Chapter 2

Literature review

2.1 Beans

Beans are one of the most inexpensive sources of energy, packed with nutritional benefits. Consumers are becoming aware of these benefits through greater access to nutrition knowledge and generally have a better understanding of ways to reduce health risks, prevent illness and improve quality of life. Beans represent one of the richest available sources of vegetable protein in the food supply, both colored and white beans are low in calories, cholesterol, fat and sodium, and high in iron, making them an excellent protein source and a great meat alternative. High levels of soluble and insoluble fiber in beans both aid in the prevention of diseases such as cancer, kidney disease, liver disease and cardiovascular disease, and help to control diabetes, obesity and intestinal disorders. As a rich source of B-vitamins, calcium, iron, phosphorous, potassium and zinc, beans offer more essential nutrients than many other food sources (Anonymous, 2007a).

Beans are also an excellent source of folate (folic acid), a B-vitamin, which is essential during pregnancy. Beans may also act as a healthy appetite suppressant. They digest slowly, causing a low, sustained increase in blood sugar, delaying hunger. They are quickly being recognized as a natural alternative to enhance weight-loss

programs. The other major factor in the growing popularity of beans is increasing multiculturalism in countries around the world. All pulses, but especially beans, are being sampled by consumers in countries where they have not been a traditional staple food. This has led to new bean recipes and a growth in restaurants which serve them. Increased ethnicity has brought culinary customs which have resulted in the growth of bean consumption (Anonymous, 2002a).

Beans have many potentially protective components for disease prevention. Eating beans regularly may help lower risk of colon cancer; reduce blood cholesterol, as well as Low Density Lipoprotein (LDL) or "bad cholesterol," leading causes of heart disease; lower risk of type 2 diabetes; improve diabetes control for existing type 1 and 2 diabetics and strengthen immune system through improved nutrition status to combat Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) (Anonymous, 2007a).

Nutritional facts of soybean, black bean, red kidney bean and mung bean were shown in Tables 2.1 – 2.4 from USDA National Nutrient Database for Standard Reference (Anonymous, 2006a).

Soybeans, mature seeds, raw

Table 2.1 Nutritional values for the edible portion of raw mature soybean (*Glycine max*) seeds

Nutrient	Units	Value per 100 grams
Proximates		
Water	g	8.54
Energy	kcal	416

Table 2.1 (Continued)

Nutrient	Units	Value per 100 grams
Energy	kj	1742
Protein	g	36.49
Total lipid (fat)	g	19.94
Ash	g	4.87
Carbohydrate, by difference	g	30.16
Fiber, total dietary	g	9.3
Minerals		
Calcium, Ca	mg	277
Iron, Fe	mg	15.70
Magnesium, Mg	mg	280
Phosphorus, P	mg	704
Potassium, K	mg	1797
Sodium, Na	mg	2
Zinc, Zn	mg	4.89
Copper, Cu	mg	1.658
Manganese, Mn	mg	2.517
Selenium, Se	mcg	17.8
Vitamins		
Vitamin C, total ascorbic acid	mg	6.0
Thiamin	mg	0.874
Riboflavin	mg	0.870
Niacin	mg	1.623
Pantothenic acid	mg	0.793
Vitamin B-6	mg	0.377
Folate, total	mcg	375
Folic acid	mcg	0
Folate, food	mcg	375

Table 2.1 (Continued)

Nutrient	Units	Value per 100 grams
Folate, DFE	mcg_DFE	375
Vitamin B-12	mcg	0.00
Vitamin A, IU	IU	0
Vitamin A, RAE	mcg_RAE	0
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.85
Vitamin K (phylloquinone)	mcg	47.0
Lipids		
Fatty acids, total saturated	g	2.884
14:0	g	0.055
16:0	g	2.116
18:0	g	0.712
Fatty acids, total monounsaturated	g	4.404
16:1 undifferentiated	g	0.055
18:1 undifferentiated	g	4.348
Fatty acids, total polyunsaturated	g	11.255
18:2 undifferentiated	g	9.925
18:3 undifferentiated	g	1.330
Cholesterol	mg	0
Phytosterols	mg	161
Amino acids		
Tryptophan	g	0.530
Threonine	g	1.585
Isoleucine	g	1.770
Leucine	g	2.972
Lysine	g	2.429
Methionine	g	0.492
Cystine	g	0.588

Table 2.1 (Continued)

Nutrient	Units	Value per 100 grams
Phenylalanine	g	1.905
Tyrosine	g	1.380
Valine	g	1.821
Arginine	g	2.831
Histidine	g	0.984
Alanine	g	1.719
Aspartic acid	g	4.589
Glutamic acid	g	7.068
Glycine	g	1.687
Proline	g	2.135
Serine	g	2.115

From: USDA National Nutrient Database for Standard Reference (Anonymous. 2006a)

Beans, kidney, red, mature seeds, raw

Table 2.2 Nutritional values for the edible portion of raw mature red kidney bean (*Phaseolus vulgasris*) seeds

Nutrient	Units	Value per 100 grams
Proximate		
Water	g	11.75
Energy	kcal	337
Energy	kj	1408
Protein	g	22.53
Total lipid (fat)	g	1.06
Ash	g	3.37

Table 2.2 (Continued)

Nutrient	Units	Value per 100 grams
Carbohydrate, by difference	g	61.29
Fiber, total dietary	g	15.2
Sugars, total	g	2.23
Minerals		
Calcium, Ca	mg	83
Iron, Fe	mg	6.69
Magnesium, Mg	mg	138
Phosphorus, P	mg	406
Potassium, K	mg	1359
Sodium, Na	mg	12
Zinc, Zn	mg	2.79
Copper, Cu	mg	0.699
Manganese, Mn	mg	1.111
Fluoride, F	mcg	2.2
Selenium, Se	mcg	3.2
Vitamins		
Vitamin C, total ascorbic acid	mg	4.5
Thiamin	mg	0.608
Riboflavin	mg	0.215
Niacin	mg	2.110
Pantothenic acid	mg	0.780
Vitamin B-6	mg	0.397
Folate, total	mcg	394
Folic acid	mcg	0
Folate, food	mcg	394
Folate, DFE	mcg_DFE	394
Vitamin B-12	mcg	0.00

Table 2.2 (Continued)

Nutrient	Units	Value per 100 grams
Vitamin B-12, added	mcg	0.00
Vitamin A, IU	IU	0
Vitamin A, RAE	mcg_RAE	0
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.22
Vitamin E, added	mg	0.00
Vitamin K (phylloquinone)	mcg	5.9
Lipids		
Fatty acids, total saturated	g	0.154
4:0	g	0.000
6:0	g	0.000
8:0	g	0.000
10:0	g	0.000
12:0	g	0.000
14:0	g	0.000
16:0	g	0.136
18:0	g	0.018
Fatty acids, total monounsaturated	g	0.082
16:1 undifferentiated	g	0.000
18:1 undifferentiated	g	0.082
20:1	g	0.000
22:1 undifferentiated	g	0.000
Fatty acids, total polyunsaturated	g	0.586
18:2 undifferentiated	g	0.228
18:3 undifferentiated	g	0.358
18:4	g	0.000
20:4 undifferentiated	g	0.000

