* (Sutherland et al., 2009) and *Lactobacillus rhamnosus* (Parkar et al., 2008). In addition to phenolics, coumaric acid, caffeic acid, gallic acid and catechins, green kiwifruit extract has been suggested to have a growth-promoting effect on probiotic bacteria.

Stimulation of growth rates by gallic acid and catechin and an increase in cell density could be related to the ability of certain probiotic bacteria to metabolize phenolic compounds (Alberto et al., 2001). Reguant, Bordons, Arola, & Rozes (2000) studied the influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine and reported that catechin and quercetin are beneficial to *Oenococcus oeni* activity. Their conclusion was based on the fact that phenolic compounds serve as oxygen scavengers and reduce the redox potential of wine. Lowering the oxygen concentration to a certain level could create a favourable environment for the growth of lactic acid bacteria which are microaerophilic. Further evidence was provided by Barthelmebs et al. (2000), who identified that *Lactobacillus plantarum* displays substrate-inducible decarboxylase activities on *p*-coumaric, caffeic, and ferulic acids which could serve as nutrients.

The inhibitory effect of green kiwifruit extract at the high end of the range could be due to the complex composition of the extract. High concentrations of phenolics and organic acids and the interplay between these compounds could exert an inhibitory effect on certain strains of bacteria such as *Bifidobacterium* (Naturalea) and *Bifidobacterium* (Biofarm). The biphasic effect green kiwifruit extract on *Bifidobacterium* (Yoplait) is yet to be identified. The inhibitory effect of green kiwifruit extract could be more predominant than the stimulatory effect in lower concentrations. With an increase in concentration, the inhibition effect could be diluted and thus masked by a stimulatory effect. The significant inhibition of *B. longum* by green kiwifruit extract will be discussed later.

4.1.3 Effect of Feijoa Extract on Growth of Probiotic Bacteria

Results from the study of functional properties of feijoa extract on probiotic bacteria showed a biphasic effect, similar to those observed with green kiwifruit extract.

Significant growth of probiotic bacteria, except for *B. longum*, could be obtained in the presence of feijoa extract at a minimum of 2 $g.l^{-1}$. *L. acidophilus* was inhibited at the highest concentration (30 $g.l^{-1}$) tested. The effect of feijoa extract was biphasic for *Bifidobacterium* (Naturalea) and *L. casei* where lower concentrations were inhibitory and higher concentrations were stimulatory.

Similar studies on the effect of feijoa extract are available but the results of this study on the effect obtained from a fruit usually associated with New Zealand, though less frequently studied than kiwifruit, are a significant finding. The growth stimulation exerted by feijoa extract may be explained when compared with the effect of similar compounds present in other fruit.

Like blueberry extract, phenolic compounds are present in feijoas (Mullen et al., 2002; Weston, 2010), although the effect of the compounds on probiotic bacteria has not yet been investigated. However, it can be speculated from research on blueberry extract by Molan et al. (2009) that the phenolic compounds serve as nutrients, thus increasing the population size of lactobacilli and bifidobacteria.

Similarly, other phenolic compounds such as gallic acids and catechin present in feijoa extract could activate and increase growth of the probiotic bacteria used in this study, as was observed with *Lactobacillus hilgardii* (Alberto et al., 2001). The latter were in agreement with the findings of Hara (1997) where catechins were suggested to exert a growth-stimulating effect.

Flavonols, also present in feijoa extract, are not metabolized by some bacteria but probiotic bacteria and, in particular bifidobacteria, have the ability to utilize compounds not easily metabolized by other bacteria. These probiotic bacteria may utilize flavonoids as an energy source, through the action of glycosidase. Beta-glycoside flavonoids may be hydrolyzed into an aglycone form which is easily absorbed by the bacteria (Laparra, Glahn, & Miller, 2009; Marotti, Bonetti, Biavati, Catizone, & Dinelli, 2007).

Anthocyanins, present in feijoas, could also contribute to the growth of probiotic bacteria as it does with gut flora (Aura et al., 2005). Compounds such as 2-amino-3-carboxy-1,4-naphthoquinone were also reported to play an important role in the growth stimulation of bifidobacterial population in the intestinal microflora (Mori et al., 1997). Kaneko, Mori, Iwata, & Meguro (1994) have also reported similar findings where 2-

amino-3-carboxy-1,4-naphthoquinone exhibited a growth-stimulating effect in all bifidobacteria tested, namely *B. longum*, *B. bifidum*, *B. adolescentis*, and *B. breve*. They also compared conventional growth stimulators such as fructo-oligosaccharies and galacto-oligosaccharides and, in particular, naphthoquinone stimulated growth at an extremely low concentration (0.5 nM). This would suggest that naphthoquinone does not function as a substrate for bifidobacteria (Kaneko et al., 1994; Mori et al., 1997). Based on the above, the stimulation of the growth of probiotic bacteria could be attributed to the presence of napthoquinone in feijoa extract which appears to serve as a growth factor rather than a simple substrate.

4.1.4 Inhibitory Effect of Blueberry and Strawberry Extracts on Pathogenic Bacteria

In this study, both blueberry and strawberry extract exerted growth-suppressing effects on all pathogens at the highest concentration of 30 $g.l^{-1}$. However, a biphasic effect at a lower concentration of the blueberry extract stimulated the growth of *S. enteritidis* and *Y. enterocolitica*. Strawberry exerted a biphasic effect on all pathogenic bacteria.

The inhibitory effect of the blueberry extract could be due to the antimicrobial compounds present in plants which are produced as a natural defence mechanism against microbial infections. Juices and extracts from berries possess antimicrobial properties against Gram-negative and Gram-positive bacteria. Antimicrobial compounds such as peptides, lectins, phenolic compounds, terpenoids, essential oils and various other compounds are potentially involved in this phenomenon (Cowan, 1999). Raw and processed fruits, as well as waste products remaining after processing (peel, seeds, stems, and flesh) are good sources of these ingredients. Moreover, the juices and extracts from berries contain antibacterial activities against Gram-negative and Gram-positive bacteria (Cavanagh et al., 2003; Sagdic et al., 2006; Vattem et al., 2005).

Most of the pathogens challenged by berry extracts in this study were enteric bacteria which are Gram-negative. Similar inhibition by berry extracts was observed with Gram-negative bacteria in contrast to growth enhancement in the Gram-positive *Lactobacillus* species (Puupponen-Pimia¨ et al., 2002).

The difference in the effect of berry extract was possibly related to differences between Gram-negative and Gram-positive bacterial cell surface structures. In particular, the outer membrane of Gram-negative bacteria functions as a preventive barrier against hydrophobic compounds (Helander et al., 1998).

Phenolic compounds in berries such as ellagitannins have been shown to inhibit many human pathogens (Badjakov et al., 2008; Chung, Wong, Wei, Huang, & Lin, 1998; Rauha et al., 2000). Ellagitannins present in strawberries, cloudberries and raspberries was the inhibitory compound against *Salmonella*, *E. coli* CM871 and especially *Typhimurium* (R. Puupponen-Pimia et al., 2001). This acid is the main phenolic compound in the hydrolyzed berry extracts of the genera *Robus* and *Fragaria* (strawberry) (Häkkinen et al., 2000). Ellagic acid is a hydrolysis product from ellagitannins, which, together with gallotannins, form the predominant group of tannins in these berries (Macheix et al., 1990). However, the strawberry extract contained only small amounts of ellagitannins and this may explain the moderate antimicrobial effects against *Salmonella* bacteria (Puupponen-Pimia¨ et al., 2005).

Badjakov et al. (2008) supplied evidence of the antimicrobial effect of phenolic compounds in strawberries, raspberries and crowberries. Flavonoids have also been shown to exert antimicrobial activity (Middleton et al., 2000). The ester linkage between gallic acid and polyols is associated with the inhibitory effects against a variety of food-borne bacteria, such as *E. coli*, *Salmonella enteritidis*, *Salmonella paratyphi* and *Staphylococcus aureus* (Chung, Stevens, Lin, & Wei, 1993).

Another phenolic component in berries found to inhibit Gram-negative pathogens was anthocyanins (R. Puupponen-Pimia et al., 2001). These studies demonstrated the inhibitory action and wide-spectrum effect of phenolic compounds in berries on Gram-negative pathogens such as *Campylobacter*, *Helicobacter*, *Salmonella*, *Escherichia* and on Gram-positive *Staphylococcus, Bacillus,* and *Clostridium*. However, *Listeria* was not inhibited by berry extract in these studies. This is contrary to what was observed in the present study but it could be due to the different varieties of the berry used and also to the particular strain of *Listeria*.

Puupponen-Pimia¨ et al. (2005) proposed that the antimicrobial effect of fruit extract on pathogens could have been influenced by the organic acid content of berries such as citric, malic and benzoic acids (Viljakainen et al., 2002). However, the acid

concentration and the pH of the berry extract can be eliminated as a significant inhibitory effector in this study.

In a separate experiment in the current study (Appendix 2), the pH of the medium decreased slightly after the addition of the fruit extract but, in general, the pH values were above pH 6.0 where organic acids should be present in a salt form such as citrate rather than in an acid form. After the addition of the fruit extract, the pH values of the medium were not inhibitory to any of the bacteria used.

A proposed mechanism of the inhibitory action of phenolic compounds in berries includes destabilization of cytoplasmic membrane, inhibition of microbial enzymes and deprivation of the substrates required for microbial growth. The phenolic compounds may also possess an anti-adherence effect on bacteria, which prevents their adherence to epithelial cells necessary for colonization and infection by many pathogenic bacteria. Complexation of metal ions by tannins (Scalbert, 1991) and inhibition of DNA replication by flavonoids have also been suggested (Cushnie & Lamb, 2005).

4.1.5 Inhibitory Effect of Green Kiwifruit Extract on Pathogenic Bacteria

Green kiwifruit extract demonstrated a biphasic effect on all pathogens. Concentrations, lower than approximately 4 g/L, were growth-stimulatory whilst higher concentrations were growth-inhibitory.

The inhibitory effect of the green kiwifruit extract in this study is similar to those of Weir et al. (2008), where *Escherichia coli* and *Salmonella typhimurium* were significantly inhibited by Zyactinase. Moreover, Molan et al. (2007) demonstrated a strong antimicrobial activity of aqueous green kiwifruit extract against both Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) and Gram-negative (*Salmonella typhymurium* and *Escherichia coli*) pathogens.

The catechin and kaempferol content of green kiwifruit may also contribute to the antimicrobial property of the extract as observed in other studies (Kataoka et al., 2001; Scalbert, 1991).

Inhibition of pathogens in the presence of high concentrations of green kiwifruit extract in this study would suggest that inhibitory compounds such as polyphenols may be present in concentrations high enough to cause inhibition. A synergistic effect between several phenolic compounds could also account for the increased inhibition at these high concentrations. The stimulatory effect at lower concentrations was probably due to polyphenols in low proportions when compared to those of stimulatory compounds. Since polyphenolic compounds are present in green kiwifruit extract, the mechanism of inhibition formulated for berry extract could also be proposed for green kiwifruit extract.

4.1.6 Inhibitory Effect of Feijoa Extract on Pathogenic Bacteria

This study demonstrated that feijoa extract had a unique effect on *B. cereus* and *L. monocytogenes*. The extract exerted biphasic effect on the other pathogens and, in low concentrations (<15 $g.l^{-1}$), was stimulatory but inhibitory in high concentrations (>15 $g.l^{-1}$). *B. cereus* and *L. monocytogenes* were inhibited by all concentrations of fruit extract used in this bioassay.

The antimicrobial effect of feijoa extract could similarly be attributed to the phenolic compounds present in the extract. Flavone, one of the active compounds present in feijoa fruit (*Feijoa sellowiana*), showed a high antimicrobial activity against bacterial strains. *Helicobacter pylori*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and *Proteus vulgaris* especially exhibited a significantly high sensitivity to flavone (Basile et al., 2010).

Pro-anthocyanidins are the other compounds present in feijoas which could explain the inhibitory effect of the extract. Puupponen-Pimia¨ et al. (2002) proposed that the antimicrobial effect of fruit extracts could be due to complex phenolic polymers such as pro-anthocyanidins, ellagitannins and tannins. Vuotto et al. (2000) reported similar results using an aqueous extract of feijoas. Growth suppression in Gram-positive and Gram-negative bacteria was obtained in the study by Vuotto et al. (2000), although the extract was more bactericidal against the Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Enterobacter aerogenes* and *Enterobacter cloacae*.

Another extract of feijoa skin and fruit were also identified as possessing wide-spectrum antimicrobial properties (Basile et al., 1997; Motohashi et al., 2000). A methanol extract of feijoa showed strong activity against Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*; and the Gram-positive organism *Staphylococcus epidemidis*. *Candida albicans* was also found to be inhibited by this extract (Nakashima, 2001).

4.1.7 *Bifidobacterium longum*

Results in this study have shown that all the fruit extracts inhibited *B. longum* and that some apparent increase in growth, at certain concentrations, was negligible and not significant.

In the present study, the response of *B. longum* to the fruit extracts was significantly different to those of other probiotic bacteria where growth stimulation has been observed. The lack of growth in the presence of fruit extract may be explained by low activity of the enzyme β-glucosidase in this species. In the study of Marotti et al. (2007) on β-glucosidase activity of *Bifidobacterium* species, *B. longum* exhibited very low activity of β-glucosidase. This enzyme hydrolyzes β-glucosidic bonds of flavonoids into aglucons, daidzein, genistein, and glycitein (Hur, Jr., Beger, Freeman, & Rafii, 2000). When β-glucosidase activity is low or absent, this bacterium would not be able to utilize the glucosides from flavonoids as an additional carbon source.

This study determined not only the lack of growth of *B. longum* in the presence of a fruit extract but also a significant inhibition by green kiwifruit and blueberry extract. This observation suggests that the effect of the fruit extract on *B. longum* accrues not only from the lack of utilization of their compounds but also the presence of bioactive compounds which were inhibitory. Such an effect of more than one bioactive compound could explain the stimulation of growth when strawberry and green kiwifruit extract are provided to the bacteria. Furthermore, synergistic interactions between bioactive compounds have been put forward by other researchers (Rosendale et al., 2008; Williamson, 2001).

4.2 Combined Fruit Extracts

The analytical comparisons adopted from Rosendale et al. (2008), indicated that combined fruit extracts in the present study elicited a dynamic different to what was obtained from each individual fruit extract present on its own. The combination of blueberry and feijoa extracts did not enhance the growth of all probiotic bacteria and the results were similar to the effect of combined strawberry and green kiwifruit extracts, with the exception of *B. longum*.

