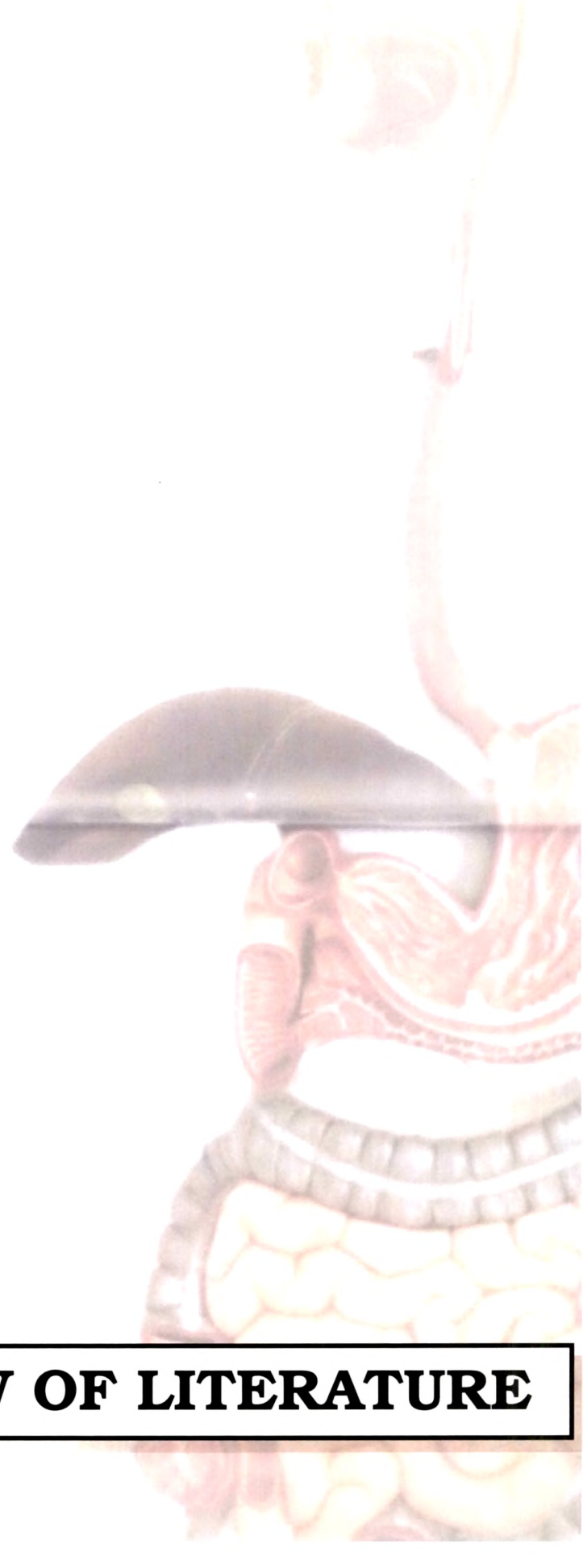


# REVIEW OF LITERATURE

# CHAPTER 2

## REVIEW OF LITERATURE

The present study has been undertaken with the broad objective of "HPLC analysis of selected foods for inulin content, acceptability trials of inulin incorporated recipes and its health benefits in institutionalized elderlies".

The relevant review of literature has been discussed under following heads:

Section 2.1 Global and regional food consumption pattern and trends

Section 2.2 Changing trends in dietary pattern and importance of dietary fibre

Section 2.3 Probiotics and prebiotics

a) As functional foods and their health claims
b) Probiotics and its types and benefits
c) Prebiotics and its types and benefits
d) Synbiotics and its types

Section 2.4 Inulin, a prebiotic

a) Chemistry and structure of inulin
b) Content of Inulin in Foods
c) Consumption Pattern of inulin
d) Safety and tolerance of inulin in diet
e) Caloric value of inulin
f) Legal, regulatory and dietary fiber status
g) Technological functionality of inulin
h) Inulin in yoghurt
i) Inulin type fructans and its health claims

Section 2.5 Modulation of gut microbiodata and inulin type fructans

a) Concept of balanced microflora
b) gastrointestinal health, its composition and significance
c) Changes in the intestinal Flora
d) Role of *Bifidobacteria* and lactobacillus
e) Factors affecting intestinal flora

f) Utilization of various non digestible oligosaccharides by gut bacteria

g) Mechanism of action of inulin

Section 2.6 Health implications of inulin, inulin along with probiotics (synbiotics)

**Section 2.1 Global and regional food consumption pattern and trends**

The world food situation is currently being redefined by new driving forces. Income growth, climate change, high energy prices, globalization and urbanization are transforming food consumption, production and markets. Changes in food availability, rising commodity prices, and new producer-consumer linkages have crucial implications for the livelihoods of people of the world. Many parts of the developing world have experienced high economic growth in recent years. Developing Asia, especially China and India, continues to show strong sustained growth. High income growth in low-income countries readily translates into increased consumption of food. The composition of food budgets is shifting from the consumption of grains and other staple crops to vegetables, fruits, meats, dairy and fish. The demand for ready –to- cook and ready –to – eat foods is also rising, particularly in urban areas. Consumers in Asia, especially in the cities, are also being exposed to non-traditional foods. Due to diet globalization, the consumption of wheat and wheat – based products, temperate- zone vegetables, and dairy products in Asia has increased. In India, cereal consumption remained unchanged between 1990 and 2005, while consumption of oil crops almost doubled; consumption of meat, milk, fish, fruits and vegetables also increased (Table 2.1). In other developing countries, the shift to high- value demand has been less obvious Brazil, Kenya, and Nigeria, the consumption of some high value products declined, which may be due to growing inequality of some of these countries. Wheat, coarse grains (including maize and sorghum) and rice are staple foods for the majority of the world's population. Cereal supply depends on the production and availability of stocks. In 2006, global cereal stocks – especially wheat- were at their lowest levels since the early 1980's Stocks in China, which constitute about 40 percent of total stocks, declined significantly from 2000 to 2004 and have not recovered in recent years. As opposed to cereals the production of high values agricultural commodities such as vegetables, meat, milk is growing at the fast rate (FAO 2007)

Today's shifting pattern is expected to be reinforced in future. Increasing urbanization will also have consequences for the dietary patterns and lifestyles of individuals, not all of which are positive. Changes in diets, patterns of work and leisure, often referred to as the ''nutrition transition'' are already contributing to the causal factors underlying non-communicable diseases even in the poorest countries. Moreover, the pace of these changes seems to be accelerating, especially in the low-income and middle-income countries.

**Table 2.1: Change in Food Consumption Quantity, Ratios 2005/ 1990**

| Type | India | China | Brazil | Kenya | Nigeria |
|---|---|---|---|---|---|
| **Cereals** | 1.0 | 0.8 | 1.2 | 1.1 | 1.0 |
| **Oil Crops** | 1.7 | 2.4 | 1.1 | 0.8 | 1.1 |
| **Meat** | 1.2 | 2.4 | 1.7 | 0.8 | 1.1 |
| **Milk** | 1.2 | 3.0 | 1.2 | 0.9 | 1.3 |
| **Fish** | 1.2 | 2.3 | 0.9 | 0.4 | 0.8 |
| **Fruits** | 1.3 | 3.5 | 0.8 | 1.0 | 1.3 |
| **Vegetables** | 1.3 | 2.9 | 1.3 | 1.0 | 1.3 |

**Source: Data from FAO 2007a.**

**Section 2.2 Changing trends in dietary pattern and importance of dietary fibre**

The dietary changes that characterize the ''nutrition transition'' include both quantitative and qualitative changes in the diet. The adverse dietary changes include shifts in the structure of the diet towards a higher energy density diet with a greater role for fat and added sugars in foods, greater saturated fat intake (mostly from animal sources), reduced intakes of complex carbohydrates and dietary fibre, and reduced fruit and vegetable intakes (Drewnowski and Popkin 1997).

The importance of dietary fiber and its major role in promoting health and limiting the development of many chronic diseases is very well known.

Fiber intake through the consumption of foods rich in this dietary component, such as fresh vegetables, fruits, whole grains, and nuts, is associated with reductions in plasma

and LDL-cholesterol, attenuating glycemic and insulin response, increasing stool bulk, and improving laxation.

Moreover, through its physiologic responses, dietary fiber consumption has established the basis for associating high-fiber diets in epidemiological studies with reduced risk of most of the major dietary problems such as obesity, coronary disease, diabetes, gastrointestinal disorders, including constipation, inflammatory bowel diseases like diverticulitis and ulcerative colitis, and colon cancer. Recent epidemiological data show that a diet high in fiber generally reflects a healthier life style (Kritchevsky 2000) and fiber intake can be viewed as a marker of a healthy diet. Despite the healthful influence dietary fiber can have on reducing the risk of chronic disease, the intake remains low in many populations worldwide (Tungland and Meyer 2002).

However, increasing fiber consumption in the diet has been a difficult challenge, as fiber sources usually used in foods have not made high-fiber foods with high quality taste properties. It is important from a food product development standpoint that high-fiber foods, made using high fiber ingredients, not only supply fiber, but also provide enhanced functional properties to make high-fiber foods taste better, thus encouraging continued high fiber intake.

This has lead to the design and development of functional foods. The design and development of functional foods is a key issue, as well as a scientific challenge, which should rely on basic scientific knowledge relevant to target functions and their possible modulation by food components (Fig.2.1). Functional foods have been the topic of considerable interest in the food and nutrition industry for years (Benkouider C 2005). These functional food products result from: technological innovation at the processing level, such as cholesterol lowering spreads, xylitol-sweetened chewing gum, and dairy products fermented with specific lactic acid

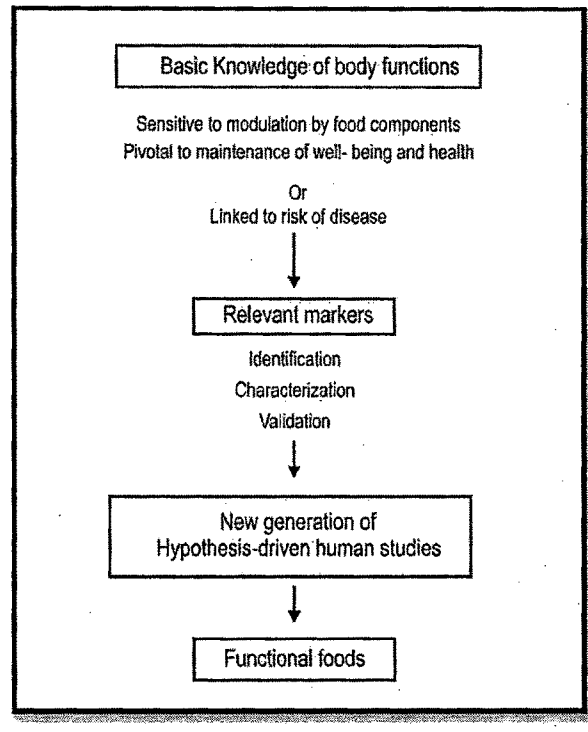



**Fig.2.1 Stepwise Process of the Design and Development of Functional Foods**

bacteria; technological innovation at the production level such as genetically modified crop seeds (e.g., Golden rice) and planting materials derived through conventional breeding (e.g., orange fleshed sweet potato); or crops that naturally contain components that have a physiological function (e.g., soy bean and oats, which are good for cardio-vascular health). Some examples of functional food include (Williams et al 2006):

- Oats (beta-glucan) Heart health
- Eggs with increased omega-3 content achieved by altered chicken feed Heart health
- Margarine fortified with plant sterols - Improved cholesterol levels
- Fermentation with specific bacteria to yield bioactive peptides - Lowers blood pressure
- Chewing gum sweetened with xylitol instead of sugar Helps prevent dental caries
- Golden rice Pro-vitamin A
- Orange-fleshed sweet potato Pro-vitamin A

The gut is an obvious target for the development of functional foods, acting as it does as the interface between diet and the metabolic events which sustain life. The key processes in digestive physiology which can be regulated by modifying diet are satiety, the rate and extent of macronutrient breakdown and absorption from the small bowel, sterol metabolism, the colonic microflora, fermentation, mucosal function and bowel habit and the gut immune system. The intestinal microflora is the main focus of many current functional foods (Salminen et al 1998).

## Section 2.3 Probiotics and prebiotics

### *a) As functional foods and their health claims*

A wide variety of foods are characterized as functional food with a variety of components affecting a various of body functions relevant to either a state of well-being and health and/or to the reduction of risk of a disease. According to the European concept (FUFOSE), the following features characterize a functional food: conventional/everyday food or food ingredient; naturally occurring in foods; proven beneficial effect on target functions beyond nutritive value/basic nutrition; and convincing human nutrition intervention studies demonstrating enhanced well-being and health and/or reduced risk of a disease and/or improved quality of life including physical, psychological, and behavioral performances (Roberfroid 2000, Diplock 1999)

If a functional food demonstrates a positive modulation of target functions after (long-term) consumption of the potential functional food components, such a modulation can be translated into claims based on its effect. If the effects concern a target function or a biological activity without direct reference to a particular disease or pathological process, claim will be made for an "enhanced function." But, if the benefit is clearly a reduction of the risk of a disease or pathological process, claim will be made for a "disease risk reduction" (Fig 2.2).

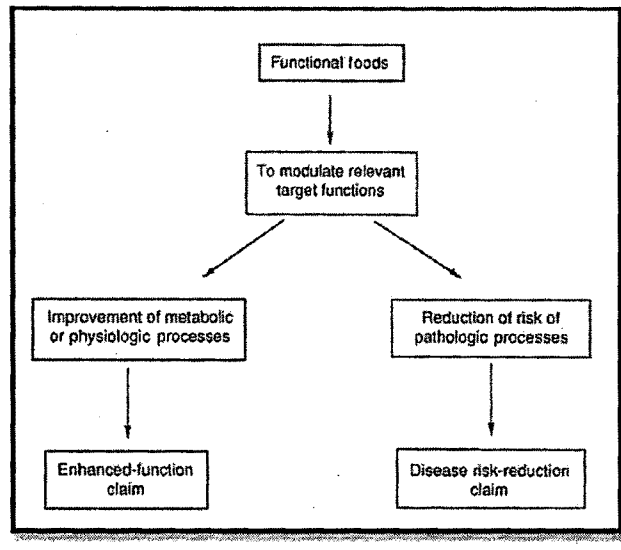



**Fig.2.2 Scientific Basis for Enhanced Structure-Function or Disease Risk-Reduction Claims**

Among the functional foods one area that is rapidly expanding is the Probiotic and prebiotic category (ILSI India 2009). The strength of experimental evidence supporting claims of a functional effect from probiotics and prebiotics is summarized in Table 2.2 and Table 2.3.

**Table 2.2: Strength of the Evidence for Improvement of Body Functions by Probiotics and Prebiotics**

| Functional effects | Strength of evidence[1] Probiotics | Prebiotics |
|---|---|---|
| Lactose intolerance | Strong | Unknown |
| Immunostimulation | Preliminary | Unknown |
| Fecal mutagenesis | Preliminary | Unknown |
| Hypocholesterolemia | No effect | Preliminary |
| Hypolipidemia | Unknown | Promising |
| Colonic flora | Preliminary | Strong |
| Calcium bioavailability | Unknown | Promising |

Source: Roberfroid 2004. It also relies on previous evaluations of the properties of probiotics and prebiotics.

**Table 2.3: Strength of the Evidence for Disease Risk Reduction by Probiotics and Prebiotics**

| | Strength of evidence[1] | |
|---|---|---|
| **Disease risk reduction** | **Probiotics** | **Prebiotics** |
| Diarrhea | Promising | Unknown |
| Constipation | Unknown | Promising |
| Colon cancer | Preliminary | Preliminary |
| Osteoporosis | Unknown | Unknown |
| Lipid-associated chronic disease | Probably no reduction | Unknown |

Source: Roberfroid 2004. It also relies on previous evaluations of the properties of probiotics and prebiotics.

***b) Probiotics and its types and benefits***

Probiotics—a word derived from Latin and Greek meaning literally "for life"—has been defined in many ways since it was first coined 50 years ago. FAO/WHO joint report (2001) defines Probiotics are "living micro-organisms which when administered in adequate amount confer health benefits of the host". This definition fits well in with that of functional foods. Probiotics are usually bacterial components of the normal human intestinal flora, for example *Lactobacilli* and *Bifidobacteria,* that produce as end products of metabolism lactate and short chain fatty acids such as acetate and butyrate. Certain specific probiotic strains have well defined and proven clinical effective for the treatment and/or prevention of diseases of intestinal and extra intestinal origin (Table 2.4). The first deliberate use of LAB for health reasons was by Metchnikoff early in the 20th century as a possible antidote to the aging process, proposed by him to be at least partly due to toxins produced by putrefactive intestinal bacteria (that is, not LAB). Today, probiotics are familiar to the public as the components of bioyoghurts and dietary supplements, are widely available, and extensively purchased. In 1997, Europeans spent the equivalent of almost $900 million on probiotic yoghurts and milks, and this market is growing rapidly. Eight products containing probiotic bacteria are readily available to the public, the most common being yoghurt.

**Table 2.4: Microorganisns Used as Probiotics in Humans and Animals**

| *Lactobacillus Species* | *Bifidobacteria Species* | *Other Lactic Acid Bacteria* | *Non Lactic Acid Bacteria* |
|---|---|---|---|
| *L. acidophilus* | *B. adolecentis* | *Enterococcus faecalis* | *Saccharomyces cerevisiae* |
| *L. brevis* | *B. animalis* | *Enterococcus faecium* | *Saccharomyces boulardii* |
| *L. casei* | *B. bifidum* | *Lactococcus lactis* | |
| *L. fermentum* | B. breve | *Leuconostoc mesenteroides* | |
| *L. gallinarum* | *B. infantis* | *Pediococcus acidilactici* | |
| *L. gasseri* | *B. longum* | *Sporolactobacillus inulinus* | |
| *L. johnsonii* | | *Streptococcus* | |
| *L. plantarum* | | *salivarus* | |
| *L. reuteri* | | | |
| *L. rhamnosus* | | | |

**Source: Balcázar JL 2007**

The standard type of yoghurt consists of milk (usually from the cow, but milk from the goat or sheep may also be used) fermented by bacteria that convert lactose into lactic acid. Lactic acid gives the yoghurt its characteristic sharp taste (usually modulated with sweeteners and flavoring) and also denatures and precipitates casein, resulting in a semisolid consistency. So-called "bioyoghurts" are produced in a similar way, but fermentation is carried out with different probiotic strains, usually *L acidophilus.* Drinks containing probiotic bacteria include fermented milks and fortified fruit juices. A third source of probiotics is supplements consisting of freeze dried bacteria in capsule, tablet, or powder form. The constituents and relative merits of these types of probiotic products are listed below

**Positive health effects of probiotics** are categorization into three classes of effects established effects, possible effects and potential risks.