Table 2.2 (Continued)

Nutrient	Units	Value per 100 grams
20:5 n-3	g	0.000
22:5 n-3	g	0.000
22:6 n-3	g	0.000
Cholesterol	mg	0
Amino acids		
Tryptophan	g	0.267
Threonine	g	0.948
Isoleucine	g	0.995
Leucine	g	1.799
Lysine	g	1.547
Methionine	g	0.339
Cystine	g	0.245
Phenylalanine	g	1.218
Tyrosine	g	0.634
Valine	g	1.179
Arginine	g	1.395
Histidine	g	0.627
Alanine	g	0.945
Aspartic acid	g	2.725
Glutamic acid	g	3.436
Glycine	g	0.880
Proline	g	0.955
Serine	g	1.226

From: USDA National Nutrient Database for Standard Reference (Anonymous, 2006a)

Beans, black, mature seeds, raw

Table 2.3 Nutritional values for the edible portion of raw mature black bean (*Phaseolus vulgaris*) seeds

Nutrient	Units	Value per 100 grams
Proximate		
Water	g	11.02
Energy	kcal	341
Energy	kj	1425
Protein	g	21.60
Total lipid (fat)	g	1.42
Ash	g	3.60
Carbohydrate, by difference	g	62.36
Fiber, total dietary	g	15.2
Sugars, total	g	2.25
Minerals		
Calcium, Ca	mg	123
Iron, Fe	mg	5.02
Magnesium, Mg	mg	171
Phosphorus, P	mg	352
Potassium, K	mg	1483
Sodium, Na	mg	5
Zinc, Zn	mg	3.65
Copper, Cu	mg	0.841
Manganese, Mn	mg	1.060
Selenium, Se	mcg	3.2
Vitamins		
Vitamin C, total ascorbic acid	mg	0.0

Table 2.3 (Continued)

Nutrient	Units	Value per 100 grams
Thiamin	mg	0.900
Riboflavin	mg	0.193
Niacin	mg	1.955
Pantothenic acid	mg	0.899
Vitamin B-6	mg	0.286
Folate, total	mcg	444
Folic acid	mcg	0
Folate, food	mcg	444
Folate, DFE	mcg_DFE	444
Vitamin B-12	mcg	0.00
Vitamin B-12, added	mcg	0.00
Vitamin A, IU	IU	0
Vitamin A, RAE	mcg_RAE	0
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.22
Vitamin E, added	mg	0.00
Vitamin K (phylloquinone)	mcg	6.0
Lipids		
Fatty acids, total saturated	g	0.366
4:0	g	0.000
6:0	g	0.000
8:0	g	0.000
10:0	g	0.000
12:0	g	0.000
14:0	g	0.001
16:0	g	0.343
18:0	g	0.022
Fatty acids, total monounsaturated	g	0.123

Table 2.3 (Continued)

Nutrient	Units	Value per 100 grams
16:1 undifferentiated	g	0.000
18:1 undifferentiated	g	0.123
20:1	g	0.000
22:1 undifferentiated	g	0.000
Fatty acids, total polyunsaturated	g	0.610
18:2 undifferentiated	g	0.332
18:3 undifferentiated	g	0.278
18:4	g	0.000
20:4 undifferentiated	g	0.000
20:5 n-3	g	0.000
22:5 n-3	g	0.000
22:6 n-3	g	0.000
Cholesterol	mg	0
Amino acids		
Tryptophan	g	0.256
Threonine	g	0.909
Isoleucine	g	0.954
Leucine	g	1.725
Lysine	g	1.483
Methionine	g	0.325
Cystine	g	0.235
Phenylalanine	g	1.168
Tyrosine	g	0.608
Valine	g	1.130
Arginine	g	1.337
Histidine	g	0.601
Alanine	g	0.905
Aspartic acid	g	2.613

Table 2.3 (Continued)

Nutrient	Units	Value per 100 grams
Glutamic acid	g	3.294
Glycine	g	0.843
Proline	g	0.916
Serine	g	1.175

From: USDA National Nutrient Database for Standard Reference (Anonymous, 2006a)

Mung beans, mature seeds, raw

Table 2.4 Nutritional values for the edible portion of raw mature mung bean (*Vigna radiata*) seeds

Nutrient	Units	Value per 100 grams
Proximates		
Water	g	9.05
Energy	kcal	347
Energy	kj	1453
Protein	g	23.86
Total lipid (fat)	g	1.15
Ash	g	3.32
Carbohydrate, by difference	g	62.62
Fiber, total dietary	g	16.3
Sugars, total	g	6.60
Minerals		
Calcium, Ca	mg	132
Iron, Fe	mg	6.74
Magnesium, Mg	mg	189

Table 2.4 (Continued)

Nutrient	Units	Value per 100 grams
Phosphorus, P	mg	367
Potassium, K	mg	1246
Sodium, Na	mg	15
Zinc, Zn	mg	2.68
Copper, Cu	mg	0.941
Manganese, Mn	mg	1.035
Selenium, Se	mcg	8.2
Vitamins		
Vitamin C, total ascorbic acid	mg	4.8
Thiamin	mg	0.621
Riboflavin	mg	0.233
Niacin	mg	2.251
Pantothenic acid	mg	1.910
Vitamin B-6	mg	0.382
Folate, total	mcg	625
Folic acid	mcg	0
Folate, food	mcg	625
Folate, DFE	mcg_DFE	625
Vitamin B-12	mcg	0.00
Vitamin B-12, added	mcg	0.00
Vitamin A, IU	IU	114
Vitamin A, RAE	mcg_RAE	6
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.51
Vitamin E, added	mg	0.00
Vitamin K (phylloquinone)	mcg	9.0
Lipids		

Table 2.4 (Continued)

Nutrient	Units	Value per 100 grams
Fatty acids, total saturated	g	0.348
4:0	g	0.000
6:0	g	0.000
8:0	g	0.000
10:0	g	0.000
12:0	g	0.000
14:0	g	0.000
16:0	g	0.250
18:0	g	0.071
Fatty acids, total monounsaturated	g	0.161
16:1 undifferentiated	g	0.000
18:1 undifferentiated	g	0.161
20:1	g	0.000
22:1 undifferentiated	g	0.000
Fatty acids, total polyunsaturated	g	0.384
18:2 undifferentiated	g	0.357
18:3 undifferentiated	g	0.027
18:4	g	0.000
20:4 undifferentiated	g	0.000
20:5 n-3	g	0.000
22:5 n-3	g	0.000
22:6 n-3	g	0.000
Cholesterol	mg	0
Phytosterols	mg	23
Amino acids		
Tryptophan	g	0.260
Threonine	g	0.782
Isoleucine	g	1.008