The lack of growth-stimulation is the opposite of the significant growth-enhancement properties of the probiotic bacteria, except for *B. longum*, when each extract was present on its own. The most interesting effect on the probiotic bacteria was that of the combined strawberry and green kiwifruit extract on *B. longum*. This combination showed a 'desirable' effect on the growth of *B. longum* in contrast to the significant inhibition by green kiwifruit as a single extract (i.e. on its own). As suggested earlier, the lack of growth in the presence of green kiwifruit extract on its own (as with other individual extracts) may not be due solely to low β-glucosidase activity in this species but also to the actual effect of bioactive compounds. This bioactive effect seems to be strongly demonstrated in these results which would suggest that an interplay between bioactive compounds in green kiwifruit extract and strawberry extract would have occurred.

The exact interaction is difficult to elucidate but may be due to the synergistic effects of bioactive compounds according to Rosendale et al. (2008) and Williamson (2001). Furthermore, a synergistic combination of bioactive compounds may relieve either the inhibition or repression of the enzyme β-glucosidase required to metabolize the sugar fraction of some phenolic compounds. Further investigation of the exact mechanism is required to fully understand the effect of complex bioactive compounds on certain bacteria.

Therefore, the enhanced growth of *B. longum* could be achieved using a combined strawberry and green kiwifruit extract if the objective is to propagate this bacterium in fruit-based substrates. The addition of the combined extracts to probiotic products could contribute to the growth of *B. longum* in the hosts, although the complex metabolic processes inside the digestive tract may complicate the effect on this particular species.

Results also suggest that blueberry extract when combined with feijoa extract might not have any functional effect on probiotic bacteria as used in commercial products and that this combination would not offer any prebiotic function to the probiotic bacteria. Further experiments on what occurs within the different compounds in the extract when the two fruit extracts are combined could help explain the effects observed in this study.

It can only be speculated that the growth stimulatory compound in one extract can be deactivated or masked by the presence of compounds in the other extract. Of greater significance were the results of the two sets of combined extracts on the pathogenic bacteria. All the pathogenic bacteria exhibited increased growth in the presence of the two sets of combined extracts which were blueberry with feijoa, and strawberry with green kiwifruit. The combination of blueberry and feijoa extracts were found to be significantly undesirable if the inhibition of *S. enteritidis*, *E. coli* 0157:H7, *Y. enterocolitica*, *B. cereus*, *V. parahaemolyticus*, *S. typhimurium* and *L. monocytogenes* was the objective.

The combination of strawberry and green kiwifruit extract was significantly undesirable towards *Y. enterocolitica*. These combinations unexpectedly enhanced the growth of the pathogenic bacteria but had significantly strong stimulatory effect towards *Y. enterocolitica.*

These results clearly contradict those obtained when each fruit extract was used as a single extract. When present on its own, the highest concentration of each fruit extract was able to inhibit all pathogenic bacteria. However, in combination, blueberry, feijoa, strawberry and green kiwifruit appeared to have lost their inhibitory effect. The results were not unique to this study, as Rosendale et al. (2008) obtained similar 'undesirable' effects on *S. typhimurium* and *E. coli* from a combined extracts of propolis and broccoli sprouts. As studies on the exact effect of bioactive compounds in fruit and plants are limited, speculation can only be made that interactions between complex bioactive compounds may result in the removal of any inhibitory effects on pathogenic bacteria. Were it not for the proposed mechanism of pathogenic inhibition by fruit extract by Puupponen-Pimia¨ et al. (2005) and Viljakainen et al. (2002), it would be difficult to identify the exact nature of the interaction without further definitive studies.

Likewise, it is not possible to identify whether the effect is synergy or antagonism between compounds may have on the proposed mechanism of pathogen inhibition by

fruit extract. In addition, it is not known if the synergy or antagonism could be affecting the membrane disruption by bioactive compounds or removing the inhibitory effect on some enzymes.

4.3 The Growth Effector in This Study: pH or the Fruit Extract?

Aqueous fruit extracts are known to consist of sugars, polyphenolic compounds, and organic acids which have a relatively low pH value. The effect of each factor on microorganisms is also known. Sugars may be eliminated as a growth effector in this study even if the water activity of the fruit extract has not been determined. If the sugar content of the fruit extract, at the highest concentration of 30 g/L, was the effector, both probiotic and pathogenic bacteria should have been inhibited. However, in this study, each fruit extract was generally inhibitory towards pathogenic bacteria but stimulatory towards probiotic bacteria.

The pH values of the cultures before and after the addition of the fruit extract are shown in Appendix 2. This addition caused a slight decrease in the pH but the pH values in the presence of the fruit extract were higher than pH 6.0 which are known to be non-inhibitory to the bacteria used in the present study. If this pH was the effector, no growth would have been obtained after the incubation for the bioassay. Furthermore, the pH did not drastically decrease as a result of the fermentative properties of the bacteria. Hence, the effect on the growth of the bacteria could not have come from the pH but most probably from the bioactive compounds present in the fruit extract.

4.4 Biphasic Effects

Results from this study showed that most probiotic and pathogenic bacteria tested indicated biphasic growth in the presence of all four fruit extracts. The dual effects of the extract on bacterial growth have not been elucidated in literature. However, according to the nature of compounds, individual compounds perform differently from each other and as well as exerting different effect on various strains of bacteria. For example, the anthocyanin pigment was identified as pelargonidin 3-monoglucoside, cyanidin 3-monoglucoside and delphinidin 3-monoglucoside (Pratt et al., 1960). However, only pelargonidin 3-monoglucoside and delphinidin 3-monoglucoside were identified as inhibiting the growth of *Escherichia coli*, whilst cyanidin 3-monoglucoside

stimulated growth (Werlein et al., 2005). Therefore, with the application of anthocyanins on this bacterium at different concentrations, the dual effects might be observed.

A similar observation has been made in a study of phenolic compounds. Flavonol myricetin demonstrated strong inhibitory effects on the growth of lactic acid bacteria derived from the human gastrointestinal tract, whereas, the flavone luteolin showed bacteriostatic effects against lactic acid bacteria (R. Puupponen-Pimia et al., 2001). Esimone et al. (2002) have proposed that two antimicrobial compounds can interact antagonistically if one is bacteriostatic and the other is bactericidal.

Furthermore, different compounds are active at different concentrations. Hence, there is evidence that the sensitivity of bacteria to the fruit extract tested is dose-and strain-dependent and that some compounds exert growth stimulation but others, such as anthocyanin pigment, is inhibitory. Therefore, one may suggest that either more than one compound is involved in the dual effect or that a single active compound behaves differently at different concentrations.

4.5 Relevance of Results

Information on the growth enhancement of probiotic bacteria in the presence of blueberry, strawberry, green kiwifruit and feijoa extract could be useful in producing high cell concentrations of the probiotic bacteria to fulfil the requirement for effective probiotic products. Although, there is currently no defined standard for commercial probiotic products, a minimum probiotic cell concentration of 10^6 cfu/ml of intestinal fluid is recommended. It is ideal, therefore, to produce a product which contains and maintains higher than the target concentration above. Moreover, some research studies show that the addition of fruit extract as functional ingredients could contribute to increased biomass propagation of probiotic strains.

The addition of fruit extract into the actual product could also contribute to the longevity of the probiotic bacteria in the intestinal tract enabling them to exert a greater health-promoting effect. However, the inhibitory effect of the fruit extract on *B. longum* should also be considered if this species of probiotic strain is incorporated in the product.

Certain undesirable effects such as enhanced growth of pathogenic bacteria from combined fruit extracts may not have any application to cell biomass propagation in the food industry but could be considered with regard to the effect on the balance of microflora in the intestinal tract. Certain combinations of fruit extracts should be avoided when inhibition of certain pathogenic bacteria is desired.

Chapter 5

Conclusion

The study investigated the functional properties and effect of aqueous extracts of blueberry, strawberry, green kiwifruit and feijoa on probiotic and pathogenic bacteria. The main aim was to determine if the four fruit extracts exerted a functional-growth enhancing effect on probiotic bacteria as well as a functional-growth inhibiting effect on pathogenic bacteria.

At the highest concentration of 30 $g.l^{-1}$, strawberry extract and blueberry extract, present on its own, can significantly stimulate the growth of all probiotic bacteria tested, except that of *Bifidobacterium longum*. Both blueberry extract and strawberry extract had the greatest stimulating effect on *Lactobacillus* and *Bifidobacterium* isolates from Yoplait yoghurt. Strawberry extract exerted a growth-enhancing effect on most probiotic bacteria at concentrations higher than 2 $g.l^{-1}$. At concentrations lower than 2 $g.l^{-1}$, strawberry extract had growth-inhibiting effect or weaker growth-stimulating activity depending on species. Hence, for specific purposes, such as the propagation of cell biomass production of probiotic bacteria, strawberry extract could be incorporated at growth-stimulating concentrations.

Blueberry extract did not exert a significant biphasic effect on the probiotic bacteria, although it did enhance the growth of *Bifidobacteria* at a concentration of at least 0.63 $g.l^{-1}$ and that of *Lactobacilli* at approximately 1.25 $g.l^{-1}$.

Green kiwifruit extract is capable of enhancing the growth of the probiotic *Bifidobacteria* and *Lactobacilli*, but not *B. longum*, when present at a concentration higher than 3.75 $g.l^{-1}$. Lower concentrations of green kiwifruit extract could be inhibitory to probiotic bacteria due to its biphasic effect. Hence, to obtain the functional effect towards probiotic bacteria for industrial propagation and conditioning of the host's gut, inhibitory lower concentrations should be avoided.

However, it should be noted that the growth of *L. acidophilus* and *Bifidobacteria* isolates could be inhibited at concentrations of green kiwifruit extract of approximately 30 $g.l^{-1}$. The inhibitory concentration of green kiwifruit extract on probiotic bacteria has

not been investigated elsewhere, so this is a very significant result. Further study; however, should be carried out to identify why green kiwifruit extract exerts different effects on different probiotic species.

Feijoa extract could also enhance the growth of probiotic bacteria, except that of *B. longum*, when present at a minimum concentration of 2 $g.l^{-1}$. At lower than 2 $g.l^{-1}$, the effect of feijoa extract on probiotic bacteria is growth-inhibiting. The growth of *L. acidophilus*, could be inhibited in the presence of feijoa extract concentration higher than 15 $g.l^{-1}$. For any propagation of a probiotic food and administration to a host, which specifically targets *L. acidophilus*, high concentrations of feijoa extract is not ideal.

All four fruit extracts tested are inhibitory on all pathogens including both Gram-positive and Gram-negative bacteria. They all exert a biphasic effect on all pathogens, except for feijoa extract on *Listeria monocytogenes*. With the exception of the latter, low concentrations of feijoa extract has a stimulatory effect on all pathogens. However, at the highest concentration of 30 $g.l^{-1}$, feijoa extract can significantly inhibit all pathogens. On its own, feijoa extract alone can inhibit *L. monocytogenes* at any concentration. This is a very significant finding since feijoa extract provides a new promising functional ingredient in a product specifically designed to target *L. monocytogenes*.

Based on two criteria: 1) dual functional property (i.e. stimulation of probiotic bacteria as well as inhibition of pathogenic bacteria) and 2) significant greatest inhibition of growth of pathogens at 30 $g.l^{-1}$; green kiwifruit extract seems to be the most effective extract when present on its own.

Towards the probiotic bacteria, *B. longum*, the four fruit extracts tested do not have a stimulatory functional effect. When each fruit extract is present on its own, it has an inhibitory effect on the growth of *B. longum*.

The fruit extract appears to lose their beneficial functional effects when used in combination with each other. A combined blueberry and feijoa extract has no stimulatory effect on probiotic bacteria. Neither does the combined strawberry and green kiwifruit extract, with the exception of *B. longum*, where it exerts significant

growth stimulation. Hence, this combination has potential application as a functional ingredient when the aim is specifically to propagate *B. longum*.

The two sets of combined extracts are not functional on pathogens. They exert adverse synergistic effect on the inhibition of pathogenic bacteria. The combined extract of strawberry and green kiwifruit is identified as undesirable for inhibiting *Yersinia enterocolitica*. It has been established that an individual functional ingredient or combined functional ingredients may perform differently in food products and in the host's gut, instead of what is demonstrated in this study which used a single microorganism rather than a mixture of microorganisms.

Complex reactions could also occur between functional ingredients when they are incorporated into food products. Further investigation is required to identify the exact compounds in these fruit extracts which could generate an effective functional effect on probiotic and pathogenic bacteria. Studies using food matrix and the simulation of the human intestinal tract are also needed to verify the growth-promoting and growth-inhibiting activities of fruit extract.

References

Ahmet, C., Sezai, E., & Nihat, T. (2007). Some physical, pomological and nutritional properties of kiwifruit cv.Hayward. *International Journal of Food Sciences and Nutrition, 58*(6), 411-418.

Aiba, Y., Suzuki, N., Kabir, A. M. A., Takagi, A., & Koga, Y. (1998). Lactic acid-mediated suppression of *Helicobacter pylori* by the oral administration of *Lactobacillus salivarius* as a probiotic in a Gnotobiotic Murine Model. *The American Journal of Gastroenterology, 93*(11), 2097-2101.

Alberto, M. a. R., Farı´as, M. E., & Nadra, M. a. C. M. d. (2001). Effect of Gallic Acid and Catechin on *Lactobacillus hilgardii* 5w Growth and Metabolism of Organic Compounds. *Journal of Agricultural and Food Chemistry, 49*(9), 4359-4363.

Andrews, P. J., & Borody, T. J. (1993). Putting back the bugs: bacterial treatment relieves chronic constipation and symptoms of irritable bowel syndrome. *Medical Journal of Australia, 159*(9), 633-634.

Arai, Y., Watanabe, S., Kimira, M., Shimoi, K., Mochizuki, R., & Kinae, N. (2000). Dietary intakes of flavonols, flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *The Jounal of Nutrition, 130*(9), 2243-2250.

Armuzzi, A., Cremonini, F., Bartolozzi, F., Canducci, F., Candelli, M., Ojetti, V., et al. (2001). The effect of oral administration of *Lactobacillus* GG on antibiotic-associated gastrointestinal side-effect during *Helicobacter pylori* eradication therapy. *Alimentary Pharmacology & Therapeutics, 15*(2), 163-169.

Arrigoni, E., Marteau, P., Briet, F., Pochart, P., Rambaud, F.-C., & Messing, B. (1994). Tolerance and absorption of lactose from milk and yogurt during short-bowel syndrome in humans. *American Journal of Clinical Nutrition, 60*(6), 926-929.