**Established effects are held to include:**

- Reduction of signs of lactose intolerance
- Reduction of the duration of diarrhoea caused by: rotavirus, antibiotics or Enterotoxic *E. coli.*
- Reduction of the following bacterial enzymes: nitroreductase, -glucuronidase, azoreductase and urease.
- Effects on the immune system.

**Among the possible effects are rated:**

- Lowering of the LDL-cholesterol level.
- Competitive exclusion of enteropathogens.
- Prevention of cancer.
- Increased resistance to infections.

**The following effects are considered as potential risks:**

- Administration of the probiotics to children with an insufficiently developed Intestinal flora.
- Administration of the probiotics to patients suffering from autoimmune disease.
- Transfer of vancomycin resistance genes by micro-organisms such as specific Strains of *Enterococcus faecium.*

Probiotics are currently being applied in particular in fermented dairy products. The probiotics most applied are various strains of *Lactobacillus casei* and *Lactobacillus acidophilus* of human origin, which are lactic acid bacteria known to be capable of passing the stomach and intestine without colonizing these organs.

***Mechanism of action:*** The mode of action of a probiotic may include host microflora modulation by a) improvement of the microbial balance via interaction of orally applied viable microbes with the microflora in the digestive tract lumen, b) the modulation of host metabolic activities, e.g., by stabilizing digestive enzyme pattern, and immunomodulation, c) by activation and regulation of mucosa-associated and systemic immune system responses (Fedork and Madsen 2004). The modes of action are also strain dependent. The

intestinal microflora provides protection against a broad range of pathogens, including certain forms of *Clostridia, Escherichia coli, Shigella,* and *Pseudomonas,* as well as yeasts such as *Candida albicans. Lactobacillus casei* subsp *rhamnosus* (*Lactobacillus* GG) produces compounds that inhibit the growth of several gram-positive and gram-negative bacteria. Examples include hydrogen peroxide and *pyroglutamate.* A few other *Lactobacilli* are capable of producing similar substances. Short chain fatty acids are commonly produced. These lower the colonic pH, which favors the growth of organisms with less pathogenicity. Colonization resistance occurs through this binding, competitively inhibiting adhesion of pathogenic bacteria (Kleeman EG and Klaenhammer 1982). Probiotics may also compete for nutrients otherwise consumed by pathogenic organisms. For example, consumption of monosaccharides by a probiotic may reduce the growth of *Clostridium difficile*, which is dependent on monosaccharides for growth.

Another possible mechanism for the positive effects of a probiotic is its effect on generalized immune enhancement, most likely through its ability to bind to epithelial cells. *Lactobacillus GG* stimulates antibody production against rotavirus (Kaila and Isolauri 1992). A major factor in determining the effectiveness of a probiotic is its ability to survive the digestive process and thrive in the gastrointestinal tract. Difficulties in establishing colonization are the focus of most investigations in search of effective probiotics (Perdigon and Alvarez 1995).

Probiotics are gaining importance because of the innumerable benefits, e.g. treating lactose intolerance, hypercholesterol problem, cardiac diseases and managing cardiac problems like atherosclerosis and arteriosclerosis. With the current focus on disease prevention and the quest for optimal health at all ages, the probiotics market potential is enormous. There are many probiotic products at the market place and most have supporting evidence behind the advertized health claims. Concerns about probiotic survivability not only during food manufacturing and preparation but also after ingestions have stimulated interest in prebiotics because their inert nature ensures resistance to processing and digestion (Ljungh 2006).

One of the mechanisms to increase the number of beneficial bacteria in the gut is through the ingestion of prebiotics.

***c) Prebiotics and its types and benefits***

A prebiotic is "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health." (Gibson et al 2004). Any food that contains oligosaccharides is potentially a prebiotic but in order to be classified as a prebiotic it must fulfill the following criteria: it should neither be hydrolysed nor absorbed in the upper part of the gastrointestinal tract and it should be selectively fermented by one or limited number of potentially beneficial bacteria commensal to the colon for example *Bifidobacteria* and *Lactobacilli* which are stimulated to grow and become metabolically activated. These requirements have been classified as three prebiotic criteria (Table 2.5) (Gibson et al 2004)

**Table 2.5: Criteria for Classification of a Food Ingredient as a Prebiotic**

- Resistance to digestive processes in the upper part of GI tract
- Fermentation by Intestinal microbiota
- Selective stimulation of growth and activity of a limited number of the health promoting bacteria in that microbiota

Although probiotic and prebiotic approaches are likely to shares common mechanism of action, as their effect is impacted through increase in beneficial colonic bacteria, they differ in composition and metabolism.

Prebiotics are found naturally in some plants or are produced enzymatically from sucrose, and often are used in dietary supplements. However, the prebiotic property has been demonstrated adequately for only a few food ingredients. These includes non digestible carbohydrates, which are often described as soluble fibers, include non starch polysaccharides, resistant starches and soluble oligosaccharides. The classification of natural and also some synthetic prebiotic types is listed in Table 2.6. Major prebiotics include fructans and galactooligosaccharides (GOS). Fructans include inulin derived from chicory, whole grains, fruits (e.g., bananas), vegetables (e.g., onions, artichokes) or

fructo-oligosaccharides (FOS) hydrolyzed from chicory or enzymatically from sucrose. GOS are made from lactose as a by-product of dairy-food processing

**Table 2.6: Classification of Prebiotics**

| Classification | Origin/ Manufacturing process |
|---|---|
| **Disaccharides** | from lactose, synthetic |
| Lactulose | from lactose, synthetic |
| Lacticol | |
| **Oligosaccharides** | Legumes, vegetables, cereals |
| Fructose Oligosaccharides (FOS) | Extraction/hydrolysis |
| Soyabean Oligosaccharides | Soyabean |
| (Trans) Galactooligosaccharides | Extraction/hydrolysis |
| Inulin | From lactose, Synthetic |
| | Legumes, vegetables, cereals |
| | Extraction |
| **Polysaccharides** | Legumes, vegetables, cereals |
| Resistant Starch | Extraction |

***d) Synbiotics and its types***

Another possibility in microflora management procedures is the use of synbiotics, in which probiotics and prebiotics are used in combination. The live microbial additions (probiotics) may be used in conjunction with specific substrates (prebiotics) for growth (eg, a fructooligosaccharide in conjunction with a *Bifidobacterial* strain or lactitol in conjunction with a *lactobacillus* organism). Some synbiotics that may be suitable for human consumption are listed below:

- *Bifidobacteria* + inulin
- *Bifidobacteria* + FOS
- *Lactobacilli* + lactitol
- *Bifidobacteria* + GOS

This combination could improve the survival of the probiotic organism, because its specific substrate is readily available for its fermentation, and result in advantages to the host that the live microorganism and prebiotic offer.

## Section 2.5 Inulin, a prebiotic

### *a) Chemistry and structure of inulin*

Inulin-type fructans are a linear polydisperse carbohydrate material consisting mainly, if not exclusively, of ß-(2 ← 1) fructosyl-fructose linkages (Waterhouse and Chatterton 1993) and the linear chain of inulin is either an α-D-glucopyranosyl-[ß-D-fructofuranosyl]$_{n-1}$-ß-D-fructofuranoside ($G_{py}F_n$) or a ß-D-fructopyranosyl-[ß-D-fructofuranosyl]$_{n-1}$- ß-D-fructofuranoside ($F_{py}F_n$). The fructosyl-glucose linkage is always ß (2 ↔1) as in sucrose, but the fructosyl-fructose linkages are ß-(1 ← 2). Chicory inulin is composed of a mixture of oligo- and polymers in which the degree of polymerization (DP) varies from 2 to ~60 units with a $DP_{av}$ = 12. About 10% of the fructan chains in native chicory inulin have a DP ranging between 2 ($F_2$) and 5 ($GF_4$). The partial enzymatic hydrolysis of inulin using an endoinulinase (EC 3.2.1.7) produces oligofructose, which is a mixture of both $G_{py}F_n$ and $F_{py}F_n$ molecules, in which the DP varies from 2 to 7 with a $DP_{av}$ = 4. Oligofructose can also be obtained by enzymatic synthesis (transfructosylation) using the fungal enzyme ß-fructosidase (EC 3.2.1.7) from Aspergillus Niger. In such a synthetic compound, the DP varies from 2 to 4 with $DP_{av}$ = 3.6 and all oligomers are of $G_{py}F_n$ type. By applying specific separation technologies, the food industry also produces a long-chain inulin known as inulin HP (DP 10 to 60) with a $DP_{av}$ = 25. Finally, mixing oligofructose and long-chain inulin has produced specific products known as Oligofructose Synergy. The different industrial products vary in $DP_{av}$, $DP_{max}$, and DP distribution, and they have varying properties (Franck A 2002).

Indeed, oligofructose and fructooligosaccharides are considered to be synonymous names for the mixture of small inulin oligomers with $DP_{max} < 10$ (Roberfroid MB 2002; Cherbut C 2002; Roberfroid et al 1998). The gross molecular formula of inulin is $GF_n$, with G being a terminal glucosyl unit, F representing the fructosyl units and "n" representing the

number of fructosyl units. The basic GF2 trimer in inulin and the shortest fructan of the inulin type is 1-kestose. The same bonds link the ensuing fructosyl units, i.e. P(2-->l) as that in 1-kestose, Figure 2.3

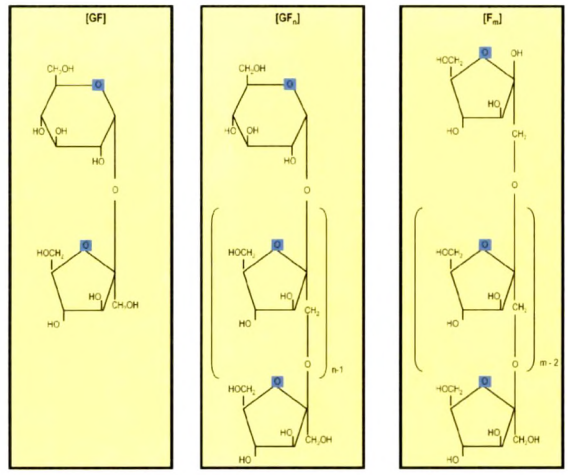



**Fig.2.3 Chemical Structure of sucrose (left), Inulin (center), Oligofructose (right)**

The plant that is most commonly used industrially for the extraction of inulin-type fructans belongs to the Compositae family, i.e., chicory. Because of the ß-configuration of the anomeric $C_2$ in its fructose monomers, inulin-type fructans resist hydrolysis by human small intestinal digestive enzymes, which are specific for α-glycosidic bonds. They have thus been classified as "nondigestible" oligosaccharides (Delzenne et al 1994; Roberfroid et al 2000).

***b) Content of Inulin in Foods***

Inulin is produced naturally in over 36,000 plants worldwide, including 1,200 native grasses belonging to 10 families (Carpita et al 1989, Marchetti 1993b). It has been estimated that as much as one third of the total vegetation on earth consists of plants that contain fructans. Inulin content in certain foods is shown in Table 2.7.

**Table 2.7: Inulin Content in Certain Foods**

| Plant Source | Inulin (g/100 g) | |
|---|---|---|
| | Range[1] | Midpoint[2] |
| Jerusalem artichoke (Tuber) – (Helianthus tuberosus) | 16.0 – 20.0 | 18.0 |
| Chicory (root) – (cichorium intybus) | 35.7 – 47.6 | 41.6 |
| Asparagus (root/tuber) – (Asparagus officinalis) | 2.0 – 3.0 | 2.5 |
| Raw | 1.4 – 2.0 | 1.7 |
| Boiled | | |
| Leek (bulb) – (Alium ampeloprasum) | 3.0 – 10.0 | 6.5 |
| Raw | | |
| Garlic (Bulb) – (Allium sativum) | 9.0 – 16.0 | 12.5 |
| Raw | 20.3 -36.1 | 28.2 |
| Dried[3] | | |
| Clobe artichoke (leaf/heart) – (Cynara scolymus) | 2.0 – 6.8 | 4.4 |
| Banana (fruit) – (Musa cavendishii L.) | 0.3 – 0.7 | 0.5 |
| Raw | 0.9 – 2.0 | 1.4 |
| Raw – Dried | 0.1 – 0.3 | 0.2 |
| Canned | | |
| Barley (cereal) – (Hordeum valgare) | 0.5 – 1.0 | 0.8 |
| Raw | 0.1 – 0.2 | 0.2 |
| Cooked | | |

Source: Van Loo et al 1995

### *c) Consumption Pattern of inulin*

There are studies reporting the average daily intake of inulin and its hydrolysis products in Western Europe estimated to be in the range of 2-12 g/person/day (*Roberfroid*, Gibson and Delzenne 1993). The U.S. consumption, estimated at 2-8 g/person/day, is slightly less based on data from the U.S. Nationwide Food Consumption Survey 1987-88 (Roberfroid et al 1993).

A USDA study by Moshfegh et al (1999) showed that American diets provide about 2.6 g of inulin and 2.5 g of oligofructose. Mean intakes varied by gender and age groups with a range from 1.3 grams for young children to 3.5 grams for teenage boys and adult males. per 1000 calories, mean intake ranged from 0.9 to 1.5 grams in American diets. Significant differences exist between variable socio-demographic categories. The primary sources of inulin in American diets are wheat and onions, Figure 2.4.

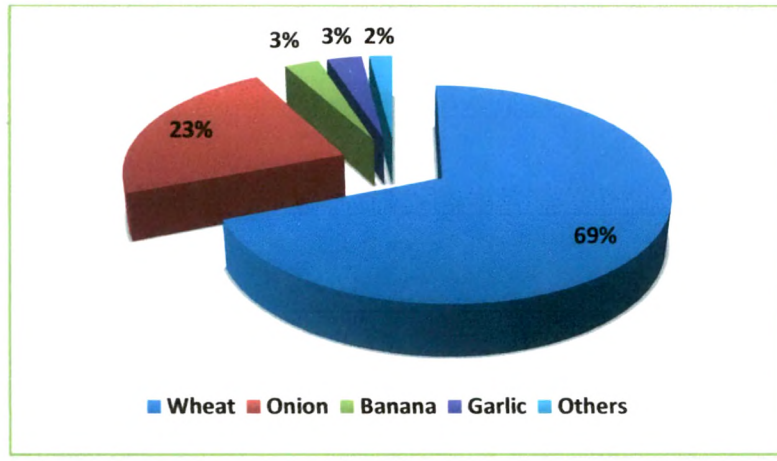



**Fig 2.4 Primary vegetable sources of inulin in the American Diet**

A study was undertaken to determine the consumption pattern of prebiotic and probiotic foods and determine the gut health of young adult females (18-25 yrs) of urban Baroda. The consumption pattern of prebiotic and probiotic Foods was studied using food frequency questionnaire and seven day estimated record method. Subjects were identified with frequent and least frequent intakes of these foods. The results revealed (Figure 2.5) that the young adult females frequently (10 to 18 food items daily, thrice a week) consumed Prebiotic and Probiotic foods (Sheth, Parnami and Bumra 2007).

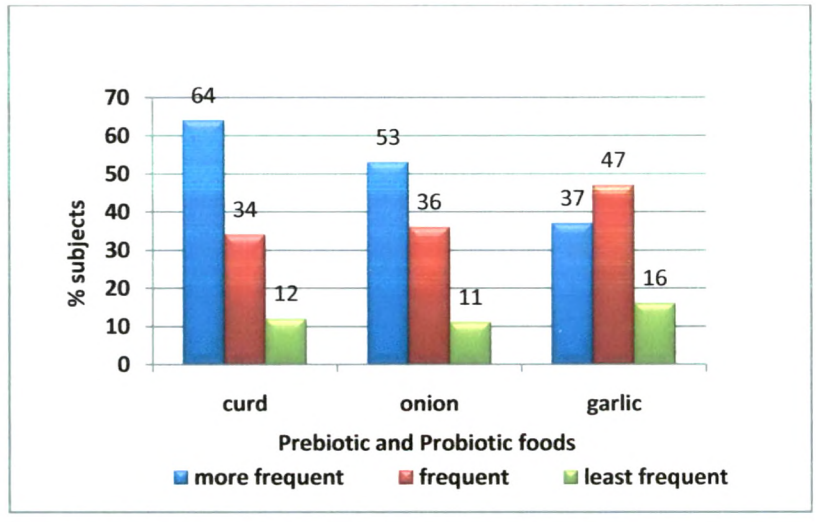



**Fig.2.5 Consumption Pattern of Curd, Onion and Garlic as Prebiotic and Probiotic foods**

Thus it was worthwhile to study selected commonly consumed cereals, pulses, roots, tubers, vegetables and fruits for their inulin content. Most foods with the exception of fruits are consumed after processing. The wheat flour is used in the preparation of several traditional food products depending upon the regional food habits viz unleavened bread *(chapati),* wheat porridge, fried flattened bread (*puri*), bread (baked).Therefore effect of processing (roasting, frying, boiling and baking) on inulin content of wheat was studied.

***d) Safety and tolerance of inulin in diet***

Estimates of current daily inulin consumption from various natural foods range from 1 to 4 grams for Americans and up to 12 grams for Europeans (Marchetti 1993b). Historically, the dietary intake of inulin has been significantly higher than current-day consumption estimates, as stated previously in other sections. Estimates of inulin intake from consumption of these foods include approximately 25 to 32 grams of inulin per day by European populations substituting Jerusalem artichokes for potatoes and approximately 160 to 260 grams of inulin per day from inulin consumption by the Australian aborigines. Human tolerance to inulin, as a class of compounds, is primarily dictated by chain length and dosage (Rumessen and Gudman-Hoyer 1998). Abdominal symptoms, primarily gas and some abdominal discomfort, increased with increasing dose and decreasing chain length.

Similar to other dietary fibers, human tolerance to inulin has been demonstrated to be greater when inulin is part of the regular diet, spread out over the course of the day, as opposed to a bolus dose. Review of the clinical study data, indicates regular consumption of 40 to 70 grams of native chicory inulin DP 2 to 60; avg. DP 9) per day by healthy adults appears to result in no significant adverse effects, especially when the consumption is in divided doses over the course of the day. This estimated consumption range is further reinforced by observations in recent review articles, reporting consumption amounts up to 40 grams of inulin daily in various food preparations do not lead to any undesired side effects (Grahn 1994, Kleessen1997).