Table 2.4 (Continued)

Nutrient	Units	Value per 100 grams
Leucine	g	1.847
Lysine	g	1.664
Methionine	g	0.286
Cystine	g	0.210
Phenylalanine	g	1.443
Tyrosine	g	0.714
Valine	g	1.237
Arginine	g	1.672
Histidine	g	0.695
Alanine	g	1.050
Aspartic acid	g	2.756
Glutamic acid	g	4.264
Glycine	g	0.954
Proline	g	1.095
Serine	g	1.176
Other		
Alcohol, ethyl	g	0.0
Caffeine	mg	0
Theobromine	mg	0
Carotene, beta	mcg	68
Carotene, alpha	mcg	0
Cryptoxanthin, beta	mcg	0
Lycopene	mcg	0
Lutein + zeaxanthin	mcg	0

From: USDA National Nutrient Database for Standard Reference (Anonymous. 2006a)

Preparation of bean milks could be done by soaking dried beans in distilled water with a ratio of 1:3 for 8 hours (Shurtleff and Aoyagi, 1984) and cleaning it twice with distilled water. The cleaned beans would then be grinded and extracted with distilled water using a ratio of 1:5 (Shurtleff and Aoyagi, 1984) in an electronic juice blender. The liquid that was extracted by the juice blender was recognized as bean milks. Afterwards, the collected bean milks would be separated into 500 ml. filled into sterile glass bottles and pasteurized at 85°C for 15 minutes (Marshall and Arbuckle, 1996).

2.2 Pasteurization

The definition of milk pasteurization by United States Department of Agriculture (USDA) is heating of every particle of milk or milk product to a specific temperature for a specified period of time without allowing recontamination of that milk or milk product during the heat treatment process. Pasteurization (or pasteurisation) is the process of heating liquids for the purpose of destroying viruses and harmful organisms such as bacteria, protozoa, molds and yeasts. The process was named after its inventor, French scientist Louis Pasteur. The first pasteurization test was completed by Pasteur and Claude Bernard on April 20, 1862 (Anonymous, 2007b).

Unlike sterilisation, pasteurization is not intended to kill all micro-organisms (pathogenic) in the food. Instead, pasteurization aims to achieve a "log reduction" in the number of viable organisms, reducing their number so they are unlikely to cause disease (assuming the pasteurised product is refrigerated and consumed before its

expiration date). Commercial-scale sterilisation of food is not common, because it adversely affects the taste and quality of the product (Anonymous, 2007b).

Pasteurization typically uses temperatures below boiling since at temperatures above the boiling point for milk casein micelles will irreversibly aggregate (or "curdle"). There are two types of pasteurization used today: high temperature/short time (HTST) and ultra-high temperature (UHT). There are two methods for HTST pasteurization: batch and continuous flow. In the batch process, a large quantity of milk is held in a heated vat at 63°C (145°F) for 30 minutes, followed by quick cooling to about 4°C (39°F). In the continuous flow process, milk is forced between metal plates or through pipes heated on the outside by hot water. UHT processing holds the milk at a temperature of 138°C (280°F) for at least two seconds. Milk simply labeled "pasteurised" is usually treated with the HTST method, whereas milk labeled "ultra-pasteurized" or simply "UHT" must be treated with the UHT method (Anonymous, 2007b).

Pasteurization methods are usually standardised and controlled by national food safety agencies (such as the USDA in the United States and the Food Standards Agency in the United Kingdom). These agencies require milk to be HTST pasteurized in order to qualify for the "pasteurised" label. There are different standards for different dairy products, depending on the fat content and the intended usage. For example, the pasteurization standards for cream differ from the standards for fluid milk, and the standards for pasteurizing cheese are designed to preserve the phosphatase enzyme, which aids in cutting (Anonymous, 2007b).

The HTST pasteurization standard was designed to achieve a 5-log reduction (0.00001 times the original) in the number of viable microorganisms in milk. This is considered adequate for destroying almost all yeasts, mold and common spoilage bacteria and also to ensure adequate destruction of common pathogenic heat-resistant organisms (including particularly *Mycobacterium tuberculosis*, which causes tuberculosis and *Coxiella burnetii*, which causes Q fever). HTST pasteurization processes must be designed so that the milk is heated evenly, and no part of the milk is subject to a shorter time or a lower temperature (Anonymous, 2007b).

Pasteurization is typically associated with milk. HTST pasteurized milk typically has a refrigerated shelf life of two to three weeks, whereas ultra pasteurized milk can last much longer when refrigerated, sometimes two to three months. When UHT pasteurization is combined with sterile handling and container technology, it can even be stored unrefrigerated for long periods of time (Anonymous, 2007b).

There are two distinct purposes for the process of milk pasteurization: public health - to make milk and milk products safe for human consumption by destroying all bacteria that may be harmful to health (pathogens) and to improve the keeping quality of milk and milk products. HTST pasteurization can destroy some undesirable enzymes and many spoilage bacteria. Shelf life can be 7, 10, 14 or up to 16 days depending on the time and temperatures during processing. Minimum temperature and time requirements for milk pasteurization are based on thermal death time studies for the most heat resistant pathogen found in milk, *Coxelliae burnetii* (Anonymous, 1999).

2.3 Probiotics

Probiotic is generally defined as live preparations of individual or mixtures of bacterial species which, when ingested, have a beneficial effect on the consumer. The claimed health benefits of probiotic-containing foods include improving general gut health, lowering blood cholesterol and improving the body's natural defences. Moreover, there is growing evidence that probiotics may be useful in managing irritable bowel syndrome, lactose intolerance, chronic liver disease, pancreatitis and even certain forms of cancer. Furthermore, the increasing use of probiotic products in both humans and in livestock can reduce the requirement for use of antibiotics. Probiotics are biological control agents. The probiotic bacteria most commonly used are *Lactobacillus*, *Bifidobacterium*, *Streptococcus* and *Enterococcus* species, although some yeast is also found in probiotic products. *Saccharomyces boulardii*, *Escherichia coli*, *Lactobacillus* species and bifidobacteria, among others, have been evaluated in clinical assessments, either case series or placebo-controlled trials, of small bowel bacterial overgrowth, colorectal cancer, diarrhea in children; traveler's diarrhea and antibiotic or *Clostridium difficile*-associated diarrhea. Commercial strains are usually isolated from the intestinal microflora of the intended consumer (human, chicken, pig, for example) and selected on the basis of criteria such as resistance to stomach acids and bile salts, ability to colonize the intestine, or antagonism of potentially pathogenic micro-organisms (Zhang, 1990).