Arunachalam, K. D. (1999). Role of *Bifidobacteria* in nutrition, medicine and technology *Nutrition Research (New York, N.Y.), 19*(10), 1559-1597

Arvola, T., Laiho, K., Torkkeli, S., Mykkanen, H., Salminen, S., Maunula, L., et al. (1999). Prophylactic *Lactobacillus* GG reduces antibiotic-associated diarrhea in children with respiratory infections: a randomized study. *Pediatrics (Evanston), 104*(5), e64.

Aura, A.-M., Martin-Lopez, P., O'Leary, K. A., Williamson, G., Oksman-Caldentey, K.-M., Poutanen, K., et al. (2005). In vitro metabolism of anthocyanins by human gut microflora. *European Journal of Nutrition, 44*(3), 133–142.

Ávila, M., Hidalgo, M., Sánchez-Moreno, C., Pelaez, C., Requena, T., & Pascual-Teresa, S. d. (2009). Bioconversion of anthocyanin glycosides by Bifidobacteria and Lactobacillus. *Food Research International, 42*(10), 1453–1461.

Badjakov, I., Nikolova, M., Gevrenova, R., Kondakova, V., Todorovska, E., & Atanassov, A. (2008). Bioactive compounds in small fruits and their influence on human health. *Biotechnology and Biotechnological Equipment, 22*(1), 581-587.

Bailey, M. T., & Coe, C. L. (1999). Maternal separation disrupts the integrity of the intestinal flora in infant rhesus monkeys. *Developmental Psychology, 35*(2), 146-155.

Bailey, M. T., Lubach, G. R., & Coe, C. L. (2004). Prenatal Stress Alters Bacterial Colonization of the Gut in Infant Monkeys. *Journal of Pediatric Gastroenterology and Nutrition, 38*(4), 414-421.

Barthelmebs, L., Divies, C., & Cavin, J.-F. (2000). Knockout of the p-Coumarate Decarboxylase Gene from *Lactobacillus plantarum* Reveals the Existence of Two Other Inducible Enzymatic Activities Involved in Phenolic Acid Metabolism. *Applied and Environmental Microbiology, 66*(8), 3368–3375.

Basile, A., Conte, B., Rigano, D., Senatore, F., & Sorbo, S. (2010). Antimicrobial and antifungal properties of acetonic extract of Feijoa sellowiana fruits and its effect on *Helicobacter Pylori* growth. *Journal of Medicinal Food, 13*(1), 189-195.

Basile, A., Vuotto, M. L., Violante, U., Sorb, S., Martone, G., & Castaldo-Cobianchi, R. (1997). Antibacterial activity in *Actinidia chinensis*, *Feijoa sellowiana* and *Aberia caffra*. *International Journal of Antimicrobial Agents, 8*(3), 199-203.

BCCResearch. (2008). *The Probiotics Market: Ingredients, Supplements, Foods* Retrieved 18 May 2010, from http://www.bccresearch.com/report/FOD035B.html

BCCResearch. (2009). *Food & Beverage World Markets for Fermentation Ingredients* Retrieved 18 May, 2010, from http://www.bccresearch.com/report/FOD020C.html

Beaugerie, L. (2004). Antibiotic-associated diarrhoea. *Best Practice and Clinical Gastroenterology, 18*(2), 337-352.

Bengmark, S. (1998). Immunonutrition: role of biosurfactants, fiber, and probiotic bacteria *Nutrition, 14*(7-8), 585-594

Binder, R. G., & Flath, R. A. (1989). Volatile components of Pineapple guava. *Journal of Agricultural and Food Chemistry, 37*(3), 734-736.

Bloch, A., & Thomson, C. A. (1995). Position of the American Dietetic Association: Phytochemicals and Functional Foods. *Journal of the American Dietetic Association, 95*(4), 493-496.

Bloch, A., & Thomson, C. A. (1997). Position of the American Dietetic Association: Phytochemicals and Functional Foods. *Journal of Dietary Supplements, 1*(1), 33-45.

Block, G., Patterson, B., & Subar, A. (1992). Fruits, vegetables, and cancer prevention: A review of epidemiological evidence. *Nutrition and Cancer, 18*(1), 1-29.

Bomba, A., Jonecová, Z., Koščová, J., Nemcová, R., Gancarčíková, S., Mudroňová, D., et al. (2006). The improvement of probiotics efficacy by synergistically acting components of natural origin: a review. *Biológia, 61*(6), 729-734.

Bourlioux, P., Koletzko, B., Guarner, F., & Braesco, V. (2003). The intestine and its microflora are partners for the protection of the host. *The American Journal of Clinical Nutrition, 78*(4), 675-683.

Bradley, H. K., Wyatt, G. M., Bayliss, C. E., & Hunter, J. O. (1987). Instability in the faecal flora of a patient suffering from food-related irritable bowel syndrome. *Journal of Medical Microbiology, 23*(1), 29-32.

Brigidi, P., Vitali, B., Swennen, E., Bazzocchi, G., & Matteuzzi, D. (2001). Effects of probiotic administration upon the composition and enzymatic activity of human fecal microbiota in patients with irritable bowel syndrome or functional diarrhea. *Research in Microbiology, 152*(8), 735-741

Camilleri, M. (2001). Management of the Irritable Bowel Syndrome *Gastroenterology, 120*(3), 652-668

Cassano, A., Donato, L., Conidi, C., & Drioli, E. (2008). Recovery of bioactive compounds in kiwifruit juice by ultrafiltration. *Innovative Food Science and Emerging Technologies, 9*(4), 556–562.

Cats, A., Kuipers, E. J., Bosschaert, M. A. R., Pot, R. G. J., Vandenbroucke, C. M. J. E., & Kusters, J. G. (2003). Effect of frequent consumption of a *Lactobacillus casei*-containing milk drink in *Helicobacter pylori*-colonized subjects *Alimentary Pharmacology & Therapeutics, 17*(3), 429-435.

Cavanagh, H. M. A., Hipwell, M., & Wilkinson, J. M. (2003). Antibacterial Activity of Berry Fruits Used for Culinary Purposes. *Journal of Medicinal Food, 6*(1), 57-61.

Chan, A. O. O., Leung, G., Tong, T., & Wong, N. Y. (2007). Increasing dietary fiber intake in term of kiwifruit improve constipation in Chinese patients. *World Journal of Gastroenterology, 13*(35), 4771-4775.

Chow, J. (2002). Probiotics and Prebiotics. *Journal of Renal Nutrition, 12*(2), 76-86.

Chu, Y.-F., Sun, J., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry, 50*(23), 6910-6916.

Chung, K.-T., Stevens, S. E., Lin, W. F., & Wei, C.-I. (1993). Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. *Letters in Applied Microbiology, 17*(1), 29-32.

Chung, K.-T., Wong, T. Y., Wei, C.-I., Huang, Y.-W., & Lin, Y. (1998). Tannins and Human Health: A Review. *Critical Reviewsin Food Science and Nutrition, 38*(6), 421-464.

Cowan, M. M. (1999). Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews, 12*(4), 564–582.

Cunningham-Rundles, S., Ahrne, S., Bengmark, S., Johann-Liang, R., Marshall, F., Metakis, L., et al. (2000). Probiotics and immune response. *The American Journal of Gastroenterology, 95*(1), S22-S25.

Cushnie, T. P. T., & Lamb, A. J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents, 26*(5), 343–356.

Dawes, H. M., & Keene, J. B. (1999). Phenolic Composition of Kiwifruit Juice. *Journal of Agricultural and Food Chemistry, 47*(6), 2398–2403.

Day, L., Seymour, R. B., Pitts, K. F., Konczak, I., & Lundin, L. (2009). Incorporation of functional ingredients into foods. *Trends in Food Science & Technology, 20*(9), 388-395.

Donohue, D. C., & Salminen, S. (1996). Safety of probiotic bacteria. *Asia Pacific Journal of Clinical Nutrition, 5*, 25-28.

Duchmann, R., Kaiser, I., Hermann, E., Mayet, W., Ewe, K., & Zum, K.-H. M. (1995). Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease(IBD). *Clinical and Experimental Immunology, 102*(3), 448-455.

Dunne, C., Murphy, L., Flynn, S., O'Mahony, L., O'Halloran, S., Feeney, M., et al. (1999). Probiotics: from myth to reality. Demonstration of functionality in animal models of disease and in human clinical trials. *Antonie van Leeuwenhoek, 76*(1/4), 279–292.

Ernst, E. (2002). The risk–benefit profile of commonly used herbal therapies: Ginkgo, St. John's Wort, Ginseng, Echinacea, Saw Palmetto, and Kava. *Annals of Internal Medicine, 136*(2), 42-53.

Esimone, C. O., Nwafor, S. V., Okoli, C. O., Chah, K. F., Uzuegbu, D. B., Chibundu, C., et al. (2002). In vivo evaluation of interaction between aqueous seed extract of *Garcinia kola* heckel and ciprofloxacin hydrochloride. *American Journal of Therapeutics, 9*(4), 275-280.

FAO/WHO. (2002). *Guidelines for the Evaluation of Probiotics in Food*. London, Ontario, Canada.

Felley, C. P., Corthesy-Theulas, I., Blanco, J.-L., Sipponen, P., Kaufmann, M., Bauerfeind, P., et al. (2001). Favourable effect of an acidified milk (LC-1) on Helicobacter pylori gastritis in man. *European Journal of Gastroenterology & Hepatology, 13*(1), 25-29.

Foo, L. Y., & Porter, L. J. (1981). Structure of tannins of some edible fruits. *Journal of the Science of Food and Agriculture, 32*(7), 711 - 716.

Fukushima, Kawata, Y., Hara, H., Terada, A., & Mitsuoka, T. (1998). Effect of probiotic formula on intestinal immunoglobulin A production in healthy children. *International Journal of Food Microbiology, 42*(1-2), 39-44.

Fukushima, Yamada, A., Endo, T., & Nakano, M. (1998). Effects of a mixture of organisms, *Lactobacillus acidophilus* or *Streptococcus faecalis* on [delta] 6-desaturase activity in the livers of rats fed a fat-and cholesterol-enriched diet. *Basic Nutritional Investigations, 15*(5), 373-378

Fukushima, M., & Nakano, M. (1995). The effect of a probiotic on faecal and liver lipid classes in rats. *British Journal of Nutrition, 73*(5), 701-710.

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

Fuller, A. R. (1992). *Probiotcs - The Scientific Basis*. London: Chapmann and Hall.

Gardiner, G. E., Heinemann, C., Bruce, A. W., Beuerman, D., & Reid, a. G. (2002). Persistence of *Lactobacillus fermentum* RC-14 and *Lactobacillus rhamnosus* GR-1 but Not *L. rhamnosus* GG in the Human Vagina as Demonstrated by Randomly Amplified Polymorphic DNA. *Clinical and Diagnostic Laboratory Immunology, 9*(1), 92-96.

Gibson, G. R., Beatty, E. R., Wang, X., & Cummings, J. H. (1995). Selective stimulation of *bifidobacteria* in the human colon by oligofructose and inulin. *Gastroenterology, 108*(4), 975-982.

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

Gilliland, S. E. (1989). Acidophilus Milk Products: A Review of Potential Benefits to Consumers. *Journal of Dairy Science, 72*(10), 2483-2494.

Gilliland, S. E., & Speck, M. L. (1977). Deconjugation of Bile Acids by Intestinal *Lactobacilli*. *Applied and Environmental Microbiology, 33*(1), 15-18.

Goldin, B. R., & Gorbach, S. L. (1984). The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. *The American Journal of Clinical Nutrition, 39*(5), 756-761.

Goldin, B. R., Gualtieri, L. J., & Moore, R. P. (1996). The effect of *Lactobacillus* GG on the initiation and promotion of DMH-induced intestinal tumors in the rat. *Nutrition and Cancer, 25*(2), 197 – 204.

Greeson, J. M., Sanford, B., & Monti, D. A. (2001). St. John's wort (Hypericum perforatum): a review of the current pharmacological, toxicological, and clinical literature. *Psychopharmacology (Berlin, Germany), 153*(4), 402-414.

Guandalini, S., Pensabene, L., Zikri, M. A., Dias, J. A., Casali, L. G., Hoekstra, H., et al. (2000). Lactobacillus GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. *Journal of Pediatric Gastroenterology and Nutrition, 30*(1), 54-60.

Guarner, F., & Malagelada, J.-R. (1998). Probiotics. *International Journal of Food Microbiology, 39*(3), 237-238.

Guarner, F., & Malagelada, J.-R. (2003). Gut flora in health and disease. *Lancet, 361*(9356), 512-519.

Gurr, M. I. (1987). Nutritional aspects of fermented milk products. *FEMS Microbiology Letters, 46*(3), 337-342

Haderstorfer, B., Psycholgin, D., Whitehead, W. E., & Schuster, M. M. (2008). Intestinal Gas Production from Bacterial Fermentation of Undigested Carbohydrate in Irritable Bowel Syndrome. *The American Journal of Gastroenterology, 84*(4), 375 - 378.

Häkkinen, S. H., Kärenlampi, S. O., Heinonen, I. M., Mykkänen, H. M., & Törrönen, A. R. (1999). Content of the Flavonols Quercetin, Myricetin, and Kaempferol in 25 Edible Berries. *Journal of Agricultural and Food Chemistry, 47*(6), 2274–2279.

Häkkinen, S. H., Kärenlampi, S. O., Mykkänen, H. M., Heinonen, I. M., & Törrönen, A. R. (2000). Ellagic acid contents in berries: Influence of domestic processing and storage. *European Food Researchand Technology, 12*(1), 75-80.

Hammad, E. M. A.-F., Nemer, N. M., & Kawar, N. S. (2000). Efficacy of Chinaberry tree (*Meliaceae*) aquous extracts and certain insecticides against the pea leafminer (Diptera: *Agromyzidae*). *Journal of Agricultural Science, Cambridge, 134*, 413-420.

Hara, Y. (1997). Influence of tea catechins on the digestive tract. *Journal of Cellular Biochemistry, 67*(s27), 52-58.

Harrigan, W. F. (1998). *Laboratory Methods in Food Microbilogy* (3 ed.). London: Academic Press.

Hata, Y., Yamamoto, M., Ohni, M., Nakajima, K., Nakamura, Y., & Takano, T. (1996). A placebo-controlled study of the effect of sour milk on blood pressure in hypertensive subjects. *American Journal of Clinical Nutrition, 64*(5), 767-771.