**e) Caloric value of inulin**

Longer chain native oligosaccharides (Inulin) and shorter chain synthetic fructooligosaccharides (Neosugar)-GF2,GF3,GF4 reach the large intestine virtually intact and, as such, were considered not to be a major source of energy (Oku et al 1984). Furthermore, in the rat model, there appear to be no hydrolytic enzymatic adjustments in the small intestine to long-term ingestion of these factors. Nilsson and others (1988 a,b) used oral intubation to give fructans with a DP of about 9 or DP 16 to rats and found that both proceeded as undigested material through the gastrointestinal tract to the colon. However, due to the bacterial fermentation that occurs in the colon, these oligosaccharides do contribute to the energy pool. The caloric value of a fructosyl unit of oligofructose is calculated at 30 to 40% of a digested fructose molecule or between 1- 1. 5 kcal/g (Roberfroid et al 1993). Ranhotra and coworkers (1993) reported a caloric value for oligofructose of 1.48 kcal/g. They determined usable energy value based on efficiency of conversion of gross food energy to net energy (carcass energy) using young rats as the test model. Molis et al1996 further defined the energy value of fructooligosaccharides (44% GF2; 46% GF3; and 10% GF4) working with six healthy human subjects. Calculated mean energy value of the fructooligosaccharide was 9.5+0.6 kJ/g (range: 8.3-11.7 kJ/g) or about 2 kcal/gram. For nutrition labeling purposes, Roberfoid (1999) recommends that inulin and oligofructose, as well as all nondigestible oligosaccharides that are mostly fermented in the colon, be assigned a caloric value of 1.5 kcal/g (6.3 kJ/g).

**f) Legal, regulatory and dietary fiber status**

Inulin derived from various natural sources, such as chicory root, dahlia, and Jerusalem artichoke is legally classified as a food or natural food ingredient, and has non-additive status. In the United States, inulin has food ingredient status and is Generally Recognized as Safe (GRAS) and can be used without any significant restrictions for all intended food categories, unless the food is standardized and the standard does not permit its use. Canada also accepts inulin as a food ingredient without restriction on use level or the foods in which it can be used, provided there are not limitations on standard of identity for a specific food.

Inulin is classified as a food ingredient according to the European Directive 95/002 on Food Additives, and is excluded from additive status. All the European Union (EU) countries list inulin as having food ingredient (non-additive) status. Other countries giving inulin food ingredient status are Norway, Finland, Denmark, Ireland, United Kingdom, Switzerland, Israel, South Africa, Australia, New Zealand, and Japan. In addition to its food ingredient status, inulin is further considered as an agricultural product in Europe as part of the EC Treaty.

Inulin and oligofructose are dietary fiber by their definition and their nutritional properties. In the United States, Canada, Australia, and some other countries that define dietary fiber by prescribing a specific analytical method are used, such as an official AOAC-type, inulin and oligofructose cannot be labeled in their entirety on the nutritional or supplemental facts panel. In Europe, according to the European Nutrition Labeling Directive 90/496, inulin and oligofructose can only be designated as being either carbohydrates or as dietary fiber. Inulin has been accepted as a food ingredient for labeling as dietary fiber in all the EU countries as well as Norway, Finland, South Africa, Ireland, Switzerland, and Portugal.

Inulin-type fructans are plant carbohydrates that, because of the ß–(2 ← 1) configuration of the fructosyl-fructose glycosidic linkages, resist digestion in the upper gastrointestinal tract but are quantitatively fermented in the colon. They are thus undoubtedly part of the dietary fiber complex, and they must be labeled as dietary fiber on consumer food products.

As a follow-up of International Life Science Institute (ILSI North America 1994 ) mini-workshop on complex carbohydrates, a workshop was held at the AOAC International meeting in Nashville in (1995) to determine if there was agreement among representative scientists as to a definition of complex carbohydrates and dietary fiber (AOAC International Workshop 1995 ). There was general agreement among the workshop participants that dietary fiber should be included in the definition of complex carbohydrates, but more importantly, they agreed that resistant oligosaccharides, namely,

inulin and oligofructose, be included in the dietary fiber complex. The results of three AOAC International surveys also supported the expansion of the dietary fiber definition to include resistant oligosaccharides (Prosky L 1995 and 1999). Further, representatives of the FDA in December of 1995 stated that they would consider inulin and oligofructose as dietary fiber if the method for their measurement would pass the scrutiny of an AOAC International collaborative study.

***g) Technological functionality of inulin***

Inulin has bland neutral taste without any off flavor or aftertaste. It can be classified into 2 types as follows

1. Standard Inulin: because it contains fructose, glucose and sucrose native inulin is slightly sweet (10% sweetness in comparison to sugar). It combines easily with other ingredients without modifying delicate flavours. It is moderately soluble in water (maximum 10% at room temperature) and brings out a rather low viscosity (less than 2 mPa for a 5%w/w solution in water) (Roberfroid 2001)
2. High Performance (HP) inulin: It is made in such a way that when mixed with water or another aqueous liquid, it forms a gel network resulting in a white creamy structure with a short spreadable texture, which can easily incorporated into foods. Such as gel is composed of a tridimensional network of insoluble sub micron crystalline inulin particles in water. Large amount of water are immobilized in this network, which assures physical stability.

The nutritional properties of inulin are based on three factors.

i. Upon ingestion, inulin is not broken down by the human digestive system and for the purpose of food labeling it is recommended that it should be given an energy value of 6′28 kJ/g (Roberfroid 1999a).
ii. Inulin is fermented by colonic bacteria causing an increase in faecal biomass, production of short-chain fatty acids and decreases in caecocolonic pH and may therefore be classed as a dietary fibre (Roberfroid 1993).
iii. Ingestion of inulin results in a significant increase of *Bifidobacteria* in the colon and inhibits the growth of less beneficial bacteria (Roberfroid et al 1998, Roberfroid 1999b, Van Loo et al 1999, Kruse et al 1999).

Using this ingredient in food formulations allows the nutritional value of the end product to be improved by increasing the dietary fibre content, reducing the calorie content and increasing the bifidus-promoting capacities. As more and more scientific data become available, the nutritional benefits of inulin became further apparent (Roberfroid 1997, Roberfroid and Delzenne 1998). This ingredients offer a unique combination of nutritional and technological advantages. Therefore it is often taken as practical illustrations of active food ingredient for 'functional foods' (Van Loo et al 1999).

The recent interest in inulin also results from its interesting technological characteristics. Inulin has a neutral taste, is colourless and has minimal influence on the organoleptic characteristics of a product. Combining inulin with high intensity sweeteners significantly improves the taste of products giving a more sugar-like sweetness. Owing to the high solubility of this ingredient over `classical fibres' inulin can be used to fortify dairy products such as milk drinks, yoghurt, cheeses and desserts, which have been traditionally difficult to fortify (Niness 1999). The functionality of inulin is based on its effect on water solutions at various solid levels. At lower concentrations it causes significant viscosity increase and can be used as a rheology modifier. At a concentration of 40-45% an inulin gel or cream is formed which is firm but with a fatty creamy feel. This cream offers many advantages. Water is tightly bound in the gel, helping to maximize freeze-thaw stability and to inhibit syneresis. In this form inulin is stable in acidic conditions or at high temperatures owing to lack of available water (Silva 1996). This has created the opportunity for use in `reduced fat' mayonnaise and salad dressings. It can be used in fat-free products to give a smooth creamy texture and taste. It allows the manufacturer to replace fat containing 37′6 kJ/g with an inulin/water combination which has an energy value of 2′09 kJ/g or less, resulting in significant calorie reduction (Wouters 1998). Inulin has been successfully applied in fat-reduced table spreads, cheese products, meat products, frozen desserts, fillings, sauces and meat replacers (Franck 2002).

Specific applications include the following.

(1) Table spreads: both fat and water form a continuum with inulin, so that inulin can both replace significant amounts of fat and stabilize the emulsion while providing a short spreadable texture.

(2) Breads: inulin cream can be used to increase water addition in doughs as well as moisture retention.

(3) Chocolate: inulin can be used as a sucrose replacer, especially when combined with fructose or polyols.

(4) Ice cream: inulin functions as an inhibitor of water crystal growth.

(5) Dairy-based mousses: the incorporation of 1-5% inulin enables the product to retain its structure for a longer time and gives a rich fat-like mouthfeel.

Due to the unique functional properties, inulin manages water effectively, affects rheology and improves texture in foods and acts synergistically with high water binding hydrocolloids.This has allowed inulin to be used across all food product application areas, particularly in low and no fat and low and no sugar systems.

Purified, analytical-grade inulin occurs as spherical crystals with radial striation. Its average molecular weight is between 5,600 and 6,300 fluctuations depending on the degree of polymerization of the molecules used in the measurement. However, refined native inulin powder from chicory is white, amorphous, and slightly hygroscopic; has a specific gravity of about 1.35 and an average molecular weight of about 1,600. It is neutral in odor and taste. Commercial inulin contributes a marginally sweet taste due to a small amount of naturally occurring mono-and disaccharides.

Inulin is soluble in water with the solubility dependent on the temperature of the water, degree of polymerization, distribution of the molecular chains, degree of molecular branching and how the molecule is processed. Typically, native chicory inulin is soluble to about 60 g/L. at 10°C, while at 90°C it is soluble to about 330 g/L. Under normal conditions native chicory inulin is dispersible in water but may have a tendency to clump during hydration due to its hygroscopic character. Dispersability may be improved either

through mixing with sugar and/or starch or by instituting the final product. Native chicory inulin has a water binding capacity of about 1:1.5.

**Table 2.8: Overview of Food Applications with Inulin and Oligofructose**

| Application | Functionality | Dosage level inulin (% w/w) |
|---|---|---|
| Dairy products | Sugar and fat replacement<br>Synergy with sweeteners<br>Body and mouthfeel<br>Foam stability<br>Fibre and prebiotic | 2-10 |
| Frozen desserts | Sugar and fat replacement<br>Texture and melting<br>Synergy with sweeteners<br>Fibre and prebiotic | 2-10 |
| Table spreads | Fat replacement<br>Texture and spreadability<br>Emulsion stability<br>Fibre and prebiotic | 2-10 |
| Baked goods and breads | Fibre and prebiotic<br>Moisture retention<br>Sugar replacement | 2-15 |
| Breakfast cereals | Fibre and prebiotic<br>Crispness and expansion | 2-25 |
| Fillings | Sugar and fat replacement<br>Texture improvement | 2-30 |
| Fruit preparations | Sugar replacement Synergy with sweeteners Body and mouthfeel<br>Fibre and prebiotic | 2-10 |
| Salad-dressings | Fat replacement<br>Body and mouthfeel | 2-10 |
| Meat products | Fat replacement<br>Texture and stability<br>Fibre and prebiotic | 2-10 |
| Dietetic products and meat replacers | Sugar and fat replacement<br>Synergy with sweeteners<br>Low caloric value<br>Body and mouthfeel<br>Fibre and prebiotic | 2-15 |
| Chocolate | Sugar replacement<br>Fibre and prebiotic<br>Heat resistance | 5-30 |

Native chicory inulin has a unique ability to add rheological and textural properties to food due to its ability to form discrete highly stable particle gels. Inulin gel characteristics are dependent on a number of factors including inulin solids concentration, which becomes more viscous and fat-like as inulin solids are increased, In addition to concentration, inulin chain length distribution also affects gel characteristics. Higher degrees of polymerization (long chain frictions) lower the inulin level required to form a gel. Increasing amounts of monomer and dozer content decrease viscosity, inulin gels are very creamy and fat-like, and as such can be used in fat reduction and fat-replacer systems.

***h) Inulin in yoghurt***

Yogurt is one of the best-known of the foods that contain probiotics.Yoghurt is a dairy product produced by bacterial fermentation of milk. It is made by introducing specific bacteria into milk under controlled temperature and environmental conditions, especially in industrial production. Yogurt is a product of the lactic acid fermentation of milk by addition of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp.bulgaricus.* In some countries less traditional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii ssp. lactis,* are sometimes mixed with the starter culture .The final nutritional composition of yogurt is also affected by the species and strains of bacteria used in the fermentation, the source and type of milk solids that may be added before fermentation, and the temperature and duration of the fermentation process.

Yogurt, Kefir, and similar fermented milk products are on the way to becoming major nutraceuticals aimed at treating a variety of disease conditions. Yogurt is an excellent source of protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1) and Vitamin B12, and a valuable source of folate, niacin, magnesium and zinc. The protein it provides is of high biological value, and the vitamins and minerals found in milk and dairy foods including yogurt are bioavailable. Yogurt particularly the low-fat varieties, provide an array of important nutrients in significant amounts in relation to their energy and fat content, making them a nutrient-dense food. Eating dairy products, such as

yogurt, helps to improve the overall quality of the diet and increases the chances of achieving nutritional recommendations (Terry et al 1999).

Inulin possesses unique physical and physiological characteristics making it widely useful for adding texture in food applications. In consumer tests, plain unsweetened yogurt containing inulin was preferred over samples without inulin. Yogurt with inulin was identified as being creamier in appearance, having a less chalky and more creamy texture, and was sweeter with a less sour/fermented taste and aftertaste (Spiegel et al 1994). Yogurt made with 10% inulin with a DP of 12-16 was found to increase firmness and decrease syneresis compared to yogurt made with shorter chained inulin (DP 5-8) and controls with no inulin (Terry et al 1999).

***c) Inulin-type fructans and their health claims***

However, because of their specific fermentative properties, inulin-type fructans do have characteristic features different from those of other dietary fibers. Therefore, they may contribute in a significant way to a well-balanced diet by increasing the fiber content, by improving the diversity of the fiber sources, and by specifically affecting several gastrointestinal functions (composition of intestinal microflora, mucosal functions, endocrine activities, mineral absorption) and even systemic functions (especially lipid homeostasis and immune functions) as well as by reducing the risk of miscellaneous diseases. These effects are summarized in Table 2.9.

**Table 2.9: Nutritional Effects and Potential Health Benefits of Inulin-type Fructans**

| Enhanced functions | Reduction of disease risks |
|---|---|
| Composition and activities of the gut microflora | Intestinal infections |
| Stool production | Irritable bowel diseases |
| Absorption of Ca and other minerals | Colon cancer |
| Production of gastrointestinal endocrine peptides | Osteoporosis |
| Immunity and resistance to infections | Obesity |
| Lipid Homeostatis | |

**Table 2.10: Experimental and Human Data that Substantiate Claims on Inulin-Type Fructans: Summary Presentation**

| Property or target function | Supportive evidence | Claims: Inulin-type fructans... |
|---|---|---|
| Dietary fiber | Oligo/polysaccharide<br>Resistant to digestion<br>Fermentation | Are dietary fiber |
| Bowel functions | Bulking effect | Regularize bowel functions |
| Stool production | Regulation of stool production<br>Improved stool consistency | |
| Colonic microflora | Substrates for anaerobic saccharolytic fermentation<br>Selective stimulation of growth of health-promoting bacteria (e.g., *Bifidobacteria*) | Are prebiotic |
| Bioavailability of Ca and Mg | Increased absorption of Ca/Mg | Increase Ca/Mg absorption |
| | Increased bone mineral content/density | Increase bone mineral content/density in adolescents |
| Lipid homeostasis | Reduction of triglyceridemia | Reduce triglyceridemia in slightly hypertriglyceridemic individuals |

**Table 2.11: Data on Inulin-Type Fructans that Support Hypotheses to be Tested in Human Nutrition and Clinical Intervention Studies: Summary Presentation**

| Target functions or disease risk | Supportive evidence |
|---|---|
| **Lipid homeostasis** | Reduced cholesterolemia |
| **Immunostimulation** | Improved resistance to common infections in children<br>Improved response to vaccination |
| **Gastrointestinal endocrinology** | Stimulation of production of intestinal hormonal peptides (GIP, GLP-1, PYY, ghrelin...)<br>Regulation of appetite |
| **Inflammatory bowel diseases (IBDs)** | Improved management of the diseases<br>Improved clinical symptoms<br>Improved biomarkers |
| **Colon cancer** | Animal data in different experimental models + SYNCAN + NCI comparative trial |

The effects of inulin-type fructans have been investigated in different domains of interest using a wide variety of experimental models and human trials. These domains can be divided into the following categories:

Category 1: Experimental results exist that have been evaluated and used to justify human intervention studies; confirmatory human data of these human trials are available to substantiate claims. The domains included in this category are dietary fiber and bowel functions, gut microflora, gastrointestinal absorption of minerals, and lipid (triglycerides) metabolism (Table 2.10).

Category 2: Data from different experimental models are convincing, preliminary human data are available, and sound hypotheses exist, but more human nutrition trials are necessary to substantiate claims. The domains included in this category are cholesterol metabolism, gut-associated immune functions, IBD, and colon cancer (Table 2.11).

Category 3: Recent experimental investigations have generated promising results that justify more extensive studies including, in some cases, preliminary tests in human volunteers. The domains that are included in this category are bone health, gastrointestinal endocrinology, cancer therapy, and behavior and cognitive performance (Table 2.12).

**Table 2.12: Data on inulin-type Fructans that Require More Experimental Research to Support Hypotheses to be tested in Human Nutrition and Clinical Intervention Studies: Summary Presentation**

| Property or target functions | Supportive evidence |
|---|---|
| **Gastrointestinal absorption of minerals** | Increased absorption of Fe, Cu, Zn |
| **Lipid homeostasis** | Reduction of lipid pool in obese rats |
| **Defense mechanisms** | Improved barrier functions |
| | Improved resistance to intestinal infections |
| **Cancer development** | Slowing of tumor growth |
| | Reduction of risk of metastasis |
| | Improved efficacy of cancer therapies |

Human trials with oligofructose and inulin include those with a controlled diet and crossover feeding trials, although the dose, substrate, duration, and volunteers vary (Table 2.13).

Together the evidence available today from both in vitro and in vivo experiments supports the classification of inulin-type fructans as prebiotic.

Inulin-type fructans are thus classified as functional food ingredients that target gastrointestinal functions but also, most likely via their effects on the gut and the gut microflora, systemic functions that are known to be closely related to health and well-being. microflora and selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well-being. In vitro data (Table 2.14) supporting the selective stimulation of bacterial growth by inulin have been generated in numerous studies carried out either in defined pure culture fermentation or by using human feces in both batch and continuous culture (Roberfroid et al 1998).

In addition to in vitro work, in vivo studies have also been carried out using animal models that all confirmed the bifidogenic effect of inulin-type fructans (Levrat et al 1991, Campbell et al 1997, Kleessen et al 2001).

**Section 2.6 Modulation of gut microbiodata and inulin type fructans**

The gastrointestinal functions are primary endpoints that benefit most from inulin-type fructans. One of the most promising effects is modulation of activities of the colon, an organ of the gastrointestinal tract that is recognized more and more as playing a variety of key roles in maintaining health and well-being as well as reducing the risk of diseases (Cummings JH 1997, Gibson and Roberfroid 1995, Berg RD 1996, Cummings and Macfarlane 1997, Rowland IR 1988 a,b ).

The concept of "colonic health" has thus emerged as a major target for functional food development in the area of enhanced function claims (Salmenin et al 1998).