An optimal balance of microbial organisms in the intestine is suggested to be an important aspect of maintaining good health. Certain bacteria, such as lactobacilli and bifidobacteria, that help maintain such a favorable balance (Hansen, 1985) are

consided to be probiotics. Fuller (1989) defined probiotics as the use of a live microbial feed supplement that beneficially affects the host animal by improving its intestinal microbial balance. As a person ages, the number of intestinal bifidobacteria decrease and the numbers of clostridia, streptococci and coliform increase (Stark and Lee, 1982). Both *Lactobacillus acidophilus* and *Bifidobacterium bifidum* produce antibiotics and organic acids (such as lactic acid and acetic acid) that are inhibitory toward Gram negative bacteria (Rasic, 1983). Some lactic acid bacteria also have anticarcinogenic properties (Oda *et al.*, 1983; Reddy *et al.*, 1983). Goldin and Gorbach (1984) studied the influence of *L. acidophilus* on the activity of enzymes produced by intestinal bacteria that can convert procarcinogens into carcinogens. The studied enzymes were β-glucuronidase, nitroreductase and azoreductase. They found reduced concentrations of each enzyme when milk supplemented with *L. acidophilus* was consumed.

Intestinal bacteria are important in preventing disease. Researchers at the Institute for Medicine, Microbiology and Hygiene at the University of Cologne in France, have been studying the benefits of bacteria in the intestinal tract. They have found that intestinal bacteria, such as *Lactobacillus acidophilus*, produce peptides, which are made from amino acids, and help to increase the immunity in the body (Challem, 1995).

Verifiable mechanisms of action for probiotic organisms include colonization of the lower intestine and inhibiting the growth of any potential pathogens through 'competitive exclusion'. Some strains have been shown to adhere to the same binding sites on the gut wall as known pathogens and even to have the ability to displace

them. One example is that a strain of *Lactobacillus* has been found to prevent colonization of poultry by *Salmonella*. Probiotic microorganisms are often incorporated in foods in the form of yoghurts and yoghurt-type fermented milk. Recently, there are probiotic ice cream, cheese, infant formulas, breakfast cereals, sausages, luncheon meats, chocolates and puddings, probiotic products in capsules containing freeze-dried cell powders and in tablet form. However, most probiotic products described in the literature are consumed as yogurt or yogurtlike dairy products, and most of the reported technologic advances describe methodologies relevant to probiotic viability in milk or innovative products destined for inoculation into milk (Colum and Fergus, 2002). There are a number of problems in determining the efficacy of probiotics as a whole. Firstly, although there are a wide range of species and strains used, the efficacy of some of them remains in doubt or has not been fully proved. Added to this are the problems of variation in viability or activity of the cells in the various preparations, the use of mixtures of organisms and their differential survival and ensuring that probiotic cells have a long shelf-life and reach their site of action (Zhang, 1990).

Lactobacillus and *Bifidobacterium* species are the most commonly used probiotics in foods for human consumption given the significant health benefits. Examples where clinical studies have shown health improvement associated with consumption of probiotics include reduction in the incidence of childhood atopic eczema, decrease in rotavirus shedding in infants and reductions in antibiotic-associated diarrhea. These microorganisms share a number of common traits, such as

generally regarded as safe (GRAS) status, acid and bile tolerance and ability to adhere to intestinal cells (Ross *et al.*, 2005).

The use of microbes to fight microbes in controlling infections and diarrheal diseases is not new. Fermented milk products have been ingested for centuries. People now think that certain cultures can control toxin-producing bacteria in the gastrointestinal tract, thereby promoting good health and prolonging life. Healthy or balanced gut microflora are necessary for maintaining the health of the host, and the theory of probiotics suggests that one way to achieve this is to ingest exogenous bacteria and incorporate them into the colonic microbiota. Consumption of these organisms leads to improvement of lactose intolerance symptoms, improvement of gastrointestinal motility, control of intestinal disorders attributed to infections, anticarcinogenic condition and anticholesterolemic effects. The terms probiotics, lactic cultures and lactic acid producing bacteria have been loosely used to refer to these organisms as a group or individually (Shanahan, 2004).

An effective probiotic agent would have certain criteria such as display resistance to digestion by enteric or pancreatic enzymes, display resistance to acid and bile, and prevent the adherence, establishment, replication, or activity of an enteropathogen. The mode of delivery of the probiotic would depend on its capacity to survive and adhere to the intestinal mucosa and on its potential for colonizing (establishing itself and further replicating) in the bowel. The most commonly used and reported probiotics include two genera: *Lactobacillus (L. acidophilus, L. casei, L. bulgaricus)* and *Bifidobacterium (B. bifidum, B. longum, B. breve, B. infantis, B. animalis* and other *Bifidobacterium* species*)*. Both genera are found in normal

intestinal flora at relatively low levels in healthy human adults. When ingested in doses of 10^6 or greater, both can be found in stools in concentrations of greater than 10^6 CFU per gram, as long as regular ingestion continues. *L. acidophilus* and related strains can adhere to the intestinal mucosa. Adherence is believed necessary for adequate, long-term colonization of the gut. Selection of suitable strains for efficacy is therefore critical. Potential mechanisms by which probiotics may exert their beneficial effects are competition with other microflora for nutrients, production of acids inhibitory to certain enteropathogens, production of bacteriocins or inhibitory metabolites, immunomodulation, competition for adhesion to the intestinal mucosa. lower cholesterol, treat rheumatoid arthritis and prevent or reduce the effects of atopic dermatitis, Crohn's disease, diarrhea, and constipation as well as candidiasis and urinary tract infections (Reid, 1999).

Fermented dairy products made with traditional cultures are generally considered to lack any adverse effects. However, microbiologists and ecologists caution that introducing large numbers of viable *nontraditional* strains or strains of nonhuman origin may pose the potential for transfer of antibiotic resistance or for provoking virulence in generally benign strains. Since the efficacy of a probiotic is directly related to the number of live and active culture cells consumed, it is important to specify the potency of the colony-forming units of the culture contained in the product. Additionally, the culture should be active in terms of growth potential. There is extensive public information on probiotics products in Japan. Health claims for food probiotic products in Japan are primarily given as "improves intestinal microflora" or "improves intestinal condition" or the equivalent. Japanese consumers

believe that bifidus-containing products in particular have a role to play in the prevention of cancer and other serious diseases (Chandan, 1997).

For successful delivery in foods, probiotics must survive food processing and storage during product maturation and shelf-life. It is recommended that the probiotic culture must be present in the product at minimum numbers of 10^7 CFU/ml and even higher numbers have been recommended. In addition, the probiotic food product should be regularly consumed in sufficient quantities to deliver the relevant dose of live bacteria to the gastrointestinal tract, keeping in mind the losses in cell viability typically encountered during gastric transit. Consequently, the technological issues relating to the development of foods containing these bacteria in sufficient numbers throughout shelf-life need to be overcome, as well as means of stabilization following ingestion, i.e. during exposure to the adverse conditions of the human gastrointestinal tract (Ross *et al.*, 2005).