Havas, S., Heimendinger, J., Reynolds, K., Baranowski, T., Nicklas, T. A., Bishop, D., et al. (1994). 5-a-Day for Better Health: a new research initiative. *Journal of the American Dietetic Association, 94*(1), 32-36.

Hayatsu, H., & Hayatsu, T. (1993). Suppressing effect of *Lactobacillus casei* administration on the urinary muta.genicity arising from ingestion of fried ground beef in the human. *Cancer Letters, 73*(2-3), 173-179.

He, F., Ouwehand, A. C., Isolauri, E., Hosoda, M., Benno, Y., & Salminen, S. (2001). Differences in Composition and Mucosal Adhesion of *Bifidobacteria* Isolated from Healthy Adults and Healthy Seniors. *Current Microbiology, 43*(5), 351-354.

Helander, I. M., Alakomi, H.-L., Latva-Kala, K. s., Mattila-Sandholm, T., Pol, I., Smid, E. J., et al. (1998). Characterization of the Action of Selected Essential Oil Components on Gram-Negative Bacteria. *Journal of Agricultural and Food Chemistry, 46*(9), 3590-3595.

Herias, M. V., Hessle, C., Telemo, E., Midtvedt, T., Hanson, L. A., & Wold, A. E. (1999). Immunomodulatory effects of *Lactobacillus plantarum* colonizing the intestine of gnotobiotic rats. *Clinical and Experimental Immunology, 116*(2), 283-290.

Herrmann, K., & Nagel, C. W. (1989). Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Critical Reviews in Food Science and Nutrition, 28*(4), 315 - 347

Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B., & Kromhout, D. (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. *The Lancet (British edition), 342*(8878), 1007-1011.

Hilliam, M. (1998a). Functional Foods in Europe. *The World of Ingredients*, 45-47.

Hilliam, M. (1998b). The Market for Functional Foods *International Dairy Journal, 8*(5-6), 349-353

Holzapfel, W. H., Haberera, P., Snelb, J., Schillingera, U., & Veld, J. H. J. H. i. t. (1998). Overview of gut flora and probiotics. *International Journal of Food Microbiology, 41*, 85–101.

Hoque, M., Inatsu, B. Y., Juneja, V. K., & Kawamoto, S. (2007). Antibacterial Activity of Guava (*Psidium guajava* L.) and Neem (*Azadirachta indica* A. Juss.) Extracts Against Foodborne Pathogens and Spoilage Bacteria. *Foodborne Pathogens and Disease, 4*(4), 481-488.

Huffnagle, G. B., & Wernick, S. (2007). *The Probiotics Revolution*. New York: Bantam Dell.

Hughes, D. B., & Hoover, D. G. (1995). Viability and Enzymatic Activity of *Bifidobacteria* in Milk. *Journal of Dairy Science, 78*(2), 268-276.

Hur, H.-G., Jr., J. O. L., Beger, R. D., Freeman, J. P., & Rafii, F. (2000). Isolation of human intestinal bacteria metabolizing the natural isoflavone glycosides daidzin and genistin. *Archives of Microbiology, 174*(6), 422-428.

Ishibashi, N., & Shimamura, S. (1993). Bifidobacteria: Research and Development in Japan. *Food Technology, 47*(6), 126-134.

Isolauri, E., Arvola, T., Sutas, Y., Moilanen, E., & Salminen, S. (2000). Probiotics in the management of atopic eczema. *Clinical and Experimental Allergy, 30*(11), 1604-1610.

Jones, D., & Collins, M. D. (1986). Irregular, Nonsporing Gram-Positive Rods. In J. P. Butler (Ed.), *Bergey's Manual of Systematic Bacteriology* (Vol. 2, pp. 1261-1434). Baltimore, MD 21202: Williams and Wilkins.

Jonsson, S., Clausen, E., & Raa, J. (1983). Amino acid degradation by a *Lactobacillus plantarum* strain from fish. *Systematic and Applied Microbiology, 4*(1), 148-154.

Joshipura, K. J., Hu, F. B., Manson, J. E., Stampfer, M. J., Rimm, E. B., Speizer, F. E., et al. (2001). The effect of fruit and vegetable intake on risk for coronary heart disease. *Annals of Internal Medicine, 134*(12), 1106-1114.

Ka¨hko¨nen, M. P., Hopia, A. I., & Heinonen, M. (2001). Berry Phenolics and Their Antioxidant Activity. *Journal of Agricultural and Food Chemistry, 49*(8), 4076-4082.

Kabir, A. M., Aiba, Y., Takagi, A., Kamiya, S., Miwa, T., & Koga, Y. (1997). Prevention of *Helicobacter pylori* infection by Lactobacillus in a Gnotobiotic Murine model. *Gut, 41*(1), 49-55.

Kaila, M., Isolauri, E., Saxelin, M., Arvilommi, H., & Vesikari, T. (1995). Viable versus inactivated *Lactobacillus strain* GG in acute rotavirus diarrhea. *Archieves of Disease in Childhood, 72*(1), 51-53.

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

Kailasapathy, K., & Rybka, S. (1997). *L. acidophilus* and *Bifidobacterium* spp. - their therapeutic potential and survival in yogurt. *Australian Journal of Dairy Technology, 52*(1), 28-35

Kalliomäki, M., Kirjavainen, P., Eerola, E., Kero, P., Salminen, S., & Isolauri, E. (2001). Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *Journal of Allergy and Clinical Immunology, 107*(1), 129-134

Kalliomaki, M., Salminen, S., Arvilommi, H., Kero, P., Koskinen, P., & solauri, E. (2001). Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *The Lancet (British edition), 357*(9262), 1076-1079.

Kandler, O., & Weiss, N. (1986). Regular, Nonsporing Gram-Positive Rods. In J. P. Butler (Ed.), *Bergey's Manual Systematic Bacteriology* (Vol. 2, pp. 1208-1260). Baltimore, MD 21202: Williams and Wilkins.

Kaneko, T., Mori, H., Iwata, M., & Meguro, S. (1994). Growth stimulator for bifidobacteria produced by *Propionibacterium freudenreichii* and several intestinal bacteria. *Journal of Dairy Science, 77*(2), 393-404.

Kataoka, M., Hirata, K., Kunikata, T., Ushio, S., Iwaki, K., Ohashi, K., et al. (2001). Antibacterial action of tryptanthrim and kaempferol from the indigo plant (Polygonum tinctorium Lour), against *Helicobacter pylori*-infected Mongolian gerbils. *Journal of Gastroenterology, 36*(1), 5-9.

Kennedy, R. J., Kirk, S. J., & Gardiner, K. R. (2000). Promotion of a favorable gut flora in inflammatory bowel disease. *Journal of Parenteral and Enteral Nutrition, 24*(3), 189-195.

Khaw, K.-T., & Barrett-Connor, E. (1987). Dietary fiber and reduced iscremic heart disease motality rates in men and women: A 12-year prospective study. *American Journal of Epidemiology, 126*(6), 1093-1102.

Kim, H. S., & Gilliland, S. E. (1983). *Lactobacillus acidophilus* as a Dietary Adjunct for Milk to Aid Lactose Digestion in Humans. *Journal of Dairy Science, 66*(5), 959-966.

King, T. S., & Hunter, J. O. (1998). Abnormal colonic fermentation in irritable bowel syndrome. *The Lancet (British edition), 352*(9135), 1187-1189.

Kirjavainen, P. V., Salminen, S. J., & Isolauri, E. (2003). Probiotic Bacteria in the Management of Atopic Disease: Underscoring the Importance of Viability. *Journal of Pediatric Gastroenterology and Nutrition, 36*(2), 223-227.

Kirschbaum, A. B., Auer, K. v., Krieger, S., Weis, S., Rauh, W., & Hellhammer, D. (2003). Blunted Cortisol Responses to Psychosocial Stress in Asthmatic Children: A General Feature of Atopic Disease? *Psychosomatic Medicine, 65*(5), 806-810.

Klaenhammer, T. R. (2000). Probiotic Bacteria: Today and Tomorrow. *The Journal of Nutrition, 130*, 415S-416S.

Knekt, P., Jarvinen, R., Reunanen, A., & Maatela, J. (1996). Flavonoid intake and coronary mortality in Finland: a cohort study *British Medical Journal (Clinical Research ed.), 312*(7027), 478-481

Knowles, S. R., Nelson, E. A., & Palombo, E. A. (2008). Investigating the role of perceived stress on bacterial flora activity and salivery cortisol secretion: A possible mechanism underlying susceptibility

to illness. *Biological Psychology, 77*(2), 132-137.

Kruger, C. L., & Mann, S. W. (2003). Safety evaluation of functional ingredients. *Food and Chemical Toxicology, 41*(6), 793-805.

Labayen, I., Forga, L., Gonzalez, A., Lenoir-Wijnkoop, I., Nutr, R., & Martinez, J. A. (2001). Relationship between lactose digestion, gastrointestinal transit and symptoms in lactose malabsorbers after dairy consumption. *Alimentary Pharmacology & Therapeutics, 15*(4), 543-549.

Lankaputhra, W. E. V., & Shah, N. P. (1998). Antimutagenic properties of probiotic bacteria and organic acids. *Mutation Research, 397*(2), 169-182.

Laparra, J. M., Glahn, R. P., & Miller, D. D. (2009). Assessing Potential Effects of Inulin and Probiotic Bacteria on Fe Availability from Common Beans (*Phaseolus vulgaris* L.) to Caco-2 Cells. *Journal of Food science, 74*(2), 40-46.

Lee, J.-W., Shin, J.-G., Kim, E. H., Kang, H. E., Yim, I. B., Kim, J. Y., et al. (2004). Immunomodulatory and antitumor effects in vivo by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. *Journal of Veterinary Science, 5*(1), 41–48.

Levy, J. (2000). The effect of antibiotic use on gastrointestinal function. *American Journal of Gastroenterology, 95*(1), S8-S10.

Lidbeck, A., Overvik, E., Rafter, J., Nord, C. E., & Gustafsson, J.-A. (1992). Effect of *Lactobacillus acidophilus* supplements on mutagen excretion in faeces and urine in humans. *Microbial Ecology in Health and Disease, 5*(1), 59-67.

Lin, M.-Y., Savaiano, D., & Harlander, S. (1991). Influence of nonfermented dairy products containing bacterial starter cultures on lactose maldigestion in humans. *Journal of Dairy Science, 74*(1), 87-95.

Liu, R. H. (2004). Potential synergy of phytochemicals in cancer prevention: mechnism of action. *The Jounal of Nutrition, 134*(12), 3479S-3485S.

Lorca, G. L., Wadstrom, T., Valdez, G. F. d., & Ljungh, A. (2001). *Lactobacillus acidophilus* autolysins inhibit *Helicobacter pylori* in vitro. *Current Microbiology, 42*(1), 39-44.

Lourens-Hattingh, A., & Viljoen, B. C. (2001). Yogurt as probiotic carrier food. *International Dairy Journal, 11*(1-2), 1-17.

Macheix, J. J., Fleuriet, A., & Billot, J. (1990). *Fruit Phenolics*. from http://books.google.co.nz/books?hl=en&lr=&id=vHqke7F4lWYC&oi=fnd&pg=PA1&dq=Fruit+

phenolics+Macheix&ots=7Eb4-POXf7&sig=_M3M3ga2fl2eLe21U57G5Xrylbg#v=onepage&q=&f=false

Malina, M., Suomalainen, H., Saxelin, M., & Isolauri, E. (1996). Promotion of IgA Immune Response in Patients with Crohn's Disease by Oral Bacteriotherapy with *Lactobacillus* GG. *Annals of Nutritions and Metabolism, 40*(3), 137-145.

Manderson, K., Pinart, M., Tuohy, K. M., Grace, W. E., Hotchkiss, A. T., Widmer, W., et al. (2005). In vitro determination of prebiotic properties of oligosaccharides derived from an orange juice manufacturing by-product stream. *Applied and Environmental Microbiology, 71*(12), 8383-8389.

Marotti, I., Bonetti, A., Biavati, B., Catizone, P., & Dinelli, G. (2007). Biotransformation of Common Bean (*Phaseolus vulgaris* L.) Flavonoid Glycosides by Bifidobacterium Species from Human Intestinal Origin. *Journal of Agricultural and Food Chemistry, 55*(10), 3913-3919.

Marshall, V. M. (1996). Bioyogurt: how healthy? *Dairy industries international, 61*(1), 28-29.

Marteau, Gerhardt, M. F., Myara, A., Bouvier, E., Trivin, F., & Rambaud, J. C. (1995). *Bifidobacteria* and *Lactobacilli* ingested in fermented dairy products can metabolize bile salts in the human small intestine. *Microbial Ecology in Health and Disease, 8*(151-157).

Marteau, Vrese, M. d., Cellier, C. J., & Schrezenmeir, J. (2001). Protection from gastrointestinal diseases with the use of probiotics. *American Journal of Clinical Nutrition, 73*(2), 430S-436s.

McDonough, F. E., Wong, N. P., Hitchins, A., & Bodwell, C. (1985). Alleviation of lactose malabsortion from sweet acidophilus milk. *The American Journal of Clinical Nutrition, 42*(2), 345-346.

Meisel, H. (1997). Biochemical properties of bioactive peptides derived from milk proteins: potential nutraceuticals for food and pharmaceutical applications. *Livestock Production Science, 50*(1-2), 125-138.

Middleton, E., Kandaswami, C., & Theoharides, T. C. (2000). The Effects of Plant Flavonoids on Mammalian Cells:Implications for Inflammation, Heart Disease, and Cancer. *Pharmacological Reviews, 52*(4), 673–751.

Midolo, P. D., Lambert, J. R., Hull, R., Luo, F., & Grayson, M. L. (1995). In vitro inhibition of *Helicobacter pylori* NCTC 11637 by organic acids and lactic acid bacteria. *Journal of Applied Microbiology, 79*(4), 475-479.

Milner, J. A. (2000). Functional foods: the US perspective. *The American Journal of Clinical Nutrition, 71*(6), 1654S-1659S.

Mital, B. K., & Garg, S. K. (1992). Acidophilus milk products: Manufacture and therapeutic. *Food Review International, 8*(3), 347-389.

Molan, A. L., Kruger, M., De, S., & Drummond, L. (2007). Concurrent Session 1: Functional Foods I: The ability of kiwifruit to positively modulate key markers of gastrointestinal function. *Asia Pacific Journal of Clinical Nutrition, 16*(3), S29-S123.

Molan, A. L., Lila, M. A., Mawson, J., & De, S. (2009). In vitro and in vivo evaluation of the prebiotic activity of water-soluble blueberry extracts. *World Journal of Microbiology and Biotechnology, 25*(7), 1243-1249.