**Table 2.13: Summary Presentation of Major Studies Demonstrating the Selective Stimulation of Bacterial Growth by Inulin-Type Fructans in Healthy Human Feeding Trials**

| Study Design | Observations | Investigators |
|---|---|---|
| 23 subjects fed oligofructose (8 g/day) for 2 weeks | Selective increase in fecal *Bifidobacteria* | Mitsouka et al (1987) |
| 10 subjects fed oligofructose (4 g/day) for 2 weeks | Selective increase in fecal *Bifidobacteria* | Williams et al (1994) |
| 12 subjects fed oligofructose (4 g/day) for 25 days | Selective increase in fecal *Bifidobacteria* | Buddington et al (1994) |
| 8 subjects on a controlled diet were fed oligofructose (15 g/day) for 15 days Subsequently 4 of these subjects were fed inulin (15 g/day) for 15 days | Oligofructose selectively increased fecal *Bifidobacteria* and decreased bacteroides, Clostridia, and fusobacteria. Inulin selectively increased *Bifidobacteria* and decreased Gram positive cocci | Gibson et al (1995) |
| 20 subjects were fed 12.5 g/day oligofructose for 12 days | Significant increase in fecal *Bifidobacteria* | Bouhnik et al (1996) |
| 10 female elderly subjects were given inulin (20 and 40 g/ day) for 19 days | Selective increase in fecal *Bifidobacteria* and significant decrease in bacteroides | Kleessen et al (1997) |
| 40 subjects fed 2.5,10, and 20 g/day oligofructose for 7 days | Selective agars showed that *Bifidobacteria* were most increased by 10 and 20 g doses of oligofructose compared to 2.5 g and that the optimum dose of oligofructose was found to be 10 g/day | Bouhnik et al (1999) |
| Chicory inulin hydrosylate fed to 8 subjects in a controlled feeding study, 8 g/day | Selective agars showed an increase in fecal *Bifidobacteria* | Menne et al (2000) |
| 8 subjects fed up to 34 g/day inulin for a period of 2 months | Selective increase in fecal *Bifidobacteria* that lasted for the whole 2 months period | Kruse et al (1999) |
| 8 young volunteers fed oligofructose (5 g/day) for 3 weeks | Selective increase in fecal *Bifidobacteria* | Rao et al (2001) |
| 8 subjects fed biscuits containing high molecular weight inulin 21 days | FISH* revealed a selective increase in fecal *Bifidobacteria* | Touhy et al (2001) |
| 19 elderly patients fed oligofructose (8 g/day) for 3 weeks | Selective increase in fecal *Bifidobacteria* | Guigoz et al (2002) |
| 12 adult volunteers were given long chain inulin (9 g/day) for 2 weeks | Quantification of all bacteria, *Bifidobacteria*, the *Eubacterium rectale-Clostridium coccoides* group (Erec group), Bacteroides, and eubacteria were counted with FISH probes. A significant increase in *Bifidobacteria* and a significant decrease in Erec group was observed | Harmsen et al (2002) |

*FISH-Flourescence *in situ* hybridization (It is a methodology applicable to enumerate bacteria in fecal microbiota)

**Table 2.14: Summary Description of Studies Carried out to Demonstrate the *In Vitro* Selectivity of Inulin-Type Fructans in Both Pure Culture, Mixed Batch Culture, and Mixed Continuous Culture Fermentation**

| Aims of Study | Observations | References |
|---|---|---|
| Batch culture using fecal inocula to study fermentation of inulin-type fructans, starch, polydextrose, fructose, and pectin | *Bifidobacteria* most increased with inulin-type fructans while populations of E. *coli* and Clostridia were maintained at relatively low levels | Wang and Gibson (1993) |
| Examining the growth of *Bifidobacteria* on different types of oligofructose in pure culture. Eight species tested as well as species of Clostridia, bacteroides, enterococci, and E. *coli* | Linear oligofructose had more of a bifidogenic effect than larger MW molecules and branched chain varieties. *Bifidobacteria* species showed a preference for inulin-type fructans compared to glucose | Gibson and Wang (1994b) |
| Continuous culture fermentation to study fermentation of oligofructose | Selective culturing showed *Bifidobacteria*, and to a lesser extent *Lactobacilli* , preferred oligofructose to inulin and sucrose. Bacteroides could not grow on oligofructose | Gibson and Wang (1994b) |
| Species of *Bifidobacteria (longutn, breve, pseudocatenulatum, adolescentis)* were tested in pure culture for their ability to ferment inulin-type fructans | *B. adolescentis* was seen to grow best and was able to metabolize all types of inulin-type fructans | Marx et al (2000) |
| Batch culture using fecal inocula to study fermentation of oligofructose, branched fructan, levan, maltodextrin | FISH revealed that branched fructan had the best prebiotic effect, followed by oligofructose | Probert and Gibson (2002) |
| The ability of *Bifidobacteria* and *Lactobacilli* to grow on MRS agar containing oligofructose was investigated. | 7/8 *Bifidobacteria* and 12/16 *Lactobacilli* were able to grow on agar containing oligofructose | Kaplan and Hutkins (2000) |

***a) Concept of balanced microflora***

The composition of the symbiotic colonic microflora is a key player in maintaining the colon (and thus the whole body) health. That composition is largely determined by the flora that establishes at and immediately after birth, is mostly "individual," can be modulated by specific compounds in the diet, and may change during the lifetime, becoming more and more complex as we age (Blaut 2002).

To support health and well-being and to reduce the risk of various diseases, we hypothesized that the gut (and especially the colon) microflora must remain a "balanced microflora," i.e., a microflora composed predominantly (in numbers) of bacteria recognized as potentially health-promoting (such as *Lactobacilli, Bifidobacteria, fusobacteria*) to prevent, impair, or control the proliferation of potentially pathogenic/harmful microorganisms (including some species of *E. coli, clostridia, veillonellae*, or *Candida*). The colonic microflora is a complex "ecosystem" with a wide variety of potential interactions among the different populations of microorganisms.

***b) Gastrointestinal health, its composition and significance***

The intestinal mucosa is the main site of interaction with external environment and therefore has an important role in maintaining good health. Formation of the gut is one of the first outcomes of multicellularity. It appears on the first impression to be quite a simple organ as it is an epithelial tube comprising different cells surrounded by a layer of muscle. However, the human gastrointestinal track is a highly dynamic ecosystem. The total area of the mucosal surface of the human gastrointestinal tract is 300 $m^2$ which makes it the largest surface area in the body that interacts with the external environment. the gut houses an enormous microbial community with total estimates in the region of $10^{14}$ microorganisms. The distal large intestine is the area of highest colonization with more than 500 different culturable species and up to 100 billion microbial inhabitants. The total number of microorganisms present in the gastrointestinal tract varies according to location (F 2.6). For example stomach contents (per gram) could be less than $10^3$ CFU, reaching $10^4$- $10^7$ in the small intestine and $10^{10}$-$10^{12}$ per gram in the colon where the microbial numbers are highest. The end product of digestion (feces) is approximately 60 % composed of bacteria.

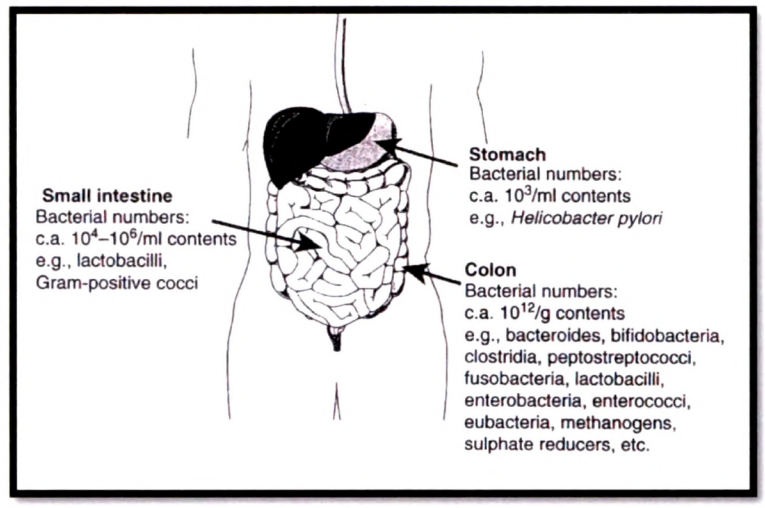



**Fig.2.6 Basic Gut Anatomy** Different regions within the gut are colonized by different types of microbial community, in terms of both species diversity and actual numbers

The gut microbiota can be thought of as a microbial organ within a human organ as the processes performed by this diverse population are extensive; it can communicate with itself ( bacteria: bacteria) and with the host ( bacteria : human ). It is also site of energy consumption, transformation, and distribution. The gastrointestinal microflora is a very complex community.

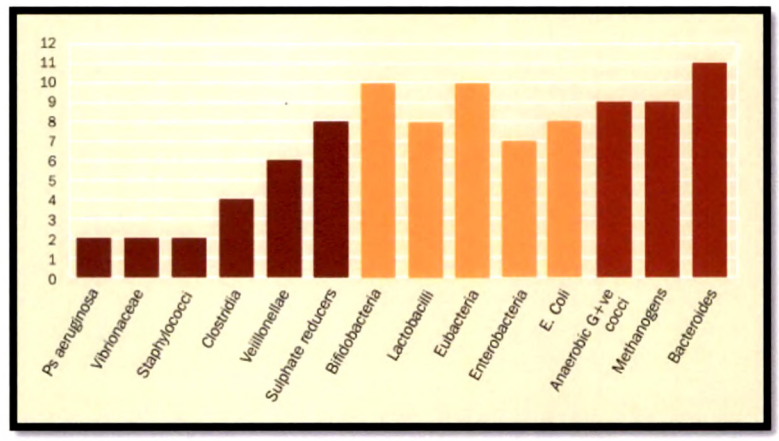



**Fig. 2.7 The Microbial Concentrations in Human Intestine (Gibson et al 1998)**

Bacterial counts per millilitre intestinal contents increase in a distal direction in the gastro-intestinal tract from $10^3$–$10^4$ in the stomach to $10^6$–$10^7$ in the distal ileum and $10^{11}$–$10^{12}$ in the colon (Table 2.15 and 2.16, Figure 2.7). Gram-positive bacteria are the dominant type in the stomach, whereas the bacteria in the distal small intestine and the colon are predominantly of the Gram-negative type. The numbers of

anaerobic bacteria increase distally. The most important intestinal bacteria are *bacteroides, Bifidobacteria, Enterobacteraceae, Lactobacilli, Gram-positive cocci, Clostridium species* and *eubacteria.* In addition, streptococci and various types of moulds and yeasts are also found in the intestines.

**Table 2.15: Composition of Human Gastro-Intestinal Flora**

| Bacterial flora | Stomach | Jejunum | Ileum | Faeces |
|---|---|---|---|---|
| Total bacterial count (/ml) | 0-10 | $0\text{-}10^5$ | $10^3\text{-}10^7$ | $10^{10}10^{12}$ |
| *Aerobic or facultative bacteria* | | | | |
| *Enterobacteria* | $0\text{-}10^2$ | $0\text{-}10^3$ | $10^2\text{-}10^6$ | $10^{10}\text{-}10^{12}$ |
| *Streptococci (including Peptostreptococcus)* | $0\text{-}10^3$ | $0\text{-}10^4$ | $10^2\text{-}10^6$ | $10^3\text{-}10^{10}$ |
| *Staphylococci* | $0\text{-}10^2$ | $0\text{-}10^3$ | $10^2\text{-}10^5$ | $10^4\text{-}10^7$ |
| *Lactobacilli* | $0\text{-}10^3$ | $0\text{-}10^4$ | $10^2\text{-}10^5$ | $10^6\text{-}10^{10}$ |
| *Fungi* | $0\text{-}10^2$ | $0\text{-}10^2$ | $10^2\text{-}10^3$ | $10^2\text{-}10^3$ |
| *Anaerobic bacteria* | | | | |
| *Bacteroides* | Rare | $0\text{-}10^2$ | $10^3\text{-}10^7$ | $10^{10}\text{-}10^{12}$ |
| *Bifidobacteria* | Rare | $0\text{-}10^3$ | $10^3\text{-}10^5$ | $10^8\text{-}10^{12}$ |
| *Gram-positive cocci* | Rare | $0\text{-}10^3$ | $10^3\text{-}10^3$ | $10^8\text{-}10^{11}$ |
| *Clostridium spp.* | Rare | rare | $10^3\text{-}10^4$ | $10^6\text{-}10^{11}$ |
| *Eubacteria* | Rare | rare | rare | $10^9\text{-}10^{12}$ |

**Table 2.16: Density and Nature of Bacteria in the Human Gastro-Intestinal Tract**

| Site | Density | Type |
|---|---|---|
| Stomach and proximal ileum | $10^3 - 10^4$ /ml | predominantly Gram-positive |
| Terminal ileum | $10^6 - 10^7$ /ml | predominantly Gram-negative |
| Colon | $10^{11} - 10^{12}$/ml | predominantly Gram-negative |

***c) Changes in the intestinal Flora***

During life, the microbiota changes dramatically in composition as depicted in Figure 2.8. The gastro-intestinal tract is a sterile environment at birth and bacterial colonization begins during the delivery process. The primary source is maternal vaginal and fecal floras that are usually ingested at the time of delivery (Stark and Lee 1982).

Again, there is a characteristic shift in the composition of the intestinal microflora when ageing. In elderly persons, *Bifidobacteria* decrease or disappear, while *Lactobacill, Enterococci, Enterobacteria* and *Clostridia* increase. This in turn may lead to increased pathogenic and toxic burdens, cancer, and disorders of liver function (Woodmansey EJ et al 2004). These changes, along with a general reduction in species diversity in most bacterial groups, and changes to diet and digestive physiology such as intestinal transit time, may result in increased putrefaction in the colon and a greater susceptibility to disease.

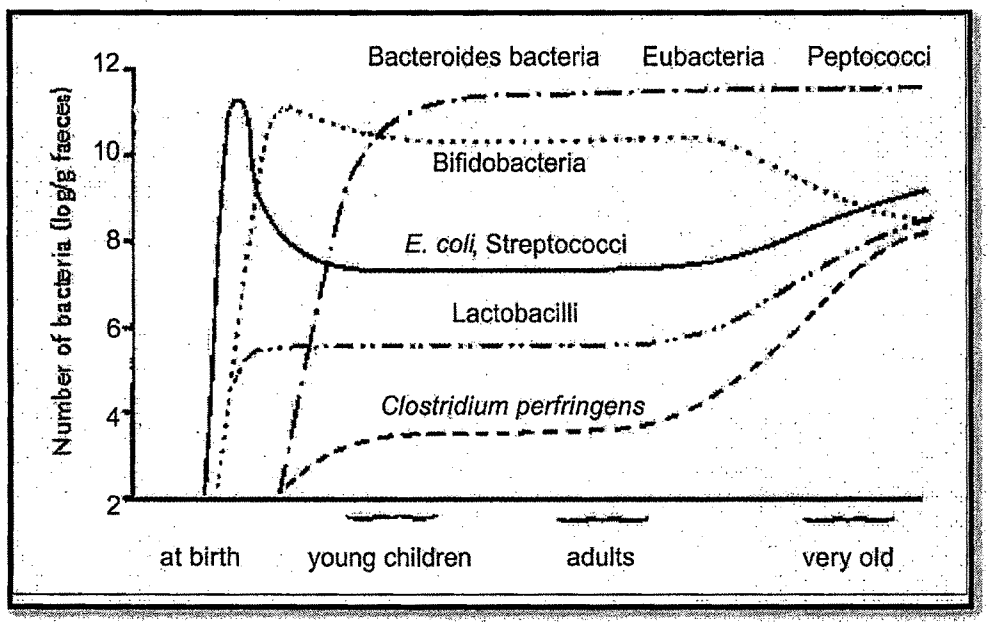



**Fig. 2.8 Changes with Age in Number of Bacteria in Faeces (MITSUOKA 1982)**

The age-related changes in the GI bacterial community in elderly humans are likely related to changes in nutritional habits, since it is well recognized that diet/dietary components codetermine the spectrum of intestinal microflora (Apajalahti et al 2001). In particular, aging is associated with the decrease in daily fiber intake (Kwok et al 2004.) related to the prolongation of mastication, the decline in olfactorial and gustatorial sensitivity, and a decrease in cognition. However, aging is also associated with alterations of GI physiology and function, such as gastric hypochlorhydria, alterations in intestinal motility, and decreased colonic transit time. Changes in GI tract physiology during the aging process may allow specific bacterial populations to take advantage of novel ecological niches, thereby altering the composition of the GI

microbiota, in turn affecting intestinal homeostasis and function (Husebye et al 2001.).

Recent studies have shown that the gut microbiota changes in old age, with an increased number of bacterial groups represented in the predominant elderly gut microbiota. This change in species "evenness" coincides with parallel changes in immune function, diet, and lifestyle and may contribute to disease susceptibility and severity in old age. The intestinal microbiota may thus be identified as an important target for improving health through reduced disease risk. A balanced intestinal flora is a precondition for a fairly stable ecosystem in which both host-related factors and antagonistic interactions among intestinal bacteria play a role (Figure 2.9).The following host related factors are relevant in this respect to gastric acid secretion, bile and pancreatic juice, intestinal motility and peristalsis, rejection of epithelial cells, secretion of immunoglobulins, lysosomal and macrophagic activity.

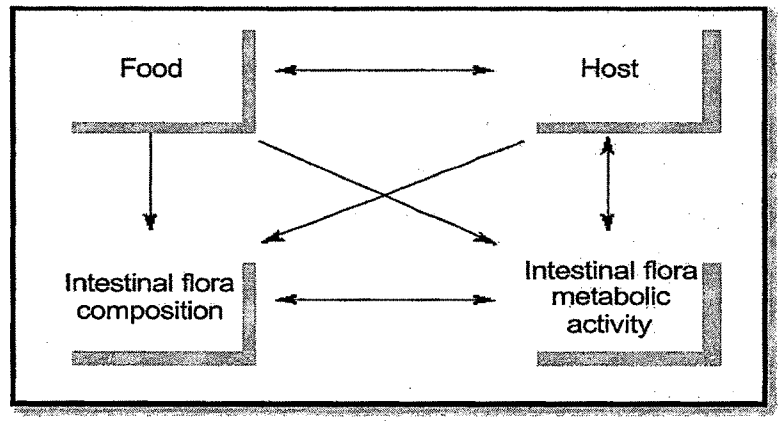



**Fig. 2.9 Schematic Presentation of Interactions between Food, Intestinal Flora and Host**

***d) Role of Bifidobacteria and lactobacillus***

The large intestine is the most heavily colonized region of the digestive tract, with up to $10^{11}$ bacteria for every gram of intestinal content (Gibson and Roberfroid 1995). Gut bacteria are comprised of one hundred different species that include both beneficial and potentially deleterious bacteria in a balance that affects how food is digested and energy is obtained, (Figure 2.10). When the main types of generally recognized beneficial bacteria, *Bifidobacteria* and *Lactobacilli* are at optimum levels they constitute approximately one-third of the bacterial population in the gastrointestinal tract. In some cases the numbers of beneficial bacteria may be so low

they are undetectable. The numbers of *Bifidobacteria* are regarded as a marker of the stability of the human intestinal microflora (Mutai and Tanaka 1987).