Ideally, microbial probiotics should have a beneficial effect and not cause any harm to the host. Therefore, all strains must have been studied comprehensively prior to use in humans or animals and thus are given GRAS status. The predominant species used as probiotic agents belong to the group of LAB (lactic acid bacteria). Due to their long history of safe use in foods, most species of LAB are considered as commensal microorganisms with no pathogenic potential. The LAB, a group of Gram-positive bacteria, consist of several species including the genera *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, *Bifidobacterium* and *Weissela*. Within the LAB group, the genus *Lactobacillus* is the most widely encountered for probiotics. These include *L. acidophilus*, *L. bulgaricus*, *L. casei*, *L. fermentum*, *L.*

plantarum, L. reuteri, L. rhamnosus and *L. salivarius*. These *Lactobacillus* species possess several important properties and therefore can be used as effective probiotic organisms. These features are efficient adherence to intestinal epithelial cells to reduce or prevent colonisation of pathogens, competitive growth, nonpathogen and production of metabolites to inhibit or kill pathogen (Chukeatirote, 2003).

Probiotics have also proved to be effective therapeutic agents in cases in which the exact cause of the diarrhea was not identified. Thus, patients with chronic kidney failure, there is often a bacterial overgrowth in the small intestine, resulting in high blood dimethylamine and nitrosodimethylamine concentrations. These toxic compounds were significantly lower in patients treated with 2 strains of *L. acidophilus*, resulting in a significantly better quality of life for these patients (Bezkorovainy, 2001).

The intestinal microfloras within a given individual are remarkably stable, although major differences may exist among different persons. Nevertheless, administration of probiotics to either newborns or adults results in certain changes in the microbial profiles and metabolic activities of the feces. Admittedly, such changes are minor; yet, when applied to pathologic situations, they are often sufficient to beneficially alter the course of disease. In most situations, probiotic administration results in an increase in fecal counts of bifidobacteria and lactobacilli, a decrease in fecal pH and a decline in those bacterial enzyme activities that are associated with the development of colon cancer (Bezkorovainy, 2001).

Hekmat and McMahon (1992) reviewed that gastrointestinal microorganisms play a role in the metabolism of cholesterol. Evidence to support this was reported that serum cholesterol was significantly reduced in people who ingested acidophilus milk. It has been concluded from these studies that consumption of *L. acidophilus* interferes with cholesterol absorption from the intestine (Hekmat and McMahon, 1992). The animals receiving the acidophilus also showed lower gains in serum cholesterol levels (Tenney, 1996).

The area which encompasses the digestive tract is large which leads to a high degree of exposure to harmful substances that enter the body. Evidence seems to point to the possibility of *L. acidophilus* in the prevention of cancer, mainly colon cancer. The acidophilus produces metabolites that help inhibit the growth of bacteria that can produce carcinogens. The effects of the acidophilus seem to be more pronounced in individuals who eat meat. When a supplement was given to vegetarians, there was only a small decrease in enzymes known to turn substances into carcinogens. But in the group who eat meat there was a two to four fold decrease in the enzymes. The change occurred over a period of one to two weeks and continued as long as the *L. acidophilus* supplements were given (Tenney, 1996). In addition, *L. acidophilus* has the ability to assimilate cholesterol during growth, thus providing the potential to decrease serum cholesterol in persons consuming the cells (Brashears and Gilliland, 1995). These potential benefits illustrate the need to provide consumers with a readily available product containing viable and active cells of *L. acidophilus*. Currently, one source of *L. acidophilus* provided to consumers is nonfermented acidophilus milk. This product is prepared by adding cells from a frozen concentrated culture of the organism to pasteurized low fat

milk and then storing the milk at refrigeration temperature (Brashears and Gilliland, 1995).

The concept of using *Lactobacillus* species for disease treatment and prevention as well as health restoration and maintenance is not new. However, in recent times, there has been a renewal of interest in the use of probiotics (as distinct from antibiotics) (also termed biotherapeutic agents), driven in large part by consumers and the lay press. Probiotics have also been used therapeutically (Reid, 1999).

Probiotic supplements have also demonstrated the ability to protect against the *Campylobacter* pathogen (Anonymous, 2004). *Campylobacter*, a Gram-negative bacterium caused by contaminated food or water, is characterized by diarrhea, abdominal pain, nausea, vomiting, fever and malaise. In recent studies, *L. acidophilus* was able to survive HCl in the human stomach and inhibited the growth of *Helicobacter pylori* (Michetti *et al.*, 1999). This is significant because *H. pylori* have been implicated in the formation of peptic ulcers. The numbers of *Enterobacteriaceae* (*Escherichia coli, Klebsiella, Salmonella, Shigella* and *Proteus*), very common and sometime deadly pathogens, decreased considerably following lactobacillus and bifidus supplementation (Alander *et al.*, 1999). Probiotics can also play a positive role in people with milk allergies. Probiotic bacteria can influence immune responses both specifically by stimulating antibody production and nonspecifically by enhancing phagocytosis of pathogens and modifying cytokine production. Probiotic bacteria appear to modulate the nonspecific immune response differently in healthy and hypersensitive subjects. This is seen as stimulating the immune system in healthy

subjects and down-regulating the immune system in milk-hypersensitive subjects (Pelto *et al.*, 1998). Numerous clinical trials have established that supplementing with probiotic microorganisms such as *Lactobacillus* and *Bifidobacterium* can help balance the intestinal microflora, stimulate immune function, possibly lower blood fats, lessen the incidence of lactose intolerance and reduce the risk of gastrointestinal tract infections (Collins and Gibson, 1999 and Vaughan *et al.*, 1999).

The ingested probiotic microbes may benefit their host through many proposed mechanisms, including the production of antimicrobial factors, competition for nutrients, competitive exclusion of potentially deleterious organisms from adhesion sites, degradation of toxins or eucaryotic toxin receptors and immunomodulation. Irrespective of their functionality, probiotics must remain ecologically competent within the intestinal tract while maintaining physiologic activities beneficial to the host (Colum and Fergus, 2002). As scientific knowledge and biotechnologic proficiency advance at an accelerating pace, the requirement for informed legislation and for mechanisms of effectively delivering these therapies to the sites of their intended function may limit the applications of probiotics (Colum and Fergus, 2002).