Mori, H., Sato, Y., Taketomo, N., Kamiyama, T., yoshiyama, Y., Meguro, S., et al. (1997). Isolation and Structural Identification of Bifidogenic Growth Stimulator Produced by *Propionibacterium freudenreichii*. *Journal of Dairy Science, 80*(9), 1959–1964.

Motohashi, N., K., M., S., Y., T., S., S., S, S., et al. (2000). Biological activity of feijoa peel extracts. *Anticancer Research, 20*(6B), 4323-4330

Mukai, T., Asasaka, T., Sato, E., Mori, K., Matsumoto, M., & Ohori, H. (2002). Inhibition of binding of *Helicobacter pylori* to the glycolipid receptors by probiotic *Lactobacillus reuteri*. *FEMS Immunology and Medical Microbiology, 32*(2), 105-110.

Mullen, W., Stewart, A. J., Lean, M. E. J., Gardner, P., Duthie, G. G., & Crozier, A. (2002). Effect of Freezing and Storage on the Phenolics, Ellagitannins, Flavonoids, and Antioxidant Capacity of Red Raspberries. *Journal of Agricultural and Food Chemistry, 50*(18), 5197-5201.

Nakamura, Y., Masuda, O., & Takano, T. (1996). Decrease of tissue angiotensin-I-converting enzyme activity upon feeding sour milk in spontaneously hypertensive rats. *Bioscience Biotechnology and Biochemistry, 60*(3), 488-489.

Nakamura, Y., Yamamoto, N., Sakai, K., & Takano, T. (1995). Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme. *Journal of Dairy Science, 78*(6), 1253–1257.

Nakashima, H. (2001). Biological activity of feijoa peel extract. *Kagoshima University Research Center for the Pacific Islands, 34*, 169-175.

Niedzielina, K., Kordeckia, H., & Birkenfeldb, B. (2000). A controlled, double-blind, randomized study on the effcacy of *Lactobacillus plantarum* 299V in patients with irritable bowel syndrome. *European Journal of Gastroenterology & Hepatology, 13*(10), 1143-1147.

O'Sullovan, M. G., Thorton, G., O'Sullivan, G. C., & Collins, J. K. (1992). Probiotic bacteria: Myth or Reality? *Trends in Food Science and Technology, 3*, 309-314.

Oberhelman, R. A., Gilman, R. H., Sheen, P., Taylor, D. N., Black, R. E., Cabrera, L., et al. (1999). A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. *The Journal of Pediatrics, 134*(1), 15-20.

Oberman, H. (1985). Fermented milks. In B. J. B. Wood (Ed.), *Microbiology of Fermented Foods* (pp. 167-196). London: Elsevier Science Publishers.

Oda, M., Hasegawa, H., Komatsu, S., Kambe, M., & Tsuchiya, F. (1983). Agricultural and Biological Chemistry *Agricultural and Biological Chemistry, 47*(7), 1623-1625

Olano-Martin, E., Gibson, G. R., & Rastall, R. A. (2002). Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides. *Journal of Applied Microbiology, 93*(3), 505-511.

Orrhage, K., Brismar, B., & Nord, C. E. (1994). Effect of supplements with *Bifidobacterium longum* and *Lactobacillus acidophilus* on the intestinal microbiota during administration of Clindamicin. *Microbial Ecology in Health and Disease, 7*(1), 17-25.

Orrhage, K., Sillerstrom, E., Gustafsson, J.-A., Nord, C. E., & Rafter, J. (1994). Binding of mutagenic heterocyclic amines by intestinal and lactiacid bacteria. *Mutation Research, 311*(2), 239-248.

Ouwehand, A. C., Isolauri, E., He, F., Hashimoto, H., Benno, Y., & Salminen, S. (2001). Differences in *Bifidobacterium* flora composition in allergic and healthy infants. *Journal of Allergy and Clinical Immunology, 108*(1), 144-145.

Ouwehand, A. C., Salminen, S., & Isolauri, E. (2002). Probiotics: an overview of beneficial effects. *Antonie van Leeuwenhoek, 82*(1-4), 279-289.

Pant, A. R., Graham, S. M., Allen, S. J., Harikul, S., Sabchareon, A., Cuevas, L., et al. (1996). *Lactobacillus* GG and acute diarrhea in young children in the tropics. *Journal of Tropical Petdiatrics, 42*(3), 162-165.

Parkar, S. G., Stevenson, D. E., & Skinner, M. A. (2008). The potential influence of fruit polyphenols on colonic microflora and human gut health. *International Journal of Food Microbiology, 124*(3), 295–298.

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

Perdigon, G., Macias, M. E. d., Alvarez, S., Oliver, G., & Holgado, A. A. d. R. (1986). Effect of perorally administered Lactobacilli on macrophage activation in mice. *Infection and Immunity, 53*(2), 404-410.

Pessi, T., Sütas, Y., Hurme, M., & Isolauri, E. (2000). Interleukin-l0 generation in atopic children following oral *Lactobacillus rhamnosus* GG. *Clinical and Experimental Allergy, 30*(12), 1804-1808.

Peterson, R., Klopfensteiny, T. J., Ericksonz, G. E., Folmer, J., Hinkleyyy, S., Moxleyzz, R. A., et al. (2007). Effect of Lactobacillus acidophilus Strain N P51 on *Escherichia coli* 0157:H7 Fecal Shedding and Finishing Performance in Beef Feedlot Cattle. *Journal of Food Protection, 70*(2), 287-291.

Plein, K., & Hotz, J. (1993). Therapeutic effects of *Saccharomyces boulardii* on mild residual symptoms in a stable phase of Crohn's disease with special respect to chronic diarrhea-a pilot study. *Zeitschrift fur Gastroenterologie, 31*(2), 129-134.

Pothoulakis, C., Kelly, C. P., Joshi, M. A., Gao, N., O'keane, C. J., Castagliuolo, I., et al. (1993). *Saccharomyces boulardii* inhibits *Clostridium difficile* toxin A binding and enterotoxicity in rat ileum. *Gastroenterology (Baltimore), 104*(4), 1108-1115.

Prasad, J., Gill, H., Smart, J., & Gopal, P. K. (1998). Selection and Characterisation of *Lactobacillus* and *Bifidobacterium* Strains for Use as Probiotics. *International Dairy Journal, 8*, 993-1002.

Pratt, D. E., Powers, J. J., & Somaatmadja, D. (1960). Anthocyanins I. The influence of strawberry and grapes anthocyanins on the growth of certain bacteria. *Journal of Food science, 25*(1), 26-32.

Puupponen-Pimia, Nohynek, L., Alakomi, H.-L., & Oksman-Caldentey, K.-M. (2005). The action of berry phenolics against human intestinal pathogens. *BioFactors, 23*, 243–251.

Puupponen-Pimia, R., Nohynek, L., Meier, C., KaÈhkoÈnen, M., Heinonen, M., Hopia, A., et al. (2001). Antimicrobial properties of phenolic compounds from berries. *Journal of Applied Microbiology, 90*(4), 494-507.

Puupponen-Pimia¨, R., Aura, A.-M., Oksman-Caldentey, K.-M., P. Mylla¨ rinen, M. S., Mattila-Sandholm, T., & Poutanen, K. (2002). Development of Functional Ingredients for Gut Health. *Trends in Food Science and Technology, 13*(1), 3-11.

Puupponen-Pimia¨, R., Nohynek, L., Hartmann-Schmidlin, S., Ka¨hko¨nen, M., Heinonen, M., tta¨-Riihinen, K. M. a., et al. (2005). Berry phenolics selectively inhibit the growth of intestinal pathogens. *Journal of Applied Microbiology, 98*(4), 991–1000.

Rauha, J.-P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., et al. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology, 56*(1), 3-12.

Raza, S., Graham, S. M., Allen, S. J., Sultana, S., Trop, L., & Hart, C. A. (1995). Lactobacillus GG promote recovery from acute nonbloody diarrhea in Pakistan. *The Petdiatric infectious Disease Journal, 14*(2), 107-111.

Reguant, C., Bordons, A., Arola, L., & Rozes, N. (2000). Influence of phenolic compounds on the physiology of *ênococcus úni* from wine. *Journal of Applied Microbiology, 88*(6), 1065ÿ1071.

Rimm, E. B., Willett, W. C., Hu, F. B., Sampson, L., Colditz, G. A., Manson, J. E., et al. (1998). Folate and Vitamin B6 From Diet and Supplements in Relation to Risk of Coronary Heart Disease Among Women. *The Journal of the American Medical Association, 279*(5), 359-364.

Robinson, R. K., & Samona, A. (1992). Health aspects of 'bifidus' products: A review. *International Journal of Food Sciences and Nutrition, 43*(3), 175-180

Romero-Rodriguez, M. A., Vazquez-Orderiz, M. L., Lopez-Hernandez, J., & Simal-Lozano, J. (1994). Composition of babaco, feijoa, passionfruit and tamarollo produced in Galicia (North-west Spain). *Food Chemistry, 49*(1), 23-27.

Rosendale, D. I., Maddox, I. S., Miles, M. C., Rodier, M., Skinner, M., & Sutherland, J. (2008). High-throughput microbial bioassays to screen potential New Zealand functional ingredients intended to manage the growth of probiotic and pathogenic gut bacteria. *International Journal of Food Science and Technology 43*(12), 2257-2267.

Rossi, E. A., Giori, G. S. d., Holgado, A. P. d. R., & Valdez, G. F. d. (1994). In vitro effect of *Enterococcus faecium* and *Lactobacillus acidophilus* on cholesterol. *Microbiology Aliments Nutrition, 12*(3), 267-270

Ruzickova, V. (1996). The effect of acidification on the virulent strains of *Salmonella enteriditis* in a defined medium (Abstract). *Journal of Veterinary Medecine, 41*(1), 25-31.

Ryan, T., Wilkinson, J. M., & Cavanagh, H. M. A. (2001). Antibacterial activity of raspberry cordial in vitro. *Research in Veterinary Science, 71*(3), 155-159.

Sagdic, O., Aksoy, A., & Ozkan, G. (2006). Evaluation of the antibacterial and antioxidant potentials of cranberry (gilaburu, Viburnum opulus L.) fruit extract. *Acta Alimentaria (Budapest), 35*(4), 487-492.

Salminen, Isolauri, E., & Onnela, T. (1995). Gut flora in normal and disordered states. *Chemotherapy, 41*(1), 5-15.

Salminen, Isolauri, E., & Salminen, E. (1996). Probiotics and stabilisation of the gut mucosal barrier. *Asia Pacific Journal of Clinical Nutrition, 5*(1), 53-56.

Salminen, & Wright, A. V. (1998). Current Probiotics—Safety Assured? *Microbial Ecology in Health and Disease, 10*, 68–77.

Salminen, E., Elomaa, I., Minkkinen, J., Vapaatalo, H., & Salminen, S. (1988). Preservation of intestinal integrity during radiotherapy using live *Lactobacillus acidophilus* cultures. *Clinical Radiology, 39*(4), 435-437.

Salonen, J. T., Nyyssönen, K., Salonen, R., Lakka, H. M., Kaikkonen, J., Porkkala-Sarataho, E., et al. (2000). Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study: a randomized trial of the effect of vitamins E and C on 3-year progression of carotid atherosclerosis. *Journal of Internal Medicine, 248*(5), 377-386.

Sánchez-Moreno, C., Jiménez-Escrig, A., & Saura-Calixto, F. (2000). Study of low-density lipoprotein oxidizability indexes to measure the antioxidant activity of dietary polyphenols. *Nutrition Research (New York, N.Y.), 20*(7), 941-953.

Sanders, M. E. (1998). Overview of Functional Foods: Emphasis on Probiotic Bacteria. *International Dairy Journal, 8*(5-6), 341-347.

Santis, A. D., Famularo, G., & Simone, C. D. (2000). Probiotics for the hemodynamic alterations of patients with liver cirrhosis. *The American Journal of Gastroenterology 95*(1), 323-324

Sawada, H., Furushiro, M., Hirai, K., Motoike, M., Watanabe, T., & Yokokura, T. (1990). Purification and characterization of an antihypertensive compound from *Lactobacillus casei*. *Agricultural and Biological Chemistry, 54*(12), 3211-3219.

Saxelin, M., Tynkkynen, S., Mattila-Sandholm, T., & Vos, W. M. d. (2005). Probiotic and other functional microbes: from markets to mechanisms. *Current Opinion in Biotechnology, 16*(2), 204-216.

Scalbert, A. (1991). Antimicrobial properties of tannins *Phytochemistry (Oxford), 30*(12), 3875-3883

Schotsmans, W., Molan, A., & MacKay, B. (2007). Controlled atmosphere storage of rabbiteye blueberries enhances postharvest quality aspects. *Postharvest Biology and Technology, 44*(3), 277-285.

Schultz, M., & Sartor, R. B. (2000). Probiotics and inflammatory bowel diseases. *American Journal of Gastroenterology, 95*(1), S19-21

Shah, N. P. (2000). Probiotic Bacteria: Selective Enumeration and Servival in Dairy Products. *Journal of Dairy Science, 83*, 894-907.

Shah, N. P. (2001). Functional Foods from Probiotics and Prebiotics. *Food Technology, 55*(11), 46-53.

Shah, N. P. (2007). Functional cultures and health benefits. *International Dairy Journal, 17*(11), 1262–1277.

Shahani, K. M., Friend, B. A., & Bailey, P. J. (1983). Antitumor activity of fermented colostrum and milk *Journal of Food Protection (USA), 46*(5), 385-386.

Shu, Q., Qu, F., & Gill, H. S. (2001). Probiotic treatment using *Bifidobacterium lactis* HN 019 reduces weanling diarrhea associared with Rotavirus and *Escherichia coli* in a piglet model. *Journal of Pediatric Gastroenterology and Nutrition, 33*(2), 171-177.

Sun-Waterhouse, D., Chen, J., Chuah, C., Wibisono, R., Melton, L. D., Laing, W., et al. (2009). Kiwifruit-based polyphenols and related antioxidants for functional foods: kiwifruit extract-enhanced gluten-free bread. *International Journal of Food Sciences and Nutrition, 60*(7), 251-264.

Sun-Waterhouse, D., Farr, J., Wibisono, R., & Saleh, Z. (2008). Fruit-based functional food I: production of food-grade apple fibre ingredients. *International Journal of Food Science and Technology, 43*, 2113-2122.

Sun, J., Chu, Y.-F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural and Food Chemistry, 50*(25), 7449-7201.