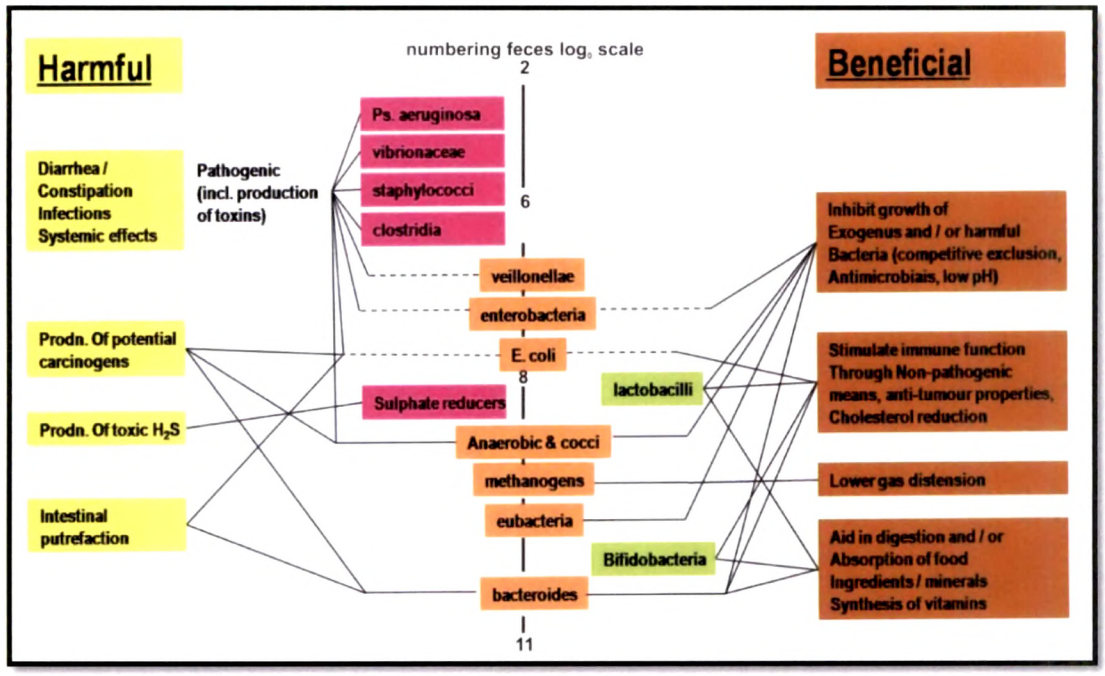



**Fig. 2.10 Schematic Overview of Colonic Microflora and Their Health Significance**

Research by Mutai and Tanaka (1987) stimulated efforts to identify the role of *Bifidobacteria* (Mitsuoka 1990a, Romond 1987), and other *lactic acid* bacteria, mainly *Lactobacilli* (Salminen et al 1993a, 1993b andTannock 1990) with regard to host health. Miller-Catchpole (1996) noted that *Bifidobacteria*, even though implicated in incidental opportunistic anaerobic infections (as in individuals with weakened immune systems), are generally regarded as safe. Stimulation of *Bifidobacteria* numbers as well as the numbers of *Lactobacilli* in the colon is beneficial to host health.

Although *Bifidobacteria* have been considered to be the most important organisms in infants, and *Lactobacilli* and E. coli are more numerous bacteria for children and adults than *Bifidobacteria*. *Bifidobacteria* also constitute one of the major organisms in the colonic flora of healthy children and adults (Mitsuoka 1990b). As a consequence to findings by Mutai and Taankk (1987), Romond (1987), Drasar and

Roberts (1989) and Mitsuoka (1990a) and a number of other researchers, scientists now generally describe to the beneficial health effects of *Bifidobacteria* in the colon, (Figure 2.11).

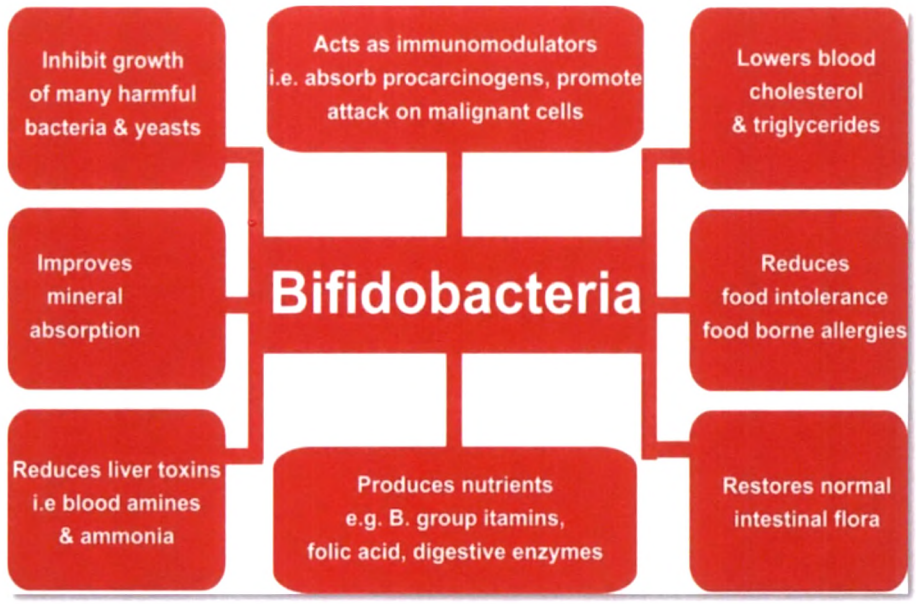



**Fig. 2.11 Colonic Bacteria and SCFA Related Health Effects of *Bifidobacteria***

These beneficial bacteria may act as wards regulating the activity of the other bacteria in the colon. The other bacteria, such as *Salmonella, Shigella, Clostridia, Staphylococcus aureaus, Candida albicans, Campylobacter jejuni, Escherichia coli, Veillonella,* and *Klebsiella,* have varying potential to cause disease and are much less numerous. However, these pathogenic bacteria can produce harmful local and systemic effects if they overgrow as a consequence of a gut microflora imbalance (Elmer et a1 1996). Research has shown beneficial bacteria, particularly *Bifidobacteria* and *Lactobacilli* keep these potential disease-causing organisms under control, preventing several disease-related dysfunctions related to an imbalances GI situation (Elmer et a1 1996).

***e) Factors affecting intestinal flora***

Factors affecting intestinal flora observed by Rasic (1994) were: animal species; habits; age; sex; climate; diet; stress; exogenous organisms; and immune mechanisms of the host. Other modifers of the gut microflora are surgery of the stomach or small intestine, kidney or liver disease, cancer, pernicious anemia, blind loop syndrome or a change in the acidity of gastric juices (Modler et al 1990). In addition, other

pathogenic disorders, such as liver cirrhosis and impaired intestinal motility may modify colonic microflora. During stages of acute infection, antibiotic therapy used to combat the bacterial invasion can also induce digestive disorders due to alteration of normal gut flora. *Bifidobacteria* have been shown to be resistant to streptomycin, but have only moderate resistance to penicillin, tetracycline, neomycin and novobiocin. *Bifidobacteria* can be completely eradicated from the colon when antibiotics such as erythromycin, spiramycin and chloramphenicol are used to combat other bacterial infections.

Reducing or eliminating more of the healthy gut microflora, like *Bifidobacteria*, has its consequences. When the human diet influences the species composition and metabolic characteristics of the intestinal microflora, toxic metabolite production is affected, such as the conversion of procarcinogens to active carcinogens (Perman 1989, Roland et al 1993, Rowland 1988). *E. coli* and *clostridia* are known to produce ammonia and amines, both liver toxins, and carcinogens and cancer promoters such as nitrosamines, phenols, cresols, indole and skatole, estrogens, secondary bile acids and aglycones. Chadwick and coworkers (1992) reported reductive enzyme activities are lower in *Bifidobacteria* and *Lactobacilli* relative to *E. coli* and *clostridia* (Drasar and Hill 1974, Kanbe 1988, Koizumi et al 1980, Mitsuoka 1982, 1990a). In addition to producing toxic metabolites, several harmful bacteria, such as *Salmonella, Shigella, Listeria, Bacteroides, Proteus, E. coli, Clostridium perfringens* and *Vibrio cholerae* also have association with diarrhea, infections, liver damage, carcinogenesis and intestinal putrefaction. It is possible the health promoting effects prompted by *Bifidobacteria* and other healthful bacteria is due to the growth inhibition of harmful bacteria, stimulation of immune functions, lowering of gas distention problems, and improved digestion/absorption of essential nutrients and synthesis of vitamins (Gibson 1995, Gibson and Roberfroid 1995, Roberfroid and Delzenne 1993).

Pathogenic effects associated with harmful intestinal microflora such as E. coli not only include colonic disorders but also have implication with possible vaginal infections and systemic disorders (Gibson and Roberfroid 1995), Figure 212. Major factors in the biology of these disorders are the overgrowth of pathogenic bacteria such as clostridia, E. coli. As well as parasites, viral infections, extensive burn injury,

post-operative stress, and antibiotic therapy. These disorders are often associated with bacterial translocation due to intestinal barrier failure (Gardner et al 1993, Gibson and MacFarlane 1994).

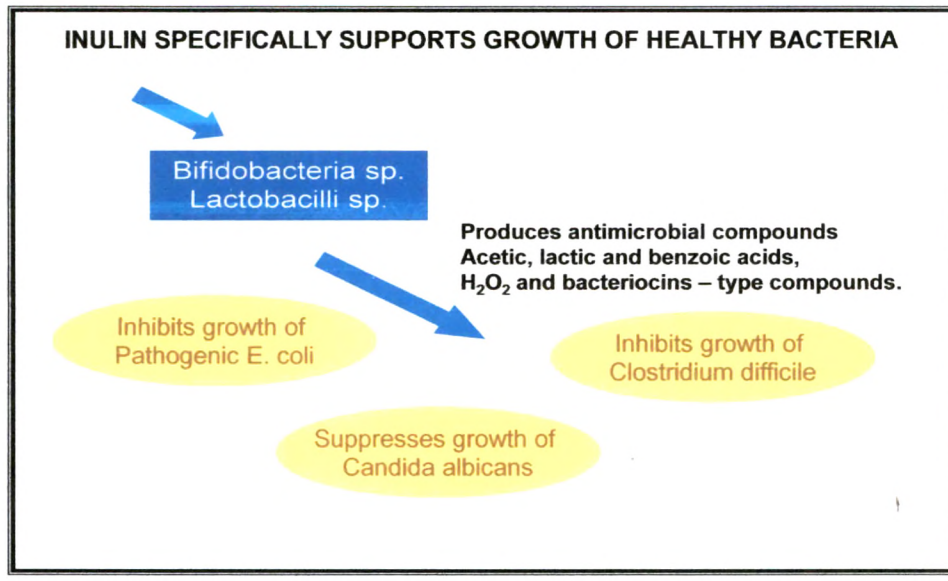



**Fig. 2.12 Pathogenic microflora suppression, from Roberfroid et al 1998**

Mechanisms related to microflora modification to a more-healthy environment vary for individual microflora groups. However, the antagonistic effects of lactic acid bacteria have been attributed to favorable competition for active sites on the colonic epithelial wall, the production of primary metabolites, such as acetic, lactic, propionic, butyric and benzoic acids, and hydrogen peroxide and the secretion of specific bacteriocins, e.g. Lactacins B, F and Lactocin 27 (Gibson and Wang 1994c, Modler et al 1990). Numerous other investigators have also reported the ability of *lactic acid bacteria* to produce antibacterial substances, which are active against certain pathogenic and putrefactive organisms (Mehta et al 1983).

Although *Bifidobacteria* do not produce hydrogen peroxide, observations by Gibson and Roberfroid (1995) hint that *Bifidobacteria* might secrete a bacteriocin-type substance that is active against *Clostridia, E. coli,* and many other pathogenic bacteria, such as *Listeria, Shigella, Salmonella,* and *Vibrio cholerae,* or antimicrobal substances. Anand (1985) tested six strains of B. bifidum for their antibacterial activity and reported that antibacterial activity differed among the strains with

inhibitory action shown by one strain against *M. flavus* followed by *Staph. aureus, B. cereus, E. coli, Ps. flourescens, S. typhosa and Sh. dysenteriae.*

Like *Lactobacill, Bifidobacteria* produce strong acids, i.e. acetic and lactic acid (Scardovi 1986), the production of these acids reduces intestinal pH. One effect of lowering the gastrointestinal pH might be the protonation of toxic ammomia ($NH_3$) to produce ammonium ion ($NH_4$+), which is non-diffusable and could result in lower blood ammonia levels and a reduced hepatic load (Levrat et al 1993, Miller-Catchpole 1989).

Acetic acid has been observed to exert a greater antimicrobial effect than lactic acid, most likely due to a greater amount of undissociated acid at intestinal pH values (5.8) common to *Bifidobacteria* and *Lactobacilli* (Modler et al 1990). Because *Bifidobacteria* produce almost two-fold more acetate than lactate, the undissociated acetic acid would be approximately 11 -fold greater than lactate. This is an important factor as the growth of many potential pathogenic bacteria is very sensitive to concentrations of undissociated acid (Modler et al 1990).

*Bifidobacteria* do not form aliphatic amines, hydrogen sulfide or nitrites (Bezkorovainy, 2001). They produce vitamins, largely of the B-group, such as biotin, thiamine, riboflavin, niacin, pyridoxine, cyanocobalamin, and folic acid (Deguchi et al 1985, Gibson et al 1995, Hartemink et al 1994). These bacteria also produce digestive enzymes such as lactase (B-galactosidase), casein phosphatase and lysozyme that may improve lactose tolerance and digestibility of dairy products (Hughes and Hoover 1991).

***f) Utilization of various non digestible oligosaccharides by gut bacteria***

Utilization of various non digestible oligosaccradies by selected human gut bacteria is shown in Table 2.17. Wang and Gibson (1993) and Gibson and Wang (1994a) while working in vitro with several *Bifidobacteria* strains, *Cl. perfringens* and *E. coli,* demonstrated that native chicory inulin allowed more rapid development of B. bifidum, *B. pseudolongum* and *B. angulatum* as compared to glucose. *B. longum* had a slower development in comparison to glucose, and the development of four other species was not significantly different. Inulin (modal 10 DP units) was demonstrated

to significantly suppress the growth of both E. coli and C. perfringens. During the fermentation of inulin, mainly $C0_2$, medial $H_2$ and relatively no $CH_4$ were produced.

McKellar et al (1993) characterizing the growth and inulinase production by *Bifidobacteria* spp. on fructooligosaccharides observed these strains to grow equally well on short-chain fructooligosaccharides and inulin as a native extract of chicory root. As for in vivo studies, Bornet and others (1997b) working with a short-chain inulin fraction observed fecal *Bifidobacteria* increases in healthy humans is dose-response related. They noted that doses of 5 and 10 g/d significantly increased colonic *Bifidobacteria* ($p<0.05$) while doses equal to or less than 2.5 g/d showed no statistically significant modification effects. In a related study, twelve elderly adults, aged 69 ± 2 yrs, ingested 8 g/d for 4 weeks and had *Bifidobacteria* counts increase from 8.52±0.26 to 9.17±0.17 log CFU/g ($p<0.05$) (Bornet et al 1997a).

However, Roberfroid et al (1998) stated that log increases in *Bifidobacteria* counts do not necessarily correlate with daily doses administered, but rather depends more on the initial number of *Bifidobacteria.* Lower initial numbers of *Bifidobacteria* have been shown to produce greater increases, irrespective of dose, within a range of 4-20 grams or more per day. An increase of *Bifidobacteria* less than one log unit is difficult to assess, and the absolute increase in number of *Bifidobacteria* is likely to be less important than the statistical significance of the increase (Roberfroid et al 1998). Gibson et al (1995) further showed humans consuming 15 grams/day Inulin (DP 2 to 60; avg. 10 units) significantly ($p<0.001$) increased the *Bifidobacteria*, from $\log_{10}$ 9.2 to log10 $_{10}$ 1 per gram in a two week period, rendering them the dominant population. The numbers of-gram-positive cocci decreased from $\log_{10}$ 6.0 to $\log_{10}$ 5,5 ($p<0.001$). In another in vivo study involving 35 elderly female subjects, mean age of 76.4 years and suffering from constipation, inulin was compared to lactose to determine effects on fecal microflora, microbial activity and bowel habit. Results showed a progressive increase in inulin ingestion from 20 g/d to 40 g/d for 19 days increased *Bifidobacteria* significantly from 7.9 to 9.2 log10/g dry feces, and decreased enterococci in number and frequency, while not changing the total bacterial counts (Kleessen et al 1997). Causey and others 2000b, in a double-blind cross-over human study involving 12 healthy male volunteers on a controlled inulin (DP 2 to 60; avg. 9 units) diet (20 g/day), showed significant increases in total anaerobes and *lactobacillus species*).

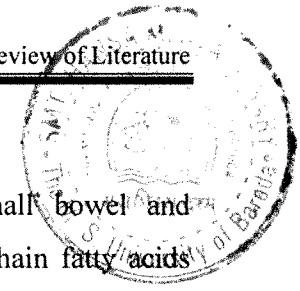
## Table 2.17: Utilization of Various NDOs by Selected Human Gut Bacteria*

| *Bacterial Species* | FOS[1)] | INU | LOL | PHGG | LAC | LAT | TOS | RAF | GLL | IMO |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Bifidobacterium spp.***[2)] | + | + | V | - | + | + | + | + | + | + |
| ***Lactobacillus acidophilus-group*** | V | + | + | - | + | + | + | - | + | V |
| *L. casei* | V | + | + | - | - | + | + | - | - | - |
| ***Bacteroides fragilis*** | + | + | + | - | + | + | + | V | + | + |
| *B. thetaitotaomicron* | + | + | + | - | + | + | + | V | + | + |
| *B. vulgates* | + | + | | - | + | + | + | V | + | + |
| *B. ovatus* | + | + | | + | + | + | + | V | - | + |
| *B. distasonis* | + | + | + | - | + | + | + | V | + | + |
| ***Eubacterium lentum*** | - | - | | | + | - | | - | - | - |
| ***E. limosum*** | - | - | | | | - | - | - | - | - |
| ***Fusobacterium necrophorum*** | - | - | | - | | - | - | - | - | - |
| ***Enterococcus faecalis*** | + | + | | | + | + | | V | | |
| *E. faecium* | + | + | | | + | + | | V | | |
| ***Propionibacterium granulosum*** | - | V | | | | | | | | |
| ***Escherichia coli*** | V | + | - | - | + | V | + | - | - | - |
| ***Peptostreptococcus prevotii*** | + | - | | | - | | | + | | |
| ***Clostridium perfringens*** | V | V | + | - | + | + | V | V | - | + |
| *C paraputrificum* | - | - | | | + | | | - | | |
| *C clostridiiforme* | V | - | + | - | + | - | | V | - | - |
| *C difficile* | - | - | | | - | - | | - | | |
| *C romosum* | + | + | + | - | + | + | | V | - | + |
| *C butyricum* | | - | + | + | | + | - | + | - | - |
| ***Megasphaera elsdenii*** | - | - | - | - | - | - | - | - | - | |
| ***Veillonella parvula*** | - | - | - | | - | - | - | - | - | - |

*Results from various studies involving oligosaccharides having comparable chemical composition were combined. When studies and/or a majority of the strains showed positive or negative results, the strain is displayed as (+) or (-), respectively. Studies having no agreement are displayed as (V). Data obtained using different methods are combined.