L. acidophilus (Anonymous, 2007c)

- *Lactobacillus acidophilus* (*L. acidophilus*)
- Kingdom: Bacteria
- Division: Firmicutes
- Class: Bacilli

- Order: Lactobacillales
- Family: *Lactobacillaceae*
- Genus: *Lactobacillus*
- Species: *Lactobacillus acidophilus*

Lactobacillus acidophilus gets its name from *lacto-* meaning milk. *-bacillus* meaning rod-like in shape, and *acidophilus* meaning acid-loving. This bacterium thrives in more acidic environments than most related microorganisms (pH 4-5 or lower) and grows best at 45°C. *L. acidophilus* occurs naturally in the human (and animal) intestine, mouth and vagina. *L. acidophilus* ferments lactose into lactic acid because the organism is a homofermentative lactic acid bacterium. Like many bacteria, *L. acidophilus* can be killed by excess heat, moisture, or direct sunlight (Anonymous, 2007c).

L. acidophilus is the most commonly used probiotic, or "friendly" bacteria. Such healthy bacteria inhabit the intestines and vagina and protect against the entrance and proliferation of "bad" organisms that can cause disease. This is accomplished through a variety of mechanisms. For example, the breakdown of food by *L. acidophilus* leads to production of lactic acid, hydrogen peroxide and other byproducts that make the environment hostile for undesired organisms. *L. acidophilus* also produces lactase, the enzyme that breaks down milk sugar (lactose) into simple sugars. People who are lactose intolerant do not produce this enzyme. For this reason, *L. acidophilus* supplements may be beneficial for these individuals (Anonymous, 2007c).

L. acidophilus can also help to fight bad bacteria and organisms that invade the body. *L. acidophilus* has been found to contain antibiotic properties. According to Dr. Khem Shahani, a professor of food science at the University of Nebraska, milk fermented by *L. acidophilus* contains an antibiotic he calls "acidophilin." It is a powerful antibiotic with similar abilities as penicillin, streptomycin and terramycin (Scheer, 1993). Detrimental bacteria invade our bodies on a daily basis. Supplementing with either yogurt containing live cultures or a freeze dried capsule may be necessary to protect the body. *L. acidophilus* can protect the digestive system from microorganisms causing infection and disease. It is a supplement that can help protect the body and work as "nature's antibiotic" (Scheer, 1993).

Other potential probiotics include a variety of *Lactobacillus* species (spp.), such as the *casei GG*, *rhamnosus*, NCFM, DDS-1 and *johnsonii* strains, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Saccharaomyces boulardii*, *Bacillus* spp. and *Escherichia coli* (Scheer, 1993).

Probiotics offer a variety of potential therapeutic uses. These include the following (Anonymous, 2006b):

- Replacing the "friendly" intestinal bacteria destroyed by antibiotics.
- Aiding digestion and suppressing disease-causing bacteria.
- Preventing and treating diarrhea, including infectious diarrhea, particularly from rotavirus (a virus that commonly causes diarrhea in children).

- Treating overgrowth of "bad" organisms in the gastrointestinal tract (a condition that tends to cause diarrhea and may occur from use of antibiotics).
- Alleviating symptoms of irritable bowel syndrome and, possibly, inflammatory bowel disease (such as Crohn's disease and ulcerative colitis).
- Preventing and/or reducing the recurrence of vaginal yeast infections, urinary tract infections and cystitis (bladder inflammation). The best scientific evidence exists for vaginal infections.
- Improving lactose absorption digestion in people who are lactose intolerant.
- Enhancing the immune response. Studies have suggested that consumption of yogurt or milk that contains specific strains of *Lactobacillus* or supplements with *Lactobacillus* or *Bifidobacterium* may improve the natural immune response. Further research is needed to confirm these early findings and to best understand how the improved immune function may or may not help in warding off infections.
- Aiding the treatment of respiratory infections such as sinusitis, bronchitis, and pneumonia. More research is needed in this area.
- Lowering risk of allergies. Examples include asthma, hay fever, food allergies to milk and skin reactions such as eczema.
- Helping to treat high cholesterol. More research is needed.
- Reducing the risk of recurring bladder tumors once this cancer has been treated. Much more research is needed in this area.

- Other conditions under investigation for use of probiotics include colon cancer, HIV related diarrhea and *Helicobacter pylori*, an organism that can lead to development of ulcers.

L. acidophilus preparations consist of dried or liquid cultures of living bacteria. These cultures are usually grown in milk but can sometimes be grown in milk-free cultures. *L. acidophilus* is available in the following forms (Anonymous, 2006b):

- Freeze-dried granules
- Freeze-dried powders
- Freeze-dried capsules
- Liquid *L. acidophilus* preparations (which must be kept refrigerated)

Recommended doses of *L. acidophilus* vary depending on the health condition being treated. The following list provides guidelines for the most common uses (Anonymous, 2006b):

- Prevention or treatment of diarrhea: 1 to 2 billion viable cells per day (some experts may recommend up to ten billion cells per day).
- Vaginal infections: 8 ounces of yogurt (with live active cultures containing one of the *Lactobacillus* or *Bifidobacterium* strains) daily or an oral daily supplement containing at least 1 to 2 billion live organisms. Clinical experience also suggests that placing yogurt with live acidophilus cultures directly to the vaginal area, using a disposable spatula and wearing a

sanitary pad, helps to relieve itching and inflammation. Similarly, *Lactobacillus* capsules or tablets may be inserted directly into the vagina.

- Cystitis: 1 to 2 capsules or tablets inserted into the vagina nightly for two weeks.
- Maintaining normal intestinal flora: 1 to 10 billion viable cells per day.

L. acidophilus is one species of bacteria essential in maintaining a healthy intestinal flora. Acidophilus is the primary friendly bacteria found in the intestinal tract and vagina. They help to protect the body from an invasion of *Candida* and other germs that invade and live in the body. *L. acidophilus* helps by adhering to the intestinal wall and preventing disease causing bacteria from taking hold. They cover the lining of the intestines leaving no space for detrimental organisms to reside. When the good bacteria are compromised, space is made for the invading organisms to take hold. They also help by eating all the food reserves and starving out the bad bacteria and allowing them to pass through without taking up residence. Acidophilus is also responsible for producing acetic acids which lower the natural pH in the intestines which discourages the growth of the other bacteria (Tenney, 1996).

L. acidophilus, along with other beneficial bacteria, produces an antibiotic like substance that works against other bacteria, viruses, protozoa and fungi. They work to protect the body from invaders. *L. acidophilus* is the most prevalent form of beneficial bacteria found in the small intestine. It is estimated that a healthy colon should contain at least 85 percent lactobacillus and 15 percent coliform bacteria. Most individuals are lacking in the necessary levels of *Lactobacillus* which contributes to digestive disorders such as gas, bloating, constipation, malabsorption of

nutrients and heart burn. Acidophilus bacteria also help by detoxifying some harmful substances in the gastrointestinal tract. They aid in the digestion of proteins which is essential for the production of essential enzymes made in the body. *L. acidophilus* helps to manufacture B vitamins such as B_1, B_2, B_3. B_{12} and folic acid (Tenney, 1996).