Sutas, Y., Hurme, M., & Isolauri, E. (1997). Oral cow milk challenge abolishes antigen-specific interferon-gamma production in the peripheral blood of children with atopic dermatitis and cow milk allergy *Clinical and Experimental Allergy, 27*(3), 277-283.

Sütas, Y., Soppi, E., Korhonen, H., Syväoja, E.-L., Saxelin, M., Rokka, T., et al. (1996). Suppression of lymphocyte proliferation in vitro by bovine caseins hydrolyzed with *Lactobacillus casei* GG-derived enzymes. *Journal of Allergy and Clinical Immunology, 98*(1), 216-224

Sutherland, J., Miles, M., Hedderley, D., Li, J., Devoy, S., Sutton, K., et al. (2009). *In vitro* effects of food extracts on selected probiotic and pathogenic bacteria *International Journal of Food Sciences and Nutrition*, 1-11.

Svarcova, I., Heinrich, J., & Valentova, K. (2007). Berry fruits as a source of biologically active compounds: The case of *Lonicera caerulea*. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub, 151*(2), 163–174.

Szeto, T. T., Tomlinson, B., & Benzie, I. F. F. (2002). Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation. *Britis Journal of Nutrition, 87*(01), 55-59.

Tamime, A. Y. (Ed.). (2005). *Probiotic Dairy Products* United Kingdom: Blackwell

Tannock, G. W., & Savage, D. C. (1974). Influences of Dietary and Environmental Stress on Microbial Populations in the Murine Gastrointestinal Tract. *Infection and Immunity, 9*(3), 591-598.

Taranto, M. P., Medici, M., Pedigon, G., Holgado, A. P. R., & Vadez, G. F. (1998). Evidence for Hypocholesterolemic Effect of *Lactobacillus reuteri* in Hypercholesterolemic Mice. *Journal of Dairy Science, 81*(9), 2336–2340.

Tasteyre, A., Barc, M.-C., Karjalainen, T., Bourlioux, P., & Collignon, A. (2002). Inhibition of in vitro cell adherence of *Clostridium difficile* by *Saccharomyces boulardii*. *Microbial Pathogenesis, 32*(5), 219-225.

Tavani, A., & Vecchia, C. L. (1995). Fruit and vegetable consumption and cancer risk in the mediterranean population. *The American Journal of Clinical Nutrition, 61*(6), 1374S-1377S.

Toit, M. d., Franza, C. M. A. P., Dicks, L. M. T., Schillinger, U., Haberer, P., Warlies, B., et al. (1998). Characterisation and selection of probiotic *Lactobacilli* for a preliminary minipig feeding trial and their effect on serum cholesterol levels, faeces pH and faeces moisture content. *International Journal of Food Microbiology, 40*(1-2), 93-104.

Trichopoulou, A., Katsuyanni, K., Stuver, S., Tzala, L., Gnardellis, C., Rimm, E., et al. (1995). Consumption of olive oil and specific food groups in relation to breast cancer risk in Greece. *Journal of National Cancer Institute, 87*(2), 110-116.

Tsai, C.-C., Hsih, H.-Y., Chiu, H.-H., Lai, Y.-Y., Liu, J.-H., Yu, B., et al. (2005). Antagonistic activity against Salmonella infection in vitro and in vivo for two Lactobacillus strains from swine and poultry. *International Journal of Food Microbiology, 102*, 185– 194.

Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., Vos, C. H. R. D., et al. (2008). Antioxidants, Phenolic Compounds, and Nutritional Quality of Different Strawberry Genotypes. *Journal of Agricultural and Food Chemistry, 56*(3), 696-704.

Vattem, D. A., Lin, Y.-T., Ghaedian, R., & Shetty, K. (2005). Cranberry synergies for dietary management of *Helicobacter pylori* infections. *Process Biochemistry, 40*(5), 1583-1592.

Venturi, A., Gionchetti, P., Rizzello, F., Johnsson, R., Zucconi, E., Brigidif, P., et al. (1999). Impact on the composition of the faecal flora by a new prohiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Alimentary Pharmacology & Therapeutics, 13*(8), 1103-1108.

Vesa, T. H., Marteau, P., & Korpela, K. (2000). Lactose intolerance. *Journal of the American College of Nutrition, 19*(90002), 165S-175S.

Viljakainen, S., Visti, A., & Laakso, S. (2002). Concentrations of Organis Acids and Soluble Sugars in Juices from Nordic Berries. *Acta Agriculturae Scandinavica Section B, Soil and Plant Science, 52*(2), 101-109.

Viljanen, M., Savilahti, E., Haahtela, T., Juntunen-Backman, K., Korpela, R., Poussa, T., et al. (2005). Probiotics in the treatment of atopic eczema/dermatitis syndrome in infants: a double-blind placebo-controlled trial. *Allergy (Copenhagen), 60*(4), 494-500.

Vrese, M. d., Stegelmann, A., Richter, B., Fenselau, S., Laue, C., & Schrezenmeir, J. (2001). Probiotics—compensation for lactase insufficiency. *American Journal of Clinical Nutrition, 73*(2), 421s-429s.

Vuorinen, H., Määttä, K., & Törrönen, R. (2000). Content of the Flavonols Myricetin, Quercetin, and Kaempferol in Finnish Berry Wines. *Journal of Agricultural and Food Chemistry, 48*(7), 2675–2680.

Vuotto, M. L., Basile, A., Moscatiello, V., Sole, P. D., Castaldo-Cobianchi, R., Laghi, E., et al. (2000). Antimicrobial and antioxidant activities of *Feijoa sellowiana* fruit. *International Journal of Antimicrobial Agents, 13*(3), 197–201.

Wagner, R. D., Warner, T., Roberts, L., Farmer, J., & Balish, E. (1997). Colonization of congenitally immunodeficient mice with probiotic bacteria. *Infection and Immunity, 65*(8), 3345-3351.

Weir, I. E., Peng, R. Y., Bian, M. L., Matharu, K., & Shu, Q. (2008). Zyactinase stimulates the probiotic gut microflora whilst inhibiting pathogenic microflora. *International Journal of Probiotics and Prebiotics, 3*(4), 231-238

Werlein, H.-D., Ku¨temeyer, C., Schatton, G., Hubbermann, E. M., & Schwarz, K. (2005). Influence of elderberry and blackcurrant concentrates on the growth of microorganisms. *Food Control, 16*(8), 729–733.

Weston, R. J. (2010). Bioactive products from fruit of the feijoa (*Feijoa sellowiana*, Myrtaceae): a review. *Food Chemistry, 121*(4), 923-1318

Williamson, E. M. (2001). Synergy and other interactions in phytomedicines. *Phytomedicine, 8*(5), 401–409.

Wittschier, N., Lengsfeld, C., Vorthems, S., Stratmann, U., Ernst, J. F., Verspohl, E. J., et al. (2007). Large molecules as anti-adhesive compounds against pathogens *Journal of Pharmacy and Pharmacology, 59*(6), 777-786.

Wright, C. E., Rees, D. D., & Moncada, S. (1992). Protective and pathological roles of nitric oxide in endotoxin shock *Cardiovascular research, 26*(1), 48-57

Xu, R.-J. (1998). Bioactive peptides in milk and their biological and health implications *Food Reviews International, 14*(1), 1-16.

Younts-Dahl, S. M., GARY, G. D. O., Galyean, M. L., Rivera, J. D., Loneragan, G. H., & Brashears, M. M. (2005). Reduction of Escherichia coli O157 in Finishing Beef Cattle by Various Doses of Lactobacillus acidophilus in Direct-Fed Microbials. *Journal of Food Protection, 68*(1), 6–10.

Ziegler, R. G. (1991). Vegetables, fruits, carotenoids and the risk of cancer. *American Journal of Clinical Nutrition, 53*(1), 251S-259S.

Appendix 1: Data of Δ_{Growth}

Single Ingredient Assays

Table 1 Summary of Δ_{Growth} of blueberry extract on the growth of probiotics bacteria.

	Extract concentration (g/l)																							
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	13.132	16.192	13.693	21.2137	44.897	24.6047	59.849	26.4247	45.2855	34.5425	50.6835	42.0472	84.8955	50.913	61.6135	47.9817	70.5895	52.0577	100.466	49.2405	73.219	32.518	82.5165	*Bifidobacterium* (Y)
	15.132	16.0912	9.9685	20.1005	16.7185	22.3155	15.9985	20.9235	14.151	45.7162	16.228	45.7162	32.904	53.2312	41.311	60.284	44.41	64.1527	42.206	67.005	36.373	69.889	29.256	*Bifidobacterium* (N)
	5.527	-0.876	4.9225	3.8645	5.305	1.201	5.0985	0.4645	6.926	5.4947	6.306	5.8867	9.292	8.451	12.5285	10.422	11.445	10.8442	13.445	12.1622	14.5565	11.723	14.289	*Bifidobacterium* (B)
	-1.651	3.223	-14.049	-1.9755	-20.178	-7.473	-21.54	-6.4382	-25.946	-9.8902	-21.425	-9.3065	-25.694	-9.3605	-21.344	-9.4795	-22.906	-2.5952	-17.186	4.4092	-6.7995	-9.1435	-2.437	*B. longum*
	7.372	12.636	11.39	22.5952	17.8915	24.4097	22.5245	25.614	20.891	41.2477	16.825	64.5485	48.193	81.517	63.291	110.5788	81.2035	151.5255	118.974	171.0703	148.585	168.449	173.8825	*L.* casei (Y)
	-9.750	2.645	-10.700	-0.0195	-1.459	-0.7347	2.962	0.772	-3.5755	2.7522	-2.909	13.7425	4.024	19.0558	25.7155	29.8146	18.2735	40.045	34.1155	61.0783	32.657	59.176	52.9405	*L. reuteri*
	-7.619	-6.9362	-5.6205	-1.456	6.9985	-0.8432	5.1445	7.159	3.0175	1.9272	8.177	22.8495	18.5625	47.5308	35.0915	44.987	33.7315	51.43	34.848	45.6888	46.384	57.1425	21.081	*L. acidophilus*
	-0.35	0.8185	4.8035	3.5345	3.2025	3.0545	5.931	1.9868	1.9165	5.6415	3.0025	12.6133	8.67	16.7733	15.71	21.1653	17.3025	25.1288	20.153	25.691	25.98	24.8105	22.968	*L. plantarum*

Y: Yoplait yoghurt
N: Naturalea yoghurt
B: Biofarm yoghurt

Table 2 Summary of Δ_{Growth} of strawberry extract on the growth of probiotics bacteria.

	Extract concentration (g/l)																							
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	31.8365	25.7563	12.1815	18.1893	11.2465	28.2335	2.1925	20.6028	3.167	39.26	14.5185	53.1333	25.7995	73.4515	70.117	71.7923	81.0715	86.9173	117.47	135.4508	176.6305	174.7655	214.841	*Bifidobacterium* (Y)
	-28.018	-10.190	-12.863	-23.693	-16.434	-18.18	-16.502	-19.555	-25.74	-18.049	-14.125	-6.8452	-0.838	0.4275	0.0185	9.0805	18.2835	30.5708	44.4575	33.4	68.4605	45.709	131.42	*Bifidobacterium* (N)
	2.0225	-1.175	-23.774	-1.1352	-21.583	-7.463	-9.5985	-3.8662	-20.28	5.3818	-12.995	12.1033	6.1865	34.8075	37.142	63.772	72.335	104.5628	131.95	123.6055	188.7345	47.2745	89.1225	*Bifidobacterium* (B)
	-2.7485	-3.325	-5.4593	-4.7945	-9.2435	-6.7935	-7.3735	-7.4752	-9.717	-6.106	-10.643	-4.7887	-9.374	-1.9112	-6.014	0.4598	-7.3985	3.8725	-3.434	7.796	2.504	3.6535	3.7505	*B. longum*
	3.646	3.6098	0.3335	7.758	5.1785	5.8895	12.0425	3.493	4.7785	12.8348	0.1505	36.3076	26.171	51.142	40.566	75.1195	67.4155	114.2643	138.68	168.2643	175.8145	59.486	1.0845	*L.* casei (Y)
	-8.2	-2.8935	-3.8005	-11.566	-12.644	-7.0275	-7.9635	1.3475	-6.441	2.0713	-3.371	15.2908	-0.4325	21.2518	10.3645	43.2595	16.976	54.4108	25.9005	72.4005	43.3775	58.7995	64.2045	*L. reuteri*
	5.6875	3.0327	6.62	5.9455	3.829	0.0268	2.908	1.74	6.6595	0.6718	2.6595	9.3283	18.869	31.3863	26.647	48.4385	33.341	69.1158	51.4235	70.6578	52.6245	68.7065	44.3945	*L. acidophilus*
	3.508	3.462	-2.935	2.0655	-3.522	1.9628	-0.015	0.501	-6.472	9.1608	-1.5655	14.8825	7.176	24.4253	9.7075	34.0463	17.203	45.3238	31.893	52.671	36.5585	53.031	38.365	*L. plantarum*

Table 3 Summary of Δ_{Growth} of green kiwifruit extract on the growth of probiotics bacteria.

	Extract concentration (g/l)																							
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	-6.5465	-1.6895	-7.710	-10.365	-16.997	-13.128	-10.42	-12.298	-22.705	-10.456	-23.68	2.489	-19.59	-6.935	-2.4005	8.494	-8.858	21.5985	4.784	29.4712	28.946	45.5155	46.544	*Bifidobacterium* (Y)
	5.843	8.6193	-6.029	14.738	-7.356	22.3853	6.4735	19.702	-1.989	23.2108	7.1845	35.8838	0.2	49.6032	1.723	59.5825	5.094	42.8735	5.094	52.8473	2.037	43.44	-28.517	*Bifidobacterium* (N)
	10.5955	-1.564	5.616	-4.3487	6.529	-3.2045	8.1895	-4.3922	7.5395	0.7198	4.4345	6.6305	10.3285	11.2765	13.582	11.2765	14.009	24.7745	14.4935	22.176	14.5375	8.6885	9.4125	*Bifidobacterium* (B)
	-9.26	-7.6095	-5.999	-10.645	-16.347	-16.466	-13.90	-19.384	-21.758	-16.862	-22.074	-15.658	-11.905	-10.107	-7.0885	3.3408	-8.35	18.935	0.294	9.0965	10.2445	-8.5855	-11.218	*B. longum*
	-5.2175	-7.398	-8.788	-7.0687	-11.541	-11.318	-9.257	-9.9782	-19.242	-8.0792	-21.397	4.2038	-3.348	9.2338	-0.2495	21.6348	9.0985	30.4458	28.614	43.1383	45.41	55.0985	56.8795	*L.* casei (Y)
	-3.236	-15.931	-1.377	-13.141	4.342	-16.320	-5.489	-18.390	-2.9075	-11.774	5.4905	1.8975	12.63	2.026	26.404	15.061	32.3685	36.0602	23.9125	49.97	60.15	75.7415	73.564	*L. reuteri*
	-16.635	-9.539	-3.074	-9.017	-8.4255	-4.142	-7.316	0.1968	-5.87	12.627	5.683	30.8485	12.532	33.5025	14.904	56.1335	27.2125	51.615	8.523	76.226	9.39	90.3215	-27.217	*L. acidophilus*
	-2.5215	-1.0155	-0.178	-2.0717	-0.3305	-4.8192	3.8055	-7.316	-0.889	-1.864	-2.167	9.161	8.6785	15.1026	14.5745	23.5848	21.85	34.8145	36.0455	36.754	44.4205	42.226	48.0755	*L. plantarum*

Table 4 Summary of Δ_{Growth} of feijoa extract on the growth of probiotics bacteria.