1) FOS = fructooligosaccharides INU inulin (including avg. DP 9-10 units), TOS = trans-galactosyloligosaccharides, GLL = 4'-galactosyllactose, IMO = isomaltooligosaccharides, RAF = raffinose, LAT = lactulose, LOL = lactitol, PHGG = partially hydrolyzed guar gum.

References: Mitsuoka et al 1987, Marchetti 1993b, Hayakawa et al 1990, Tanaka et al 1983 - Hartemink et al 1994, Hartemink et al 199, Saito et al 1992, Yanahira et al 1995, Asano et al 1994, Kitler et al 1992.



***g) Mechanism of action of inulin***

Dietary carbohydrates escaping /digestion/ absorption in the small bowel and prebiotics undergo fermentation in the colon and enhancing short chain fatty acids production. These have been associated with reduced risk of some diseases, including the irritable bowel syndrome, inflammatory bowel diseases, CVD, Daibetes and cancer (Jenkins et al 1999, Roediger 1980). Upon reaching the large intestine inulin is preferentially utilized by a group of healthy bacteria, *Bifidobacteria* and *Lactobacilli*, that are present in the ceco-colon. During the fermentation process, energy is provided for bacterial proliferation and increased cell mass. A few species of *Lactobacilli* produce carbon dioxide gas during their fermentation (Hartemik et al 1997). *Bifidobacteria* have not been found to produce hydrogen or carbon dioxide (Holdeman and Moore 1977). In addition to these fermentation products, short-chain fatty acids (SCFA), acetate, propionate, and butyrate are also formed along with L-(+)-lactate. Rats consuming inulin had significantly higher production of short chain fatty acids (SCFA) in the cecum ($p<0.05$) in comparison to other fibers tested: wheat bran, pea hull, oat husk, cocoa seed and carrot fiber (Roland et al 1995). SCFA are important anions in the colonic lumen, affecting both colonocyte morphology and function. By stimulating sodium and water absorption, SFCA act to minimize effects due to diarrhea. SCFAS may enhance ileal motility and increase intestinal cell proliferation by local action and by increasing mucosal blood flow (Scheppach 1997), Figure 2.13.

In addition to their effects on gut morphology and function, the SCFAS are absorbed through the colonic epithelial cells into the portal blood, thus becoming a source for host energy and regulators of several metabolic processes, (Figure 2.14).

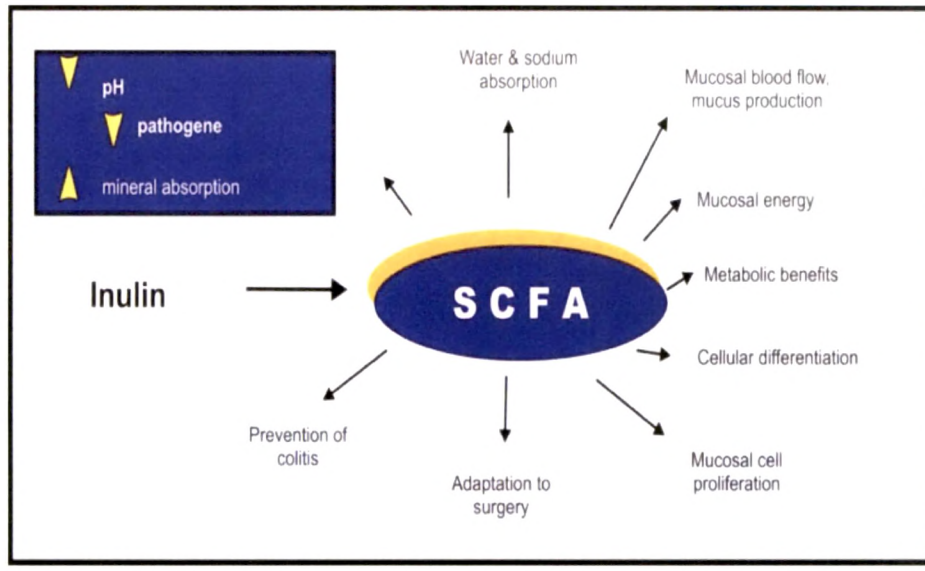



**Fig. 2.13 Factual and Hypothetical Effects of Short Chain Fatty Acids (SCFAS) on Colonic Morphology and Function, from Scheppach 1994**

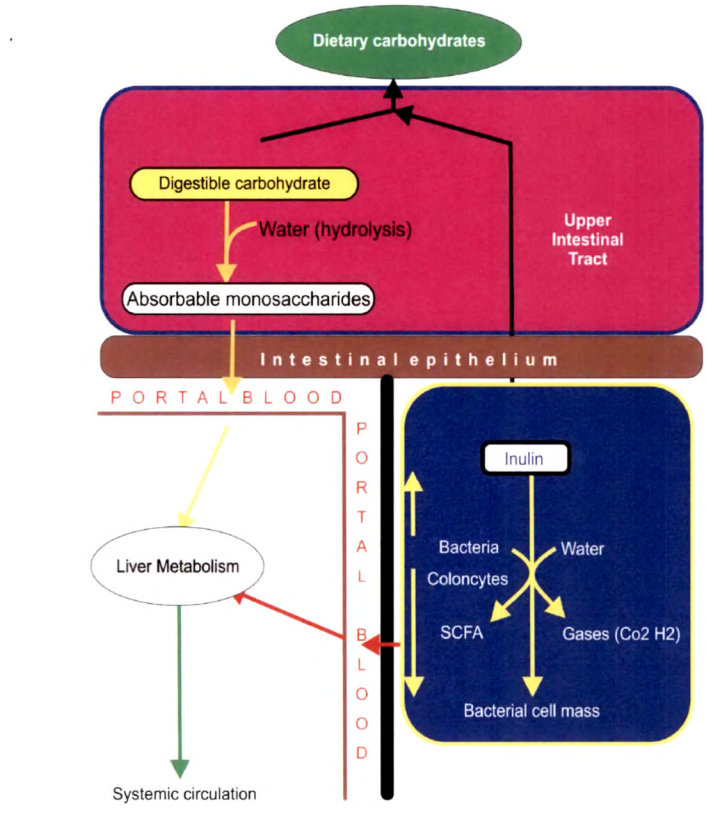



**Fig. 2.14 Schematic Diagram of the Bioavailability of Digestible Carbohydrates and Inulin**

Butyrate, remaining from colonic metabolism, propionate and Lt-l-lactate enter the liver from the portal blood and are completely metabolized. Propionate is transformed into methylmalonyl-SCoA and then succinyl-coA. L(+)-lactate is the precursor in gluconeogenesis. The small amount of butyrate reaching the liver is a precursor in lipogenesis (Roberfroid and Delzenne, 1993). About 50-75% of acetate is metabolized in the liver to produce energy, and like butyrate, serves as a lipogenic substrate. The remaining acetate fraction passes into peripheral muscle tissue where it is metabolized (Roberfroid and Delzenne1993).

The ratios of individual SCFA are likely very important, as each SCFA impacts host metabolism differently. Propionate has been demonstrated to lower cholesterol synthesis, both in vitro in isolated rat hepatocytes (Demigni et al 1995, Nishina and Freeland 1990, Wright et al 1990) and in vivo in rats (Chen et al 1984, Ullman et al 1988) and in humans (Wolever et al 1995), likely by inhibiting gluconeogenesis, stimulating glycolysis and inhibiting biosynthesis of fatty acids. Conversely, acetate stimulates gluconeogenesis (Remesy et al 1992b), inhibits glycolysis, and is a well known precursor of cholesterol (Nilsson and Belfrage 1978).

Butyrate and, less efficiently, propionate affect colonocytes at various stages of the adenoma-carcinoma-sequence. Butyrate has also been shown to be the preferred energy substrate for the colonocyte, accounting for about 70% of total energy consumption, and to be a potent differentiating agent in cell culture (Remesy et al 1992b, Roediger 1980, Scheppach 1994). Butyrate and to a lesser extent, propionate may also have a role in preventing certain types of colitis (Scheppach 1994, Scheppach et al 2001). Butyrate in particular enhances the growth of *Lactobacilli* and *Bifidobacteria* and a play a crucial role in the colon physiology and metabolism.

The SCFA (acetate/propionate) ratio resulting from colonic fermentation of inulin appears quite favorable for modulating carbohydrates and lowering cholesterol. Botham and others (1998) working in vitro with human fecal slurries, showed inulin fermentation provided the highest propionate/acetate ratio of NDOs tested and a relatively high butyrate level, (Figures 2.15 and 2.16). The work also showed that the chemical structure effects the dependability of a fiber: cellulose with B-1,4 bound

glucose as the primary structure is hardly fermented, while starch with both a-1,4 and a-1,6 linked glucose units is much more suspectable. It is also clear that amylase film is little fermented due to the limited accessibility to fermentative enzymes, whereas an amylose gel having a much better access for enzymes is fermented to a larger extent further, this ratio may be some what dose dependent as shown in vitro using rat heptocytes.

Levrat and coworkers (1991) further showed that higher inulin concentrations, up to 10% of the diet, favored higher propionate levels and a SCFA ratio of approximately 42:38:20 percent, acetate, propionate, and butyrate respectively. However, while a dose relationship for inulin is suggested from in vitro study, no dose-effect relationship has yet been determined in vivo with human volunteers (Roberfroid et al 1998). Rather, significant *Bifidobacteria*-stimulating effects have occurred at a human consumption level of 15 grams/day using native inulin extracted from chicory (Gibson et al 1995). However, based on available literature, the threshold consumption level at which these effects begin to be significant is likely about 4 grams/day.

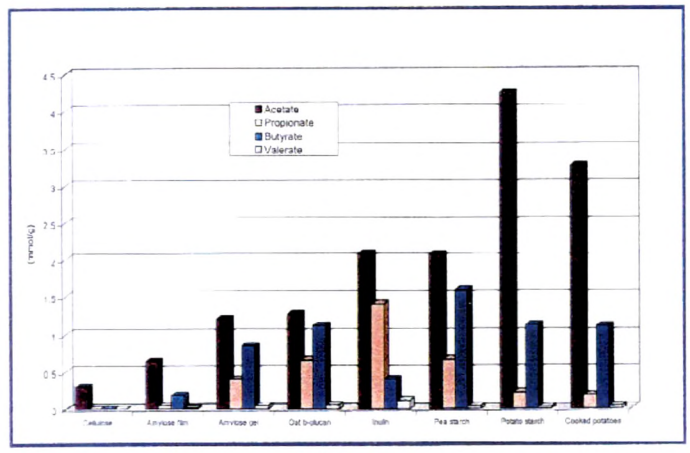



**Fig. 2.15 Short Chain Fatty Acids Levels after 24 Hours in Vitro Fermentation, Botham et al 1998**

In terms of carbon units, the overall balance from fermentation of 1 mol fructosyl unit of inulin produces about 40 percent SCFA (mol-ratio: acetate 81:propionate 13:butyrate 6), 15 percent L(+)-lactate and 5 percent $C0_2$ and up to 40 percent bacterial biomass, predominately *Bifidobacteria* (Roberfroid and Delzenne 1993). It has been shown that the activity of microbial inulinases, which are the necessary

enzymes for inulin fermentation and SCFA production and gas production, is influenced in vitro by the degree of polymerization (Roberfroid et al 1998). Inulin is reported to produce much more favorable mean SCFA/gas volume ratio (10.8) than shorter chain FOS (7.8) or non- preferential NDOs [soy polysaccharide (8.7), resistant starch (7.2), or arabic gums (8.0)].

Fiber-NDO mixtures have been shown in vitro to provide the highest SCFA/gas volume ratios (13.5), while inulin produced the highest concentration of total SCFA over the fermentation period 5.8 mmol/g inulin vs. 5.2 mmol/g fiber-NDO mixtures, (Figure 2.17), (Van Hoeij et al 1997).

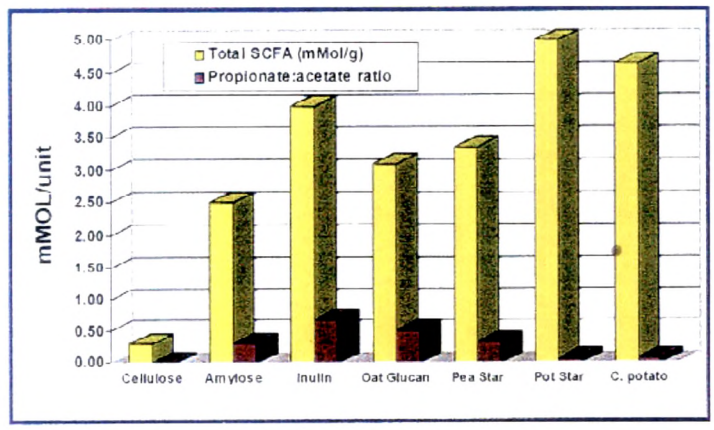



**Fig. 2.16 SCFA Levels and Ratios after 24 Hours in Vitro Fermentation, Botham et al 1998**

Favorable SCFA/gas ratio indicates that inulin only results in modest gas production while producing relatively high quantities of the SCFA, an important factor in patient tolerance for supplemented enteral nutrition formula (Van Hoeij et al 1997). The rate of fermentation also defines intestinal tolerance and SCFA-mediated systemic responses such as mineral absorption, carbohydrate and lipid effects, and osmotic taxation (Roberfroid et al 1998) (Figure 2.17).

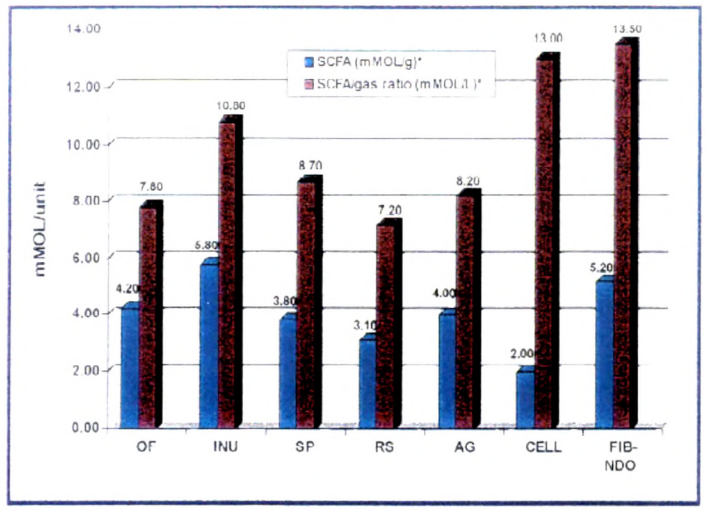



**Fig. 2.17 Short Chain Fatty Acid and Gas Production of Individual NDO, Polysaccharides, and their Mixtures, from Van Hoeij et al 1997**

**Section 2.7 Health implications of inulin, inulin along with probiotics (synbiotics)**

In addition to its important physiological and immunological functions, the colon is also involved in causation of miscellaneous diseases from acute infections and diarrhea or constipation to chronic diseases such as inflammatory bowel diseases, irritable bowel syndrome, or cancer. Through modulation of the colonic functions, inulin-type fructans thus have the potential to reduce the risk of some diseases.

**a) Heart Disease:** One of the main health concerns of the consumer is the risk of concurring cardiovascular diseases (CVD). Therefore, it is not surprising that the possibility of exploiting the diet to reduce the risk of CVD is gaining ever-increasing interest.

A fairly recent development is the use of prebiotic food ingredients, such as inulin as the possible dietary factor for lowering serum blood lipids relevant for CVD.

A commonly referenced systemic effect of inulin is related to lipid metabolism. Most animal studies have shown that inulin has an effect on blood lipid levels (Delzenne et al 2002; Mortensen et al 2002; Fava et al 2006; Delzenne and Kok 2001, Rault and Naina et al 2006) . Daily feeding of inulin (mean DP of 4.8) to rats at a 10% dose

level resulted in significant serum triglyceride lowering effect after just one week of feeding (Fiordaliso et al 1995). Serum triglycerides in the inulin-fed rats continued to remain significantly lower than controlfed rats for an additional 12 weeks of feeding. Tokunaga and coworkers (1986) had also shown that rats fed 10% or 29% fructooligosaccharide (GF2-GF4) diets experienced lowered serum triacylglycerol levels. In contrast, however, they failed to show reduced serum cholesterol levels whereas Fiordaliso's group was able to show significantly reduced serum phospolipids and total cholesterol levels in inulin-fed rats. Reduced serum triglycerides, phospholipids and total cholesterol were mainly due to a decreased number of very low density lipoprotein (VLDL) particles, and not the low density (LDL) or high density lipoprotein (HDL) fraction. Trautwein and coworkers (1998) have reported similar findings in hamsters fed 16% inulin diets for five weeks. These works have suggested that inulin feeding alters hepatic lipid metabolism which results in less VLDL production.

Since liver enzyme activity was also reduced for two of the four enzymes assayed, it is probable that SCFA produced from inulin fermentation decreased liver capacity for de novo triglyceride and fatty acid synthesis through inhibition of key enzyme activities, particularly glycerol-3-phosphate acyltransferase and fatty acid synthase (Kok et al 1996, Delzenne and Kok 2001).

A small number of well designed studies have repoted some positive outcomes with regards to the effect of prebiotics on blood lipids (Delzenne and Williams 1999, Daubioul et al 2000, Williams and Jackson 2002, Daubioul et al 2005, Beylot 2005, Fava et al 2006). In a double-blind crossover human study involving 12 slightly hypercholesterolemic men, Causey and others (2000) showed serum triglyceride reduction of 40 mg/dL (282.92 control, 243.24 inulin, $p = 0.024$) when 20 grams/day of chicory inulin (DP 2 to 60; avg. 9 units) was consumed. Total serum cholesterol was also reduced by 11 mg/dL (240.8 control; 229.8 inulin; $p = 0.086$, LDL-cholesterol also showed reduction, albeit, not significant. No change in HDL was noted, (Figure 2.18).