A number of approaches are being explored to increase viability of probiotic bacteria in commercial and experimented products, including selection of acid and bile resistant strains, use of oxygen impermeable containers, microencapsulation, two-step fermentation, stress adaptation and incorporation of micronutrients such as peptides and amino acids (Hou *et al.*, 2003). Dave and Shah (1998) have found that the viability of *L. acidophilus* was improved on an addition of cysteine in yogurt.

2.4 Prebiotics

Prebiotics promote the growth of probiotic gut microflora, exert positive effects on digestive health and regularity, improve mineral absorption, enhance immune function and promote overall health. Low in calories and suitable for diabetics, they may well be a major asset for detoxification products as well. Prebiotics such as oligosaccharides are found naturally in certain fruit and vegetables, including asparagus, bananas, chicory, garlic, onions, wheat and tomatoes. Clinical studies have shown that administering Fructo-oligosaccharides (FOS), Galacto-oligosaccharides (GOS) and inulin can increase the number of friendly bacteria such as *Bifidobacteria* and *Lactobacillus* species in the colon while simultaneously reducing the population of harmful bacteria (Anonymous, 2007d).

A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health. For a food ingredient to be classified as a prebiotic, it must: 1) be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract, 2) be a selective substrate for one or a limited number of beneficial bacteria inhabiting the colon, which are stimulated to grow, and 3) be able to alter the colonic flora in favor of a healthier composition to induce luminal or systemic effects that are beneficial to the host. Fructo-oligosaccharides are the only products presently recognized and used as food ingredients that meet all of these criteria. Transgalactosylated disaccharides and soybean oligosaccharides may also fit this classification (Chandan, 1997).

Prebiotics occur naturally in foods, but supplements provide a more concentrated source of this substance. Prebiotics are oligosaccharides, chains of sugar units linked together. Inulin is a long-chain oligosaccharide (from 2-60 sugars) and FOS is short-chain oligosaccharides (from 2-7 sugars). It is not clear at this time which type of prebiotic is the most effective (Anonymous, 2007d).

FOS are a group of "prebiotics," non-digestible food ingredients that benefit the host by stimulating the growth of beneficial microflora. Short-chain FOS is metabolized in the colon (by colonic bacteria) into short-chain fatty acids. These short-chain fatty acids produce a drop in pH, which may inhibit the growth of pathogenic bacteria, facilitate intestinal calcium absorption and act as a substrate for colonic epithelial cells. By manipulating colonic pH and microflora content, FOS may play a protective role

against colon cancer. Research points to a reduction in liver fatty acid synthesis as a possible mechanism for serum lipid reduction (Anonymous, 2007e).

FOS typically refer to short-chain oligosaccharides comprised of D-fructose and D-glucose, containing from 3 to 5 monosaccharide units. Similar molecules are obtained by partial enzymatic hydrolysis of inulins. Those are called oligofructose. FOS, also called neosugar and short-chain FOS (scFOS), are produced on a commercial scale from sucrose using a fungal fructosyltransferase enzyme (Anonymous, 2007e).

FOS are comprised of one molecule of D-glucose in the terminal position and from 2 to 4 D-fructose units. FOS containing 2 fructose residues is abbreviated GF_2 (G is for glucose, F, for fructose). Those with 3 fructoses are abbreviated GF_3, and those with 4 fructoses, GF_4. GF_2 is also called 1-kestose and GF_3 is called nystose. The linkage between fructose units in FOS is a beta-(2-1) glycosidic link. The structural formula of FOS is shown in Figure 2.1 (Anonymous, 2007e):

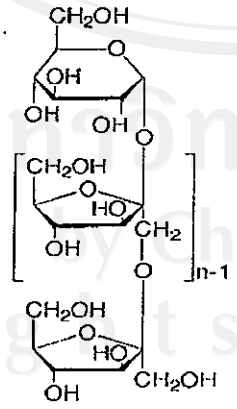

Figure 2.1 Fructo-oligosaccharides (Anonymous, 2007e)

(The top sugar is glucose. n = 2-4 fructose residues)

FOS are resistant to digestion in the stomach and small intestine. The reason for this is the presence of the beta configuration of the anomeric C_2 in the D-fructose residues. The human digestive enzymes sucrase, maltase-isomaltase and alpha-glucosidase are specific for alpha-glycosidic linkages. FOS are considered nondigestible oligosaccharides. They are, however, fermented by a limited number of colonic bacteria. This could lead to changes in the colonic ecosystem in favor of some bacteria, such as bifidobacteria, which appear to be beneficial in some respects. FOS and other nondigestible oligosaccharides are referred to as bifidogenic factors (Anonymous, 2007e).

FOS may have anticarcinogenic, antimicrobial, hypolipidemic and hypoglycemic actions in some. They may also help improve mineral absorption and balance and may have anti-osteoporotic and anti-osteopenic activities (Anonymous. 2007e).

Human studies have shown significant increases in bifidobacteria (beneficial bacteria in the gut) from ingestion of as little as 6-8 grams of short-chain FOS per day. Research has also shown decreases in pathogenic colonic bacteria from FOS ingestion. There is evidence that short-chain FOS can lower cholesterol and triglycerides, but most of this research has involved animal models. Colon tumors and indicators of cancer have also been reduced in animal models. Although animal studies have given promising results, human studies have failed to show that mineral absorption can be enhanced from FOS ingestion. Overall, the results from human studies on FOS ingestion have been disappointing.

but there is clearly a need for more research since animal studies show promising results (Anonymous, 2007e).

Ten g of FOS per day appears to be the optimal dose, since this amount produces a significant increase in bifidobacteria and is fairly well-tolerated. Since significant increases in bifidobacteria have been noted from ingestion of relatively small doses of short-chain FOS, smaller amounts may also be beneficial (Anonymous, 2007e).

The possible anticarcinogenic activity of FOS might be accounted for, in part, by the possible anticarcinogenic action of butyrate. Butyrate, along with other short-chain fatty acids, is produced by bacterial fermentation of FOS in the colon. Some studies suggest that butyrate may induce growth arrest and cell differentiation, and may also upregulate apoptosis, three activities that could be significant for antitumor activity. FOS may also aid in increasing the concentrations of calcium and magnesium in the colon. High concentrations of these cations in the colon may help control the rate of cell turnover. High concentrations of calcium in the colon may also lead to the formation of insoluble bile or salts of fatty acids. This might reduce the potential damaging effects of bile or fatty acids on colonocytes (Anonymous, 2007e).

FOS may promote the growth of favorable bacterial populations, such as bifidobacteria, in the colon. Bifidobacteria may inhibit the growth of pathogenic bacteria, such as *Clostridium perfringens* and diarrheogenic strains of *E. coli*. FOS may lower serum triglyceride levels in some. The mechanism of this possible effect is unclear. Decreased hepatocyte triglyceride synthesis is a hypothetical possibility. FOS

may also lower total cholesterol and LDL-cholesterol levels in some. Again, the mechanism of this possible effect is unclear. Propionate, a product of FOS fermentation in the colon, may inhibit HMG-CoA reductase, the rate-limiting step in cholesterol synthesis (Anonymous, 2007e).

The possible effects of FOS on blood glucose may be explained in a few ways. FOS may delay gastric emptying and/or shorten small-intestinal tract transit time. Propionate may inhibit gluconeogenesis by its metabolic conversion to methylmalonyl-CoA and succinyl-CoA. These metabolites could inhibit pyruvate carboxylase. Propionate may also reduce plasma levels of free fatty acids. High levels of free fatty acids lower glucose utilization and induce insulin resistance. Propionate may enhance glycolysis via depletion of citrate in hepatocytes. Citrate is an allosteric inhibitor of phosphofructokinase (Anonymous, 2007e).

FOS may bind/sequester minerals such as calcium and magnesium in the small intestine. The short-chain fatty acids formed from the bacterial fermentation of FOS may facilitate the colonic absorption of calcium and, possibly, also magnesium ions. This could be beneficial in preventing osteoporosis and osteopenia (Anonymous, 2007e).

Little digestion of FOS occurs in the stomach and small intestine following ingestion of FOS. FOS are fermented in the colon by bifidobacteria and some other bacteria to produce the short-chain fatty acids (SCFA) acetate, propionate and butyrate; the gases hydrogen, hydrogen sulfide, carbon dioxide and methane; and lactate, pyruvate and succinate. Some acetate, propionate and butyrate are absorbed

from the colon and transported by the circulation to various tissues where these SCFA undergo further metabolism. Many SCFA are metabolized by the colonocytes. Butyrate is an important respiratory fuel for the colonocytes. Those with ileostomies may have a microbial population colonizing their ileums. In those cases, FOS could be fermented by some of the bacteria, much as they are in the colon. FOS may also protect against colon cancer, and may have favorable lipid effects in some. FOS may also aid in calcium absorption (Anonymous, 2007e).

There is evidence that FOS can improve the microbial ecology of the gut and protect against some bacterial pathogens, particularly in the large intestine. FOS selectively stimulates the growth of bifidobacteria and also have many of the actions and benefits of dietary fibers (Anonymous, 2007e).

A fermented milk product containing FOS significantly lowered LDL-cholesterol levels in male subjects with borderline elevated levels of serum total cholesterol. This double-blind, placebo-controlled study was done for three weeks. Other studies have credited FOS with lowering both cholesterol and triglyceride levels. FOS have been shown to lower hepatic lipogenesis. In one recent study, however, 15g of FOS daily for 20 days failed to favorably affect either blood glucose levels or serum lipid concentrations in patients with type 2 diabetes (Anonymous, 2007e).

2.5 Microencapsulation

Microencapsulation is a process by which live cells are packaged within a shell material to shield them from the surrounding unfavourable environment. It is one of

the techniques reported to enhance the survival of probiotic bacteria in dairy foods. Probiotic bacteria when encapsulated have acquired protection from stomach acidity and have increased their tolerance to bile. The viability of *Bifidobacterium pseudolongum* in simulated gastric juices was improved when it was encapsulated (Talwalkar and Kailasapathy, 2003).

Microencapsulation has been suggested as an alternative method for entrapment and immobilization of whole cells or their extracts. Currently there is a limited number of reports describing the microencapsulation of microbial cells. The microencapsulation method in alginate gel is carried out in a single step process under very mild conditions and should be compatible with most living cells. Alginates are a family of polysaccharides composed of L-glucuronic acid (G) and D-mannuronic acid (M) residues, arranged in homopolymeric blocks of each type (MM, GG) and in heteropolymeric blocks which are reported to have a major impact on the properties of the different systems. Alginates have the ability to bind multivalent cations being the basis of their gelling properties, leading to the formation of covalent bonds yielding insoluble hydrogels. This anionic polysaccharide forms strong gels with divalent cations like Ca^{2+}, giving both strength and flexibility. Such crosslinking process stiffens and roughens the polymer and reduces the swelling in solvents. The soluble sodium alginate was crosslinked with calcium chloride resulting in the formation of the insoluble calcium alginate. Natural polypolymers are used both as carriers and determinants of the release rate in controlled release systems. The main advantages of natural polymers lie in their biocompatibility and biodegradability, without producing systemic toxicity on administration (Bregni *et al.*, 2000).

Calcium alginate was chosen as an encapsulation material because of its low cost, non-toxic nature and for its ability to release cells from the alginate gel under appropriate conditions. The compound is the most widely used as an immobilizing vehicle. It forms a gel when in contact with calcium and multivalent cations. Alginate beads (or microparticles) are stable in low pH conditions but swell in weak basic solutions followed by disintegration and erosion. The immobilization of *L. bulgaricus* in calcium alginate offered good protection to the organisms during frozen storage and in ice cream. Bifidobacteria immobilized in alginate were more resistant to acid pH values in mayonnaise than the free cells (Grosso and Favaro-Trindade, 2004).

It has been reported that the microencapsulation of bifidobacteria can ensure greater survival in gastric and intestinal environments. Immobilized cells exhibit many advantages over free cells, including the maintenance of stable and active biocatalysts, high volumetric productivity, improved process control, the protection of cells against damage and reduced susceptibility to contamination. Recently, yogurt products containing encapsulated lactic acid bacteria have been distributed under the brand name Doctor-Capsule (Bingrae Co., Kyunggi-do, Korea) in Korea. Among the available techniques for immobilizing living cells, entrapment in Ca alginate beads has been frequently used for the immobilization of lactic acid bacteria. Alginate has the benefits of being nontoxic to the cells being immobilized and it is an accepted food additive (Lee and Heo, 2000).

The main purpose of this study was to maintain a high viability of *L. acidophilus* in non-fermented pasteurized bean milk during storage at low temperature. Factors such

as pH values, the availability of a micronutrient growth factor, initial concentrations of the probiotic bacterium and an extrusion method to immobilize the probiotic bacterium were investigated to find ways to improve the viability of *L. acidophilus* during storage at 4°C in the bean milk. Since the survival of probiotic organisms in the gastrointestinal tract is important in delivering beneficial effects of the microorganisms, this study also evaluated the survival of *L. acidophilus* in simulated gastrointestinal conditions, mainly high-acid gastric and bile-salt conditions. The *L. acidophilus* was cultured using a drop plate technique in de Man Rogosa and Sharp (MRS) agar to compare the effect of different treatments.