	Extract concentration (g/l)																							
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	43.905	75.8305	54.126	108.6305	36.476	118.8113	53.379	80.821	79.8475	101.9848	45.449	116.3975	37.121	143.0113	71.506	160.807	103.223	225.727	134.0725	220.6943	101.0855	62.8525	59.4445	*Bifidobacterium* (Y)
	-16.43	-6.807	-10.49	-3.1992	-17.702	-2.648	-17.819	-1.1307	-31.025	-6.3832	-24.306	1.08	-3.5795	12.3135	2.4915	24.856	24.298	51.5738	74.731	220.4648	112.247	127.151	121.046	*Bifidobacterium* (N)
	-19.68	-15.796	-0.198	-23.7668	4.705	-26.2575	10.998	-29.385	17.4035	-38.0558	16.61	-30.4095	5.6405	-20.0418	14.172	-12.939	41.6945	13.3	93.962	30.3903	137.9815	32.4585	137.9815	*Bifidobacterium* (B)
	2.3965	-3.6767	-6.814	-11.943	-8.0335	-6.6857	-11.602	-10.356	-16.596	-5.7165	-13.166	-4.2595	-7.215	3.0095	2.621	8.0208	0.2175	12.0712	-1.83	-18.093	-21.917	-48.502	-46.0755	*B. longum*
	-3.395	-1.054	-1.054	-7.9147	-8.6015	-11.783	-14.731	-19.897	-23.569	-17.2163	-32.193	-17.2163	-17.634	-9.0572	-19.763	7.0748	-1.3545	28.9648	23.398	50.51	66.258	97.1655	88.494	*L.* casei (Y)
	8.1835	10.2812	-8.300	12.857	3.454	9.7075	6.8125	17.87	-10.427	8.1455	4.355	32.4368	1.9145	29.384	14.396	58.649	44.1685	111.155	80.1185	127.613	133.1685	170.018	181.695	*L. reuteri*
	6.7675	4.9696	19.957	13.5758	0.5215	5.5122	19.9835	20.4442	0.295	43.9685	28.0495	36.4278	52.0225	69.1112	77.1325	117.367	86.57	132.918	122.4795	99.57	27.852	-7.8225	-32.7475	*L. acidophilus*
	-6.371	-9.1445	1.933	-7.98	1.814	-8.9842	2.764	-11.687	-2.6235	-6.4625	-3.8305	-0.4655	7.461	6.642	12.72	14.4475	20.5005	24.8305	31.257	29.8832	38.173	27.1035	38.2895	*L. plantarum*

Table 5 Summary of Δ_{Growth} of blueberry extract on the growth of pathogenic bacteria.

	Extract concentration (g/l)																						
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	-2.179	-1.613	-2.726	-3.0628	-1.306	-1.4353	-0.072	-3.2318	-0.6355	3.2707	-4.258	5.5405	2.2815	6.9292	5.679	7.4145	4.161	7.5577	0.1335	-1.467	7.5515	-0.751	-0.455	*S. enteritidis*
	-0.045	-0.650	-0.684	-3.8857	-0.977	-1.6095	-2.9645	0.2652	-0.7665	0.913	-0.493	2.1362	0.9155	-0.5897	2.103	3.3415	1.1685	3.6747	-3.3835	-1.7577	-4.124	-7.196	-9.988	*E. coli 0157:H 7*
	5.021	3.9522	2.504	4.4135	2.626	3.8205	3.1325	3.2632	2.671	0.8222	1.909	3.6617	2.389	3.3532	1.983	2.4445	3.557	-2.8442	-9.8955	-22.848	-28.073	-39.83	-44.94	*S. typhimurium*
	18.4425	17.81	5.7855	14.0712	9.5615	14.5767	12.4185	13.6752	10.407	18.711	10.371	15.4082	16.87	13.4815	19.6275	29.4102	19.932	35.5027	22.3795	26.5835	17.0755	17.843	-53.05	*Y. enterocolitica*
	-16.449	-7.336	2.0175	-8.6215	-8.306	-8.3943	0.7645	-13.082	-5.9415	-14.565	-12.50	1.719	-19.27	13.6857	-2.713	-11.756	-3.295	1.3625	-25.076	-25.631	-48.222	-44.61	-68.02	*B. cereus*
	7.5775	7.689	7.7205	10.1632	9.8675	8.3932	10.805	10.056	10.1975	10.8435	9.158	11.0447	12.124	-0.4235	-1.9055	-8.5732	-16.09	-13.915	-12.649	-14.003	-15.222	-20.87	-25.21	*V. parahaemolyticus*
	-1.0735	-3.022	-0.771	-4.1675	-0.889	-4.0107	-1.1235	-5.5652	-2.0175	-4.2405	-2.648	-1.9337	-5.826	-9.0827	-11.583	-17.962	-26.27	-29.704	-36.836	-39.655	-34.819	-44.88	-57.40	*L. monocytogenes*

Table 6 Summary of Δ_{Growth} of strawberry extract on the growth of pathogenic bacteria.

	Extract concentration (g/l)																							
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	1.2015	1.3157	2.467	2.0937	2.357	1.9927	4.0315	1.9642	2.899	6.9462	0.895	10.2372	7.814	12.7682	10.6805	12.3477	8.392	11.163	7.4095	0.6762	-10.859	-24.761	-52.20	*S. enteritidis*
	1.117	1.6232	1.2085	1.8417	0.978	1.8665	1.3165	1.3502	0.541	2.2292	0.809	4.6397	3.0305	5.0615	5.1495	4.5127	3.9975	2.579	2.376	-1.948	-3.7915	-22.839	-41.26	*E. coli 0157:H 7*
	2.292	2.29	1.8155	2.0945	1.7825	1.802	1.5545	1.4542	1.0655	2.2245	1.292	3.8067	3.0465	3.691	3.691	1.2842	0.685	-11.764	-12.106	-37.825	-35.685	-54.444	-61.02	*S. typhimurium*
	13.3015	-1.3132	5.58	15.8915	0	-0.976	6.8695	4.3275	4.464	13.4525	3.571	31.2782	30.1335	53.734	55.803	61.0602	82.8565	85.6483	87.7495	87.0108	86.4015	40.386	41.464	*Y. enterocolitica*
	8.599	5.02	22.057	5.5945	15.2945	-10.422	6.8095	0.0312	-6.102	0.3875	-0.3085	-14.710	8.5015	-17.042	-2.561	-21.017	-33.521	-26.617	-16.53	-40.116	-21.827	-44.47	-51.65	*B. cereus*
	16.0935	15.9945	16.5695	24.8827	26.8745	27.9257	28.2445	29.5352	31.197	38.0282	32.2125	48.0632	43.317	49.4752	41.2805	48.1535	38.811	45.31	42.107	35.299	30.9025	2.473	-16.40	*V. parahaemolyticus*
	-0.708	-2.5622	-4.317	-3.948	-2.2355	-4.0632	-0.4945	-4.9252	-0.802	-4.6762	0.828	-0.4767	9.385	-2.4332	11.2415	-15.752	4.626	-28.782	-15.150	-43.348	-40.578	-49.303	-42.11	*L. monocytogenes*

Table 7 Summary of Δ_{Growth} of green kiwifruit extract on the growth of pathogenic bacteria.

	Extract concentration (g/l)																							
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	-0.566	0.3417	2.9645	1.2887	4.2285	1.8585	7.5325	1.9267	6.5805	4.865	7.6275	8.726	13.0975	10.837	18.7975	12.5215	16.396	12.222	15.3995	11.3347	11.099	0.313	-10.338	*S. enteritidis*
	1.165	1.232	1.164	2.0707	1.4685	2.347	2.5525	2.292	2.6285	3.4302	2.82	5.9087	5.1575	6.9717	7.1275	7.6642	7.092	8.9027	7.124	7.8432	6.7135	4.595	-0.1335	*E. coli 0157:H 7*
	1.68	1.54	0.397	1.3372	-0.287	1.6777	0.3895	2.151	-0.406	1.4035	-0.198	2.6257	0.7445	2.9242	1.295	1.8057	-1.703	-0.4845	-12.182	-12.350	-24.266	-24.695	-34.539	*S. typhimurium*
	2.221	2.6627	1.2385	2.587	-1.310	3.077	-0.698	3.3155	0.3605	4.101	0.2885	7.5572	2.519	9.735	7.725	11.1275	3.4365	8.545	0.294	0.9375	-10.491	-15.992	-20.159	*Y. enterocolitica*
	3.424	6.3462	2.895	6.4545	6.722	7.5362	4.993	6.5465	7.601	10.0467	1.611	15.674	1.777	18.8547	4.765	18.3827	9.983	22.2207	8.8225	21.0352	14.3765	11.0905	-11.303	*B. cereus*
	0.357	4.7912	2.746	6.8237	8.4705	5.6955	11.76	5.1087	10.811	4.2442	8.3805	4.356	7.882	6.0252	9.4515	6.0817	-2.632	-4.5417	-9.9425	-13.185	-10.523	-15.767	-21.358	*V. parahaemolyticus*
	-1.111	-3.797	-0.254	-3.983	-1.968	-3.33	-1.064	-4.697	-1.901	-1.5277	-4.296	0.7297	-3.8585	-1.5467	-3.148	-3.5637	0.0645	-0.0955	6.9955	3.4037	-6.606	0.9505	-43.82	*L. monocytogenes*

Table 8 Summary of Δ_{Growth} of feijoa extract on the growth of pathogenic bacteria.

	Extract concentration (g/l)																						
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	1.9775	3.152	2.7835	5.0957	3.105	6.4302	5.186	6.1835	5.795	8.874	5.8135	12.5887	10.421	13.9535	12.2565	14.344	11.634	14.676	9.1975	6.3365	1.442	-8.6145	-22.516	*S. enteritidis*
	0.742	1.542	0.532	2.5917	1.057	3.3317	5.7975	3.3552	2.149	5.175	2.818	8.3787	6.867	9.9255	9.932	10.6327	11.0585	13.23	11.862	8.3667	11.22	-6.9065	-7.706	*E. coli 0157:H 7*
	0.6175	-0.1405	0.5465	-0.8025	-0.576	-0.8552	-0.539	-0.8202	-0.682	0.3787	0.686	1.9597	3.0645	3.3097	5.2625	2.6855	0.7845	-5.61	-11.660	-16.358	-23.32	-29.942	-37.78	*S. typhimurium*
	2.319	11.2232	12.6325	-9.3007	5.3085	-12.426	6.5095	-12.689	23.961	-3.984	4.2315	27.041	18.52	4.713	12.954	39.7247	13.356	42.7952	11.6185	13.0227	3.6325	8.9265	-9.3945	*Y. enterocolitica*
	5.6605	10.1157	2.76	-5.5582	3.687	-6.8727	-12.564	-8.0382	2.904	-9.051	10.956	-0.1122	7.4505	-0.5442	-2.442	0.3182	6.481	-2.998	-2.0075	-9.7312	-12.73	-18.891	-15.605	*B. cereus*
	9.108	12.315	12.0805	17.7522	14.9055	20.1832	18.7425	19.8172	17.9345	26.377	18.272	32.4512	24.2965	35.4	28.21145	37.2677	27.451	38.8912	24.6575	27.0557	9.87	-4.5915	-40.19	*V. parahaemolyticus*
	-7.064	-7.186	-4.821	-7.949	-7.236	-7.287	-7.529	-8.616	-8.746	-6.698	-9.265	-2.5112	-7.4335	-3.549	-8.3765	-2.6815	-12.540	-10.455	-5.012	-4	-3.075	-12.953	-22.064	*L. monocytogenes*

Combined Ingredient Assays

Table 9 Summary of Δ_{Growth} of combined blueberry and feijoa extract on the growth of probiotics bacteria.

	Extract concentration (g/l)																						
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	-5.0325	-10.690	-10.539	-13.911	-8.525	-15.475	-24.957	-19.263	-18.82	-18.167	-24.855	-11.037	-19.091	-8.786	-16.743	3.71	5.7935	10.385	10.0895	41.429	41.218	43.041	37.753	*Bifidobacterium* (Y)
	-8.495	1.3908	15	7.5272	16.178	-2.6275	18.4615	-5.6878	18.961	-0.849	11.8235	6.9687	23.759	16.8465	39.398	26.5755	61.168	38.1482	46.1485	36.0802	52.773	24.6425	30.8195	*Bifidobacterium* (N)
	8.2995	2.7552	-1.904	5.665	-2.389	3.083	1.3815	2.6612	-3.575	7.95	-2.3925	11.771	2.064	18.5142	9.094	22.141	9.487	34.5465	16.1815	33.753	14.59	14.416	1.9015	*Bifidobacterium* (B)
	-0.711	-4.9842	-0.8885	-6.001	-3.564	-9.7822	-7.3925	-11.543	-6.781	-1.307	-14.011	-8.431	-10.904	-3.407	-6.279	3.381	1.326	8.0835	6.835	-8.0722	-4.5315	-28.572	-30.262	*B. longum*
	1.8575	7.5017	0.5795	5.9845	4.542	2.7377	10.0955	2.3172	10.55	1.527	3.661	16.8465	20.957	14.959	22.3525	29.3005	30.2685	45.6482	41.1515	55.5115	47.803	54.0855	32.356	*L.* casei (Y)
	-12.18	-17.792	-25.082	-26.060	-36.29	-28.447	-39.722	-29.689	-50.84	-30.837	-45.528	-25.804	-45.466	-21.715	-39.885	-12.062	-34.937	5.5505	-8.0585	44.967	30.9815	89.011	65.3425	*L. reuteri*
	-10.540	-12.359	-2.7945	-27.722	-0.803	-23.144	3.427	-31.392	3.021	-21.974	4.5955	-18.125	11.9825	-8.432	17.3	2.1857	27.3175	27.5502	34.6115	45.5087	36.1765	18.6	22.905	*L. acidophilus*
	-0.3455	-1.4742	1.135	0.457	3.337	-1.1552	12.9545	-2.1852	1.491	2.8525	1.228	8.1782	12.619	13.7642	18.155	12.599	23.5525	26.579	29.8595	31.7202	38.1195	30.408	39.905	*L. plantarum*

Table 10 Summary of Δ_{Growth} of combined strawberry and green kiwifruit extract on the growth of probiotics bacteria.