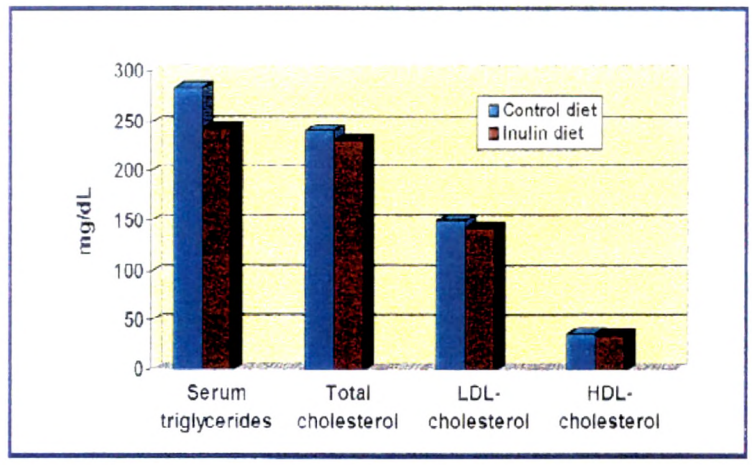



**Fig. 2.18 Effect of Inulin Ingestion on Lipid Metabolism, from Causey et al 1998a**

Another crossover study, involving twenty-one hypercholesterolemic men and women ingesting 18 g/day chicory inulin (DP 2 to 60; avg. 9 units) on a low-fat diet, showed statistically significant ($P < 0.05$) reductions for LDL-C (-4.4%) and total cholesterol (-8.7%), respectively (Davidson et al 1998). No effects on HDL cholesterol or serum triglyceride were noted. Yet, in another study, Davidson and Maki (1999) reported on the serum lipid profile of 25 adults with mild-to-moderate hypercholesterolemia who were fed 18 g/day of chicory inulin as a substitute for the sugar content of study foods. The study was a random, double-blind, crossover design with six week study periods and a six week washout period. At the end of both study periods, serum lipids (LDL-C, HDL-C, TC and TG) were not significantly different between experimental and control groups. Similar results have been reported by other researchers (Giacco et al 2004, Forcheron and Beylot 2007)

In healthy human populations the effects of inulin and oligofructose are more mixed. Canzi and others (1995, 1999) observed that daily intake of inulin (9g/d) in a rice-based ready to-eat cereal by 12 normolipidemic men resulted in significant reductions ($p<0.05$) in serum triacylglycerol and LDL- levels, 20.4 mg/dL and 8 mg/dL, respectively. However, Pederson and coworkers (1997) observed no effect with inulin in a low fat spread (14g/d) consumed by a group of 64 healthy normolipidemic women over a four-week double-blind crossover study. A study by Jackson et al

(1999), in which fifty-four healthy but slightly hyperlipidemic adults consumed 10 g/day of long-chain chicory inulin (DP 15 to 60; avg. 22 units) for an eight-week period. There were no effects on cholesterol, but fasting serum tri acylglycerols (- 19% after 8 weeks) and insulin levels (- 17% after 4 weeks and - 10% after 8 weeks) dropped significantly ($p<0.05$). Although not significant, a trend towards decrease total and LDL cholesterol was also observed. HDL cholesterol levels remained unchanged in both inulin-fed and control groups.

Possible mechanisms for the lipid lowering effects have been proposed (Fig 2.19) by various researchers. Ellegard and coworkers (1997) found that neither cholesterol absorption nor excretion from the small intestine was affected in ileostomy patients when either 17.1 g inulin or 15.5 g oligofructose was fed. They proposed that a lipid lowering effect may happen by another route, such as propionate absorption from the colon, which could suppress hepatic synthesis. Short chain fatty acids, particularly propionate, influence carbohydrate and lipid metabolism. Propionate has been demonstrated to lower cholesterol synthesis (Cheng and Lai 2000, Lopez et al 2001, Delzenne and Kok 2001, Chen et al 1984, Demigne et al 1995, Illman et al 1988; Nishina and Freeland 1990, Wright et al 1990, Wolever et al 1995). Others propose that some *Bifidobacteria* and *Lactobacilli* in fermented products are able to remove cholesterol (Van Poppel and Schaafsma 1996).

Like other NDOs, inulin could suppress serum cholesterol through an enhanced secretion of bile acids (Kim and Shin 1998, Mazur et al 1990, Trautwein et al 1998, Trauwein et al 1999, Adam et al 2001, Lopez et al 2001). In addition, inulin could decrease the serum cholesterol level by reducing hepatic cholesterol synthesis through inhibition of HMG-CoA reductase activity, with subsequent effects on concentration of B- hydroxy-B-methylglutaryl CoA (HMG-CoA) the key cholesterol intermediate.

As for the triglyceride lowering effect Delzenne and Kok (1999, 2001), in confirmation of earlier in vivo and in vitro rat studies, again reinforced the concept that short chain fatty acids produced from inulin fermentation exerts a triglyceride-lowering action primarily due to a reduction of de novo fatty acid synthesis in the

liver, through inhibition of all lipogenic enzymes. This suggested that inulin decreases lipogenic enzyme gene expression.

Yet another route inulin and oligofructose may decrease serum lipid levels is via lowered serum insulin and glucose, both known to regulate lipogenesis (Delzenne and Williams 2002, Sugatani et al 2006). Kok and coworkers (1998b) postulated that the lower glucose and insulin levels that were found after feeding a dose of oligofructose 10 g/100 g to rats contributed to the reduced hepatic fatty acid and triglyceride synthesis, and are part of the mechanism of the hypolipidemic effect of oligofructose. The researchers suggest that cecal hypertrophy resulting from SCFA is also likely to positively influence lipid metabolism by increasing the secretion of intestinal incretins, namely, glucosedependent insulinotropic polypeptide (GIP) and glucagon-like peptide-I (GLP-1). These gut hormones are known to regulate postprandial insulin release and also to have direct insulin-like actions on lipid metabolism (Cani et al 2004, Cani et al 2006, Delmee et al 2006, Cani et al 2007b). Inulin has been shown to reduce postprandial glycemia and insulinemia by 17 % and 26%, repectively (Kok et al 1996).

While the effects of inulin and oligofructose on lipid metabolism in animals tend to be more concrete and consistent, the determination of consistent lipid lowering effects for inulin and oligofructose in humans is yet to be confirmed.

**b) Obesity:** Obesity is now classically characterized by a cluster of several metabolic disorders, and by a low grade inflammation. The evidence that the gut microbiota composition can be different between healthy and or obese and type 2 diabetic patients has led to the study of this environmental factor as a key link between the pathophysiology of metabolic diseases and the gut microbiota (Ley et al 2006).

Several mechanisms are proposed linking events occurring in the colon and the regulation of energy metabolism, such as i.e. the energy harvest from the diet, the synthesis of gut peptides involved in energy homeostasis (GLP-1, PYY), and the regulation of fat storage (Fig 2.20). Moreover, the development of obesity and metabolic disorders following a high-fat diet may be associated to the innate immune

system. Indeed, high-fat diet feeding triggers the development of obesity, inflammation, insulin resistance, type 2 diabetes and atherosclerosis by mechanisms dependent of the LPS and/or the fatty acids activation of the CD14/TLR4 receptor complex. Importantly, fat feeding is also associated with the development of metabolic endotoxemia in human subjects and participates in the low-grade inflammation, a mechanism associated with the development of atherogenic markers (Cani and Delzenne 2009)

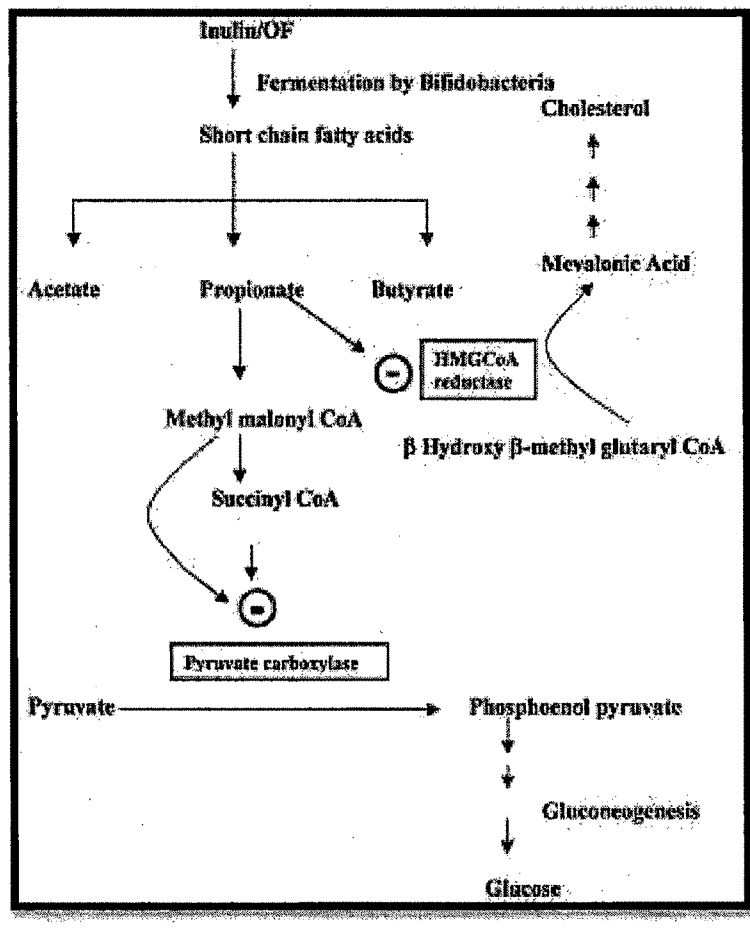



**Fig. 2.19 Inhibition of Pyruvate Carboxylase by Methyl Malonyl CoA and Succinyl CoA, and HMG CoA Reductase by Propionate Inhibition**

In experimental animals (healthy rats fed normal or high-fat diets), the addition of inulin-type fructans has been found to normalize blood lipid and glucose levels and reduce food intake and body weight, with a concomitant modulation of the levels of blood hormones involved in appetite regulation, for example, glucagon-like peptide-1

(GLP-1) and ghrelin (Cani et al 2004). The beneficial effects of oligofructose have also been seen in animals genetically ''at risk'' of the development of obesity and diabetes (Cani et al 2005a) By using genetically modified (GLP-1 receptor knockout) mice, it was possible to elucidate the mechanism behind the observed effects of oligofructose. This stems from its selective fermentation in the bowel and the corresponding formation of end-products, which in turn increase the expression of GLP-1 in the colon and its subsequent release in the portal vein. Glucagon-like peptide-1 is strongly involved in glucose homeostasis and affects appetite by increasing satiety (Cani et al 2005b).

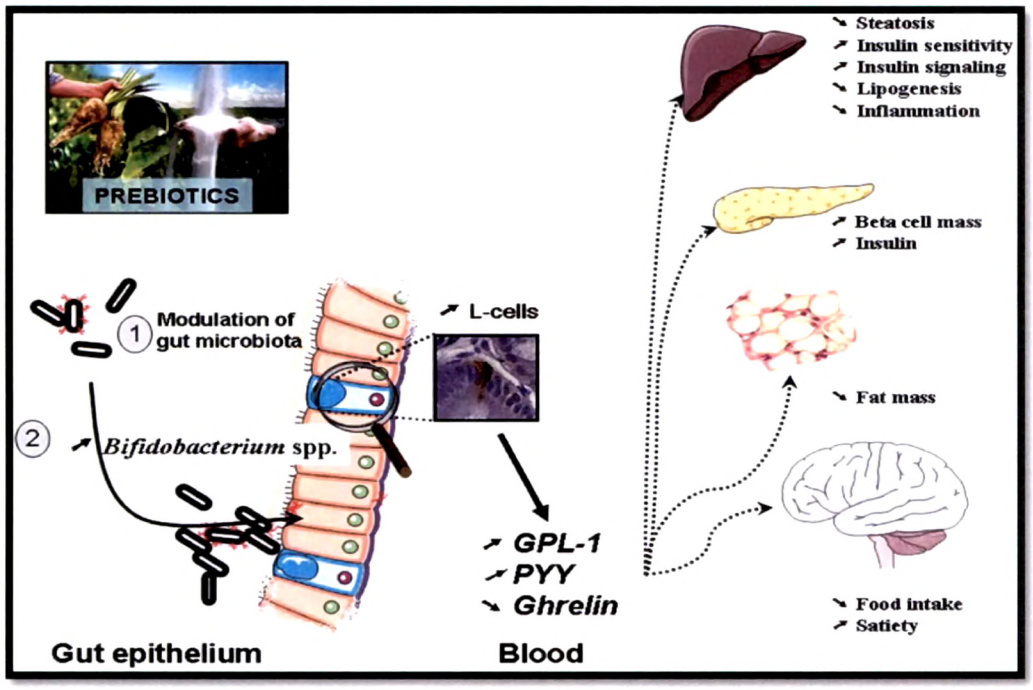



**Fig 2.20 The Modulation of Gut Microbiota by Prebiotics Treatment Modulates the Endogenous Production of Gut Peptides Associated with Energy Homeostasis**
Prebiotics change gut microbiota, increase portal plasma levels of two gut peptide GLP-1 and PYY (anorexigenic) and decrease Ghrelin (orexigenic). Prebiotics feeding promotes GLP-1 synthesis in the proximal colon by a mechanism linked to the differentiation of precursor cells into enteroendocrine L-cells. All these features are associated with a reduced food intake, body weight gain and fat mass development, a restored beta cell mass and glucose-induced insulin secretion.

Also, in human studies, inulin and oligofructose have had a favorable impact on lipid and glucose metabolism (Cani et al 2005b). In a recent randomized and placebo-controlled study, healthy adults were supplemented with 8 g of oligofructose twice a day for a period of 2 weeks. Volunteers felt more satiatedand had a lower energy intake (by almost 10%) when they received oligofructose in their diet compared with the control diet (Cani et al 2005c).

**c) Diabetes:** The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. According to the estimates of WHO, approximately 150 million people throughout the world have diabetes and the number is predicted to increase to 300 million (122% increment) by the year 2025, with the majority of cases being type 2 diabetes mellitus. In the developed countries, 42% increase from 51 million to 72 million is estimated while in developing countries, the predicted increase is over 170% from 84 million to 228 million. This indicates that by the year 2025, over 75% of the population with diabetes will be from the developing countries as compared to 62% in 1995 (King H et al 1998, Munichoodapa 2002). In the developed countries urbanization is likely to be limited and the future incidence of type 2 diabetes would be the aging population and also may be due to the increase in the population size. All these may not result in the alarming increase in the prevalence rate.

As a consequence of increasing incidence of this disease, people have begun to change their dietary habits, decreasing the glycemic index of foods they eat and supplementing their diet with active agents that can have influence on blood glucose and insulin levels. Foods containing fructans have had a long history for such use. In more recent years, others also have suggested that inulin-containing food products may be beneficial to persons with diabetic disease due to effects on reducing glucose uptake and thereby reducing postprandial hyperglycemia (Kim and Shin 1996). Yamashita and others (1984) fed 8 grams of fructooligosaccharide for 14 days to 18 diabetic subjects. By the end of the study the diabetic subjects experienced 15 mg/dL decline in fasting blood glucose levels while control subjects showed no change. The implications from this study suggest that inulin-containing products may be useful carbohydrate substitute in diabetic diets.

In eight healthy human subjects, Rumessen and others (1990) also examined the effects of fructans from Jerusalem artichoke on blood responses and after a 20 g fructan, these subjects demonstrated a lower glycemic response and insulin peak than when lactose was fed.

**d) Cancer:** Surveys indicate that 60% of the consumers are more likely to believe that high soluble fiber containing bits, vegetables and grains, has an effect on the prevention of colon cancer (Sloan 1999). The majority of colon cancer cases follow the so-called adenoma sequence: normal colon cells undergo transformation as a result of cellular mutations. Hyperproliferation and aberrant crypt foci develop and may progress to form small and then larger adenomas, which may eventually transform into cancer. This entire sequence will usually last 15-20 years. Dietary factors can influence this process in both positive and negative ways, Figure 2.21.

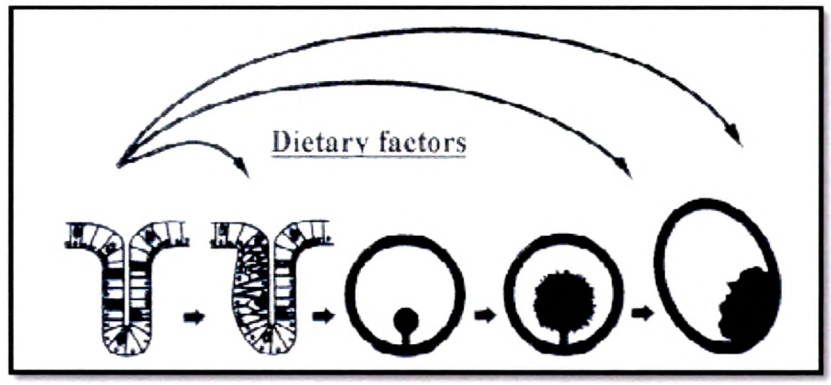



**Fig. 2.21 Normal colon mutual transformation via hyperproliferation and adenomas to carcinoma**

In a review of possible mechanistic effects for colon cancer inhibition, Reddy (1999) emphasized the importance of examining both probiotic and prebiotic activity, with possible synergistic effect when used together. Lactic cultures used in the fermentation of milk products are examples of probiotics. These products have been shown to possess antimutagenic and anticarcinogenic properties (Bodana and Rao 1990, Goldin and Gorbach 1980, Lidbeck et a1 1992).

Prebiotic factors such as inulin and oligofructose are also able to selectively stimulate growth of *Bifidobacteria* at the expense of more putrefactive bacteria (Gibson and Roberfroid 1995). Furthermore, fermentation of these oligosaccharides in the colonic regions results in production of short chain fatty acids (Roberfioid et al 1993, Wang and Gibson 1993). Butyric acid has been shown to increase apoptosis in human colonic tumor cell lines (Scheppach 1994, 1997). Apoptosis is a mechanism by which excess or redundant cells are removed during development and restricted tissue size is

maintained. Apoptosis is thus an innate cellar defense against carinogenesis. Lactic acid production can lower intestinal pH. There is evidence that increasing the numbers of *Bifidobacteria* in the colon and reducing intestinal pH has a direct impact on carcinogenesis in the large intestine (Goldin and Gorbach 1980, Hill 1988, Koo and Rao 1991).

Possible mechanisms for the anticarcinogenic and antitumorigenic effect are not completely understood. Modulation of the microflora through the stimulation of *Bifidobacteria* while keeping *E. coli or Clostridia* at low levels is one possibility. Rumney and Rowland (1995) suggested that production of toxic metabolites may be reduced by increasing the proportion of healthier colonic microflora which competes with pathogenic and putrefactive bacteria to reduce the levels of toxin and carcinogenic-producing enzymes, Figure 2.22.

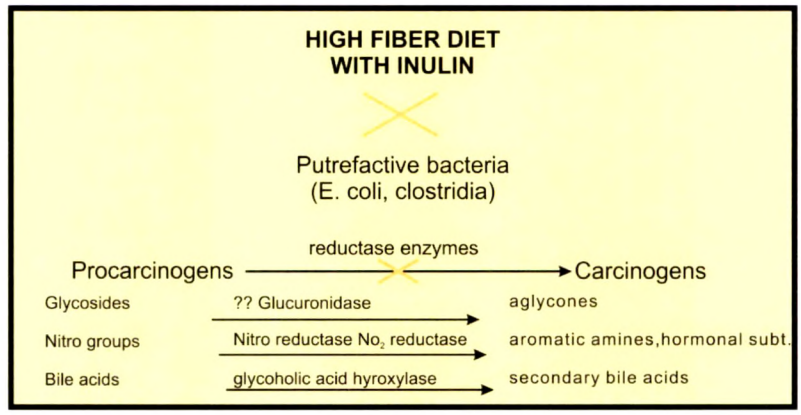



**Fig.2.22 Reductase Enzymes and their Role in Carcinogen Formation**

Toxin and carcinogenic-producing enzymes have relatively low activity in *Bifidobacteria* and *Lactobacilli* compared to other more harmful colonic microflora (Rowland 1995). These alterations in bacterial enzymes can interfere with the conversion of procarcinogens to its carcinogenic form and thus reduce cancer risk. Hidaka et al (1986) fed rats a diet supplemented with tyrosine and tryptophan as precursors to phenolic products. Fructooligosaccharides were administered at 0.4 to 10% by weight in the diet. Results indicated p-cresol levels were reduced in the fecal material. Buddington et al (1991,1996) further noted significantly reduced nitroreductase activity while using 4 g/day of FOS. In addition, the study showed

reductive enzymes I- glucuronidase and glycoholic acid hydroxylate were decreased 75% and 90%, respectively. B-glucuronidase has implications in carcinogenesis through the release of aglycones from glycosides, while glycoholic acid hydroxylate is involved with the production of secondary bile acids, potentially linking it to the increased risk of cancer associated with high-fat diets (Buddington et al 1996). Rowland and others (1998) also demonstrated decreased ammonia concentration and B-glucoronidase activity in cecal contents in the presence of *Bifidobacteria*, inulin, or both. B-glucoronidase and ammonia concentration are known contributors to carcinogenesis of the colon in experimental animal models.

Collectively, these findings are indication that both probiotics (bifdobacteria) and prebiotics (oligosaccharides) might reduce carcinogenic activity. The synbiotic com bination of *rhamnosus GG* and resistant starch has shown to decrease the activity of β glucuronidase, nitroreductase and chologylgycic hydrolase (Ling et al 1996). The synbiotic combination of RS and *B.Lactis* significantly reduces the apoptotic reponse to gene toxic carcinogen in the distal colon of rats. Differences in initial colonic microflora populations, diet, age or sex of the rats, species or strain of the probiotic, number of viable organisms reaching the colon, and initial body weight at time of introduction of the carcinogen are all critical factors to consider when determining the effectiveness of either the probiotic or prebiotic (Gallaher et al 1996).

Although data from animal studies support a role of inulin in the prevention of the diseases (not only abrerrant crypt foci but also colonic tumorformation) (Roller et al 2004), trials on human beings are still in early stages. In one large multicenter European study (SYNCAN), biomarkers of colonic cancer risk , such as cell proliferation and toxicity of feces (fecal water), improved when participants were given synbiotics (oligofructose enriched inulin plus lactobacillus rhamnosus and bifidobacterium lactis) for 12 weeks ( Rafter et al 2007). Bouhnik and others (1996) found that 12.5 g/day FOS fed to 20 healthy volunteers in two week periods resulted in increased colonic *bifidobacteria* but there were no beneficial changes in factors that are potentially involved in the pathogenesis of colon cancer. In this study, fecal total Anaerobes, pH, enzymatic activities for azoreductase, B-glucoronidase, and nitroreductase, and the concentrations of bile acids were unaffected. However, under unique conditions, Vanurikhina et al (1997) have been able to demonstrate a possible

relationship between inulin and cancer risk. This study involved 25 people exposed to radiation as a result of the Chemobyl accident in 1986. Patients who ingested 10 g/day inulin for 2 months had reduced frequency of metaphases with chromosomal aberrations due to decreased pair fragments and translocations. In these exposed patients, inulin was associated with an expressed antimutagenous effect. Based on the available research data with inulin type fructans and originating from various types of animals models, it was concluded as part of the European Commission-funded ENDO project that these compounds consistently demonstrate a reduced risk in experimentally induced carcinogenesis processes (Van Loo et al 1999). The data further support the investment for performing human nutrition studies.

**e) Immune system:** The ability of the body to mount an effective defensive response to disease declines with age, a phenomenon known as immunosenescence. Elderly people may indeed be regarded as immunocompromised (Aspinall and Andrew 2000) Cellular immunity seems to be the most seriously affected, with decreased numbers of circulating CD3+ lymphocytes and diminished activity of natural killer (NK) cells. Clearly, any strategy that can boost immunity in the elderly is to be welcomed. Various probiotic bacteria, including yoghurt organisms, L johnsonii La1, L acidophilus, L casei and B lactis Bb12, have been shown on the basis of in vitro and ex vivo models to have immunostimulatory properties, including modulation of cytokine production, increased phagocytic activity of polymorphs, adjuvant effects on specific humoral responses, T lymphocytic function, and NK activity (Meydani and Ha W-K 2000; Blum et al 2002) Several investigations show that probiotics stimulate the immune system in elderly subjects. Gill and colleagues report in a series of papers the beneficial effects of three week courses of L rhamnosus HN001 or B lactis HN019 in elderly healthy volunteers; they found significant increases in levels of a-interferon, total lymphocyte counts, circulating counts of CD4+ and CD25+ cells, and NK tumoricidal activities (Arunachalam et al 2000, Gill et al 2001ab, Sheih et al 2001, Of particular interest were the findings that improvements in immunological functions were more marked in subjects aged over 70 and in those with poor pretreatment parameters. Van de Water et al (1999) found that yoghurt consumption for one year decreased the incidence of allergies and serum IgE levels in a group of healthy elderly subject. Part of the hypothesis that inulin may play a role comes from work with

yogurt as a bearer of lactic acid producing microbes (Hitchins and McDonough 1989, Van de Water et al 1999). According to the hypothesis, ingestion of a lactobacillus culture may stimulate an immune response. Since lactic-acid producing bacteria can be stimulated when inulin is ingested, a similar immune response might be generated.

Feeding inulin has also been demonstrated to impact the immune system. Kelly-Quagliana and others (1998) used B6C3F1 mice to examine the immunomodulating properties of inulin (avg. DP 22 units). By measuring natural killer cell activity in spleenocytes and quantifying phagocytosis by peritoneal lavage microphones, they determined that inulin fed mice had increased percentage of NK cells ($p<0.0005$) and/or an increased speed of macrophage response. Causey and others (1998) provided further evidence that inulin, particularly long chain inulin molecules (average DP 22 units), stimulate the human immune system by binding to specific lectin-like receptors on leukocytes. Data indicate that long chain inulin molecules stimulate macrophage proliferation without a concomitant rise in the inflammatory marker leukotriene B4 (LT-B4). The long chain inulin also did not stimulate interleukin - 1a (IL-1a) production at 25, 50 or 100 ug/mL. It was suggested that higher concentrations of inulin may enhance production of this cytokine. Guigoz et al (2002) investigated the effects of giving the prebiotic FOS, 8 g daily for three weeks, to frail elderly subjects in a nursing home. An increase in numbers of faecal *Bifidobacteria* was accompanied by significant rises in counts of total lymphocytes, CD4+ and CD8+ cells. An unexpected finding was a fall in phagocytic activity of polymorphs and monocytes, as well as reduced expression of interleukin-6 mRNA in peripheral blood monocytes; the authors attributed these changes to a general decrease in inflammation. However, Bunout et al (2002) found that a prebiotic mixture of inulin and oligofructose did not augment the results of vaccination with influenzal and pneumococcal antigens. It remains to be demonstrated whether the apparently beneficial effects of probiotics and prebiotics on various immune parameters, as outlined above, is of practical significance. An interesting finding in this respect was made byTurchet et al (2003) who supplemented the diet of a group of healthy elderly subjects with L casei for three weeks, and reported that the duration of "winter infections" (gastrointestinal or respiratory) was decreased by 20% compared with a control group.

**f) Dental Health:**

Use of inulin/FOS as a replacement for other carbohydrates may have a role in dental health. Inulin and oligofructose have synergistic effects when combined with sweetening agents aspartame and acesulfame-potassium. The lingering high-intensity aftertaste of several high-intensity sweeteners, particularly the two mentioned, can be corrected towards a more natural sweetness profile, improving the mouthfeel and use for oral products, such as chewing gums and hard confections. Further, inulin and oligofructose provide improved elasticity and prevent drying of these oral confections. Using in vitro experiments with dental plaque, researchers have shown that low molecular weight fructans (DP<5) may serve as substrates to oral microorganisms such as certain strains of streptococci (Nilsson et al 1988). However, the in vitro acid production rate was low compared to glucose. Telemetry tests have shown that inulins used as the only bulking components in chewing gum produced no decrease in the oral pH under the critical limit of 5.7 (De Soete 2000).

**g) Skeletal Health and Menopausal Support:**

Osteoporosis is a global health problem that takes on increasing significance as life expectancy increases. Although physical activity, vitamin D, and calcium are key preventative measures, there is emerging evidence for a positive impact of inulin-type fructans and, more particularly, oligofructose-enriched inulin. A study in adolescents (Abhram et al 2005) found improvements in calcium absorption, bone mineral content, and bone mineral density after 1 year of supplementation with 8 g/d of oligofructose-enriched inulin. The difference between intervention and control groups translated into an extra 31 mg of calcium per day assimilated into bones. Animal models of the menopause (ovariectomized rats) have been used to determine the likely impact of inulin and oligofructose on osteoporosis risk. Studies have revealed improvements in calcium absorption, bone mineral content, and bone mineral density and reduction in ovariectomy-induced bone loss after supplementation with inulin-type fructans (Scholez-Ahrens et al 2002) In growing rats, oligofructose was seen to improve markers of biomechanical strength, implying enhanced resistance to fracture (Lobo et al 2006). In these studies, the best calcium absorption and retention results

were seen with oligofructose-enriched inulin, rather than oligofructose or inulin alone, possibly because of a different fermentation pattern of the former (Coudray et al 2003).

In experiments conducted by Levrat and others (1991) using rats fed a diet supplemented with a 10% inulin fraction, the cecal pool for calcium, magnesium and phosphate was improved. Furthermore, feeding of fructooligosaccharides to animals has led to reduced fecal excretion of minerals. As an example, Ohta et al (1994a) fed fructooligosaccharides to male Sprague-Dawley rats that were also consuming two levels of magnesium while maintaining constant and sufficient levels of calcium and phosphorous. Under low magnesium intake, fructooligosaccharides were able to increase magnesium absorption and reduced the occurrence of auricular and facial peripheral hyperemia and hemorrhage, both signs of magnesium deficiency. When 5% fructooligosaccharides were fed to rats on low magnesium diets, they began to absorb magnesium at approximately 3.0 mg per day, which was similar to rats fed magnesium-sufficient diets.

Inulin and oligofructose have been shown in human studies to enhance dietary calcium absorption, maintain healthy calcium balance and provide means to improve bone density (van den Heuvel et al 1999, Coudray et al 1997, Ellegard et al 1997). Further, the positive growth-promoting effects inulin has on probiotic bacteria populations could potentially enhance estrogen re-cycling which can also affect bone health (Chaitow and Trenev, 1990). As summarized by Chaitow and Trenev, sixty percent of the circulating female hormones such as estrogen are excreted via bile into the GI tract. Under normal healthy conditions, the hormones are converted by bacterial enzymes such as sulphate catalyze to a recycled form which is mostly resorbed into the bloodstream and converted to a biologically active form. However, under conditions such as those resulting from broad-spectrum antibiotic use, improper diet and stress levels, probiotic bacteria can substantially decrease potentially leading to lower estrogen recycled in to the bloodstream. If inulin as a prebiotic can boost the appropriate bacterial populations, the recycling effect might be maintained. Although cereal fiber and its associated phytate content have been found to depress the absorption and retention of several minerals (Greger 1999), it has been hypothesized that fermentable carbohydrates, like inulin, could possibly improve the metabolic

absorption of certain minerals such as calcium, magnesium and iron (Campbell et al 1997, Lopez et al 1998, Schafer and Lutz 1990, Schulz et al 1993). Ohta et al (1995b, 1997) and Baba et al (1996) formulated the hypothesis that the effects of NDO on calcium and magnesium absorption occur at the level of the large intestine, a new concept, as it is generally accepted that mineral absorption occurs mainly via the small intestine. The mechanism for this improvement is speculated to be the production of SCFA and lactate resulting from inulin fermentation in the colon, which in turn results in a reduction in luminal pH with corresponding increase in the mineral free ionic form, solubility and bioavailability. The lower luminal pH raises the concentration of ionized minerals and accelerates passive diffusion (Remesy et al 1992a).

When focussing on iron, Ohta and others (1995a) investigated the effects of fructooligosaccharides on mineral absorption in rats that were made anemic prior to the study. Male Sprague-Dawley rats were fed 5% fructooligosaccharides with either 15 mg Fe/kg diet (low iron intake) or 30 mg Fe/kg diet. Magnesium, calcium and iron absorption were measured. Fructooligosaccharide feeding reduced the cecal pH, increased iron, calcium and magnesium solubility in the cecal contents, and increased iron, calcium and magnesium absorption. Furthermore, iron levels did not affect calcium or magnesium absorption when fructooligosaccharides were included in the diet. Thus, fructooligosaccharides may be helpful in correcting iron deficiency anemia while also increasing the absorption of calcium and magnesium. Correction of iron-anemia in post-gastrectomized rats fed a 7.5% FOS diet for six weeks has also been demonstrated (Ohta et al 1998c)

Delzenne and coworkers (1995) concluded rats fed a diet containing higher levels of inulin (10% inulin) also lead to a significant increase (of about 60%) in the apparent retention of calcium magnesium, and iron. The rat study further showed inulin increased fecal excretion and decreased urinary excretion of nitrogen. Thus, inulin may provide a good means to counteract dysfunctions resulting from hyperammonemia or disturbed iron, calcium, magnesium and zinc homeostasis (Delzenne et al 1995).

**h) Opportunistic infections (Urinary tract health and Candidiasis):** Infections of the urinary tract (UTIs) and respiratory infections occur more often in women and children. Probiotics, particularly Lactobacillus acidophilus strains, have been used with some success in helping to maintain a healthy balance of colonic microflora (Elmer et al 1996, Kageyama et al 1984, Pochart et al 1992). However, since probiotic effects may be transient (Bounik et a1 1992), use of prebiotics such as inulin may provide another means to selectively modify colonic microflora populations, and establish a healthy colonic balance. Relative to lactic acid producing bacteria *(L. acidophilus),* opportunistic pathogenic microorganisms such as *Candida* (yeast infections), *clostridia* (*Cl. difficile* antibiotic associated diarrhea) and E. coli, and other members of the Enterobacteriaceae family (urinary tract infections), Figure 2.23(Causey et al 2000) are intolerant of low pH levels (5.0 and 4.5) (Wang and Gibson 1993, Borriello 1990, Hopkins et al 1997) and normal pathogenic gut microorganisms do not have highly active intracellular 2,1,B-d-fructan-fructohydrolase enzymes (Hudson et al 1993, McKellar et al 1993).

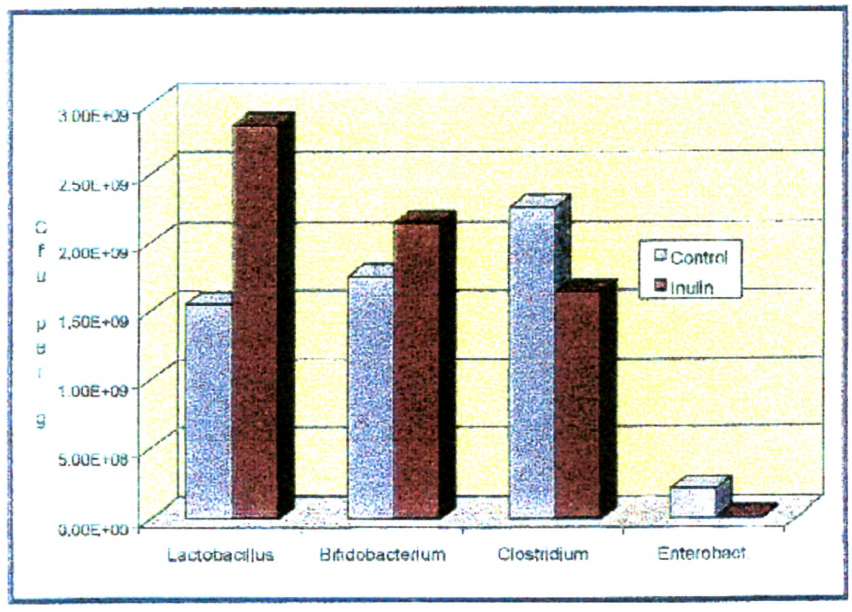



**Fig.2.23 Effect of 20 g/d Full Consumption on *Enterobacteriaceae***

Further, only five of the clinically significant yeast species can use inulin to any extent as a growth substrate (Barlett et a1 1990). As consequence, the overpower of opportunistic pathogenic microorganisms may be selectively reduced and maintained

with the use of probiotics and selective prebiotic agents, like inulin However, selective prebiotic agents function effectively only when there are populations of probiotic bacteria present to nourish. Thus, the primary application for inulin in opporrtunstic disorders is likely prevention of overgrowth rather than its therapeutical use, its therapeutic use being dependent on several variables, such as magnitude of the overgrowth condition, and specific strain(s) of organism(s) involved. In addition to yeast infections, opportunistic E. coli overgrowth can have substantial clinical significance. Consumption of inulin, by mechanisms defined in the bifidogenic section, has been shown to significantly reduce concentrations of these bacteria, minimizing the potential for their overgrowth into other areas of the body.

**i) Gastrointestinal Health:** One of the strongest health links for herbal and nutraceutical medicines is the ability to prevent and treat digestive problems. Related to this need to self-treat their digestive ailments and maintain proper GI health is a growing awareness of various nutraceutical ingredients having GI health related effects.

Fructan-containing plants have been used for centuries to promote gastrointestinal health and treat problems of the GI tract. Various species of Inula roots have been widely used