	Extract concentration (g/l)																						
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}		-9.411	-21.987	-18.010	-29.562	-37.838	-36.441	-37.752	-30.227	-43.215	-30.685	-48.437	-24.634	-35.177	-8.069	-34.244	7.6205	-18.226	30.0292	9.071	57.4552	40.036	74.367	*Bifidobacterium* (Y)
		1.986	-4.2087	-2.976	0.0145	1.2415	-3.6225	-5.4825	-0.2182	0.8675	-4.562	4.759	4.3492	8.7635	5.3595	12.8305	9.866	12.198	10.5955	19.986	13.774	22.819	12.4055	*Bifidobacterium* (N)
		2.088	3.66	4.797	8.4902	12.43	10.6182	15.04	9.4502	14.1345	13.9335	12.9795	24.2817	27.02	31.5595	37.508	32.8457	39.976	36.079	38.2125	30.362	31.8135	29.0685	*Bifidobacterium* (B)
		1.6125	-1.345	-1.122	-2.625	-0.859	-0.0487	-1.0455	-0.8447	-1.007	-1.4997	0.4065	3.303	5.3415	4.3572	10.4545	14.1225	10.475	20.5232	19.302	-4.9812	20.9675	-45.241	*B. longum*
		-3.123	-3.1092	1.977	0.3567	7.2445	-0.5782	12.631	-2.184	10.0135	6.8632	7.75	18.5467	19.548	30.5872	27.434	38.1352	32.2445	55.658	46.409	66.0762	58.049	70.2255	*L.* casei (Y)
		-20.37	-17.509	-3.639	-26.616	-12.525	-25.674	-18.382	-26.609	-26.356	-19.383	-25.392	-16.443	-18.295	-3.9755	-6.1845	8.0832	3.253	33.3982	30.4485	44.9915	52.7995	63.3135	*L. reuteri*
		-10.10	1.1815	0.1805	-3.1417	-9.375	-1.925	5.0145	-2.2625	8.09	3.7495	-1.4115	15.998	26.5835	23.731	36.6455	32.3267	31.9895	46.7535	46.7175	52.5615	50.3125	44.5355	*L. acidophilus*
		-1.743	-0.8177	5.3565	-2.0645	3.106	-2.47	6.014	-4.245	3.645	3.8257	0.5295	8.346	12.1105	13.4847	21.008	21.4982	31.8265	30.5177	44.7335	38.1137	56.872	44.1325	*L. plantarum*

Table 11 Summary of Δ_{Growth} of combined blueberry and feijoa extract on the growth of pathogenic bacteria.

	Extract concentration (g/l)																							
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	-0.045	2.957	2.8955	5.7595	3.9825	6.5945	6.5085	6.5517	5.8605	9.548	6.9825	14.1775	11.7235	17.23	14.9005	16.6737	15.7675	19.4605	15.2975	22.7375	12.9935	3.9205	-6.84	*S. enteritidis*
	-0.362	1.0362	2.2815	2.4147	3.1125	3.1287	5.0345	2.9195	4.2015	5.4405	4.8885	8.6332	9.381	10.5415	11.9925	12.689	13.2255	15.3647	15.144	16.1655	18.261	8.872	6.2715	*E. coli 0157:H 7*
	-2.4885	-2.117	-2.054	-2.065	-2.415	-2.1575	-2.200	-1.840	-2.2	-1.1442	-1.5515	-0.1125	-0.326	0.5037	0.5385	0.3417	-4.0775	-6.765	-16.505	-18.687	-25.948	-28.612	-30.17	*S. typhimurium*
	9.2895	3.5317	8.4765	3.5167	10.724	0.026	5.1485	5.5192	2.851	2.2832	-1.324	7.7712	13.1555	15.49	18.354	19.416	22.8065	27.8185	31.4615	25.3435	56.5775	30.733	52.2495	*Y. enterocolitica*
	0.737	-0.601	2.189	-3.32	-4.535	-5.9625	-1.566	-6.281	-3.996	-5.2345	-2.694	0.5107	6.386	-0.4157	7.536	3.4645	2.097	5.447	10.8315	16.5392	-3.487	-12.921	-22.236	*B. cereus*
	10.451	12.199	12.742	18.0975	18.908	19.9577	25.15	19.167	23.037	25.509	29.4895	35.122	38.0665	42.2442	44.2225	45.8637	43.13	48.8642	54.7555	50.8025	54.401	35.947	31.0315	*V. parahaemolyticus*
	15.6035	15.987	3.544	17.3532	3.822	15.6237	6.1775	15.655	5.5905	22.2492	5.104	34.2385	19.951	40.4485	30.815	51.9722	38.0775	62.5627	43.27	57.9785	36.923	45.645	17.5125	*L. monocytogenes*

Table 12 Summary of Δ_{Growth} of combined strawberry and green kiwifruit extract on the growth of pathogenic bacteria.

	Extract concentration (g/l)																						
	0.01	**0.02**	**0.03**	**0.04**	**0.06**	**0.08**	**0.12**	**0.16**	**0.24**	**0.32**	**0.47**	**0.63**	**0.94**	**1.25**	**1.88**	**2.5**	**3.75**	**5**	**7.5**	**10**	**15**	**20**	**30**	
Δ_{Growth}	2.5945	2.3315	5.3545	4.8745	7.554	5.3945	9.795	6.0322	8.4765	9.35	9.513	14.8397	15.4445	16.6422	20.4905	17.5365	17.692	20.291	14.512	14.7917	8.4015	5.275	-9.0325	*S. enteritidis*
	1.821	1.9347	0.7535	3.405	1.8265	4.2977	3.038	3.9057	2.223	7.0142	-0.2095	10.511	-1.8005	12.521	-1.312	15.4855	-0.304	18.2712	1.4355	17.9935	1.147	10.2635	-14.571	*E. coli 0157:H 7*
	-0.4355	-0.9635	-0.634	-1.695	-0.949	-0.887	-0.6385	-1.032	-0.132	-0.526	-0.0995	0.5942	1.183	1.648	1.798	0.5582	-5.571	-10.067	-17.396	-19.287	-22.8305	-25.502	-32.131	*S. typhimurium*
	9.6595	15.274	18.4935	15.0292	27.087	13.4805	25.8135	12.2107	27.743	15.8327	21.0485	25.1007	34.76	30.4835	42.55	45.0775	60.7645	67.629	92.195	93.049	135.9465	108.363	107.592	*Y. enterocolitica*
	15.2545	-16.948	-11.637	-5.6495	-26.13	-10.685	-18.748	-10.525	-15.24	-0.2277	-12.644	8.3437	-7.6675	14.1912	-14.101	5.5882	-18.554	7.7767	-7.7565	-1.4192	-16.504	-17.057	-30.016	*B. cereus*
	11.077	11.3937	5.9615	16.725	4.602	17.8407	8.2795	18.4275	11.046	25.1452	8.3745	34.2417	14.5225	37.604	19.3085	40.614	18.1065	42.7015	16.6975	39.407	8.244	22.234	-6.5465	*V. parahaemolyticus*
	-0.0165	1.1457	1.755	4.1392	3.731	7.5885	4.0825	7.5885	1.4525	14.256	-4.222	21.998	-4.82	24.9962	-2.1255	26.8357	-2.902	27.052	-0.517	7.247	-1.8915	-11.698	-25.275	*L. monocytogenes*

Appendix 2: pH Results

Probiotic Bacteria - Single Assay

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
Bifidobacterium (Yoplait)	6.83	6.11	7.50	7.10
	Strawberry			
	6.45	6.25		
	Kiwifruit			
	6.85	6.14		
	Feijoa			
	6.83	6.22		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
Bifidobacterium (Naturalea)	7.03	6.40	7.46	6.65
	Strawberry			
	6.48	6.27		
	Kiwifruit			
	6.86	6.32		
	Feijoa			
	6.82	6.39		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
Bifidobacterium (Biofarm)	6.71	6.42	7.45	7.15
	Strawberry			
	6.83	6.52		
	Kiwifruit			
	6.82	6.48		
	Feijoa			
	6.35	6.28		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
B. longum	7.01	6.00	7.43	6.34
	Strawberry			
	6.89	6.00		
	Kiwifruit			
	6.90	5.50		
	Feijoa			
	6.87	5.93		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
L. casei (Yoplait)	7.04	6.72	7.49	7.00
	Strawberry			
	6.84	6.65		
	Kiwifruit			
	6.44	6.31		
	Feijoa			
	6.56	6.34		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
L. reuteri	7.06	6.52	7.47	7.08
	Strawberry			
	6.97	6.44		
	Kiwifruit			
	6.81	6.38		
	Feijoa			
	6.75	6.38		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
L. acidophilus	6.86	5.07	7.43	6.51
	Strawberry			
	6.65	5.08		
	Kiwifruit			
	6.93	5.11		
	Feijoa			
	6.82	5.1		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
L. plantarum	6.88	5.06	7.07	6.67
	Strawberry			
	6.92	6.01		
	Kiwifruit			
	6.82	5.58		
	Feijoa			
	6.78	6.02		

Probiotic Bacteria - Combined Assay

	pH			
	T0	T16	T0	T16
	Blueberry + Feijoa		Control	
Bifidobacterium (Yoplait)	7.08	6.38	7.45	7.08
	Strawberry + Kiwifruit			
	6.99	6.31		

	pH			
	T0	T16	T0	T16
	Blueberry + Feijoa		Control	
Bifidobacterium (Naturalea)	7.01	6.75	7.44	6.67
	Strawberry + Kiwifruit			
	6.57	6.48		

	pH			
	T0	T16	T0	T16
	Blueberry + Feijoa		Control	
Bifidobacterium (Biofarm)	7.01	6.65	7.43	7.16
	Strawberry + Kiwifruit			
	6.75	6.42		

	pH			
B. longum	T0	T16	T0	T16
	Blueberry + Feijoa		Control	
	7.03	5.98	7.49	6.87
	Strawberry + Kiwifruit			
	6.86	5.73		

	pH			
L. casei (Yoplait)	T0	T16	T0	T16
	Blueberry + Feijoa		Control	
	6.90	5.48	7.51	7.05
	Strawberry + Kiwifruit			
	6.78	5.48		

	pH			
L. reuteri	T0	T16	T0	T16
	Blueberry + Feijoa		Control	
	6.85	5.75	7.49	7.09
	Strawberry + Kiwifruit			
	6.76	5.70		

	pH			
L. acidophilus	T0	T16	T0	T16
	Blueberry + Feijoa		Control	
	7.01	5.69	7.45	6.61
	Strawberry + Kiwifruit			
	6.94	5.68		

	pH			
L. plantarum	T0	T16	T0	T16
	Blueberry + Feijoa		Control	
	6.98	5.46	7.47	7.09
	Strawberry + Kiwifruit			
	6.77	5.52		

Pathogenic Bacteria - Single Assay

	pH			
S. enteritidis	T0	T16	T0	T16
	Blueberry		Control	
	7.66	5.55	7.99	6.66
	Strawberry			
	6.19	5.78		
	Kiwifruit			
	6.26	5.62		
	Feijoa			
	6.21	5.64		

	pH			
E. coli 0157:H7	T0	T16	T0	T16
	Blueberry		Control	
	7.16	5.62	7.98	6.68
	Strawberry			
	6.20	5.74		
	Kiwifruit			
	6.19	5.63		
	Feijoa			
	6.19	5.69		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
S. typhimurium	7.16	5.59	7.99	6.49
	Strawberry			
	6.31	6.03		
	Kiwifruit			
	6.18	5.79		
	Feijoa			
	6.20	5.79		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
Y. enterocolitica	7.16	6.69	7.96	7.61
	Strawberry			
	6.78	6.71		
	Kiwifruit			
	6.22	6.15		
	Feijoa			
	6.20	6.17		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
B. cereus	7.18	5.59	7.96	6.38
	Strawberry			
	6.30	6.22		
	Kiwifruit			
	6.20	6.11		
	Feijoa			
	6.74	5.77		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
Vibrio parahemolyticus	7.17	6.48	7.95	6.48
	Strawberry			
	6.98	6.09		
	Kiwifruit			
	6.25	6.12		
	Feijoa			
	6.32	6.21		

	pH			
	T0	T16	T0	T16
	Blueberry		Control	
L. monocytogenes	7.18	5.97	7.92	6.44
	Strawberry			
	7.35	6.29		
	Kiwifruit			
	6.21	6.17		
	Feijoa			
	6.22	6.15		

Pathogenic Bacteria - Combined Assay

	pH			
	T0	T16	T0	T16
S. enteritidis	Blueberry + Feijoa		Control	
	6.62	5.53	7.93	6.49
	Strawberry + Kiwifruit			
	6.28	5.90		

	pH			
	T0	T16	T0	T16
E. coli 0157:H7	Blueberry + Feijoa		Control	
	6.62	5.57	7.99	6.38
	Strawberry + Kiwifruit			
	6.21	6.08		

	pH			
	T0	T16	T0	T16
S. typhimurium	Blueberry + Feijoa		Control	
	6.61	5.66	7.98	6.46
	Strawberry + Kiwifruit			
	6.26	5.91		

	pH			
	T0	T16	T0	T16
Y. enterocolitica	Blueberry + Feijoa		Control	
	7.50	7.04	7.98	7.87
	Strawberry + Kiwifruit			
	7.34	6.13		

	pH			
	T0	T16	T0	T16
B. cereus	Blueberry + Feijoa		Control	
	6.58	5.79	7.97	6.40
	Strawberry + Kiwifruit			
	6.26	6.22		

	pH			
	T0	T16	T0	T16
Vibrio parahaemolyticus	Blueberry + Feijoa		Control	
	6.59	6.48	7.98	7.55
	Strawberry + Kiwifruit			
	6.57	6.42		

	pH			
	T0	T16	T0	T16
L. monocytogenes	Blueberry + Feijoa		Control	
	6.60	5.84	7.98	6.27
	Strawberry + Kiwifruit			
	6.27	6.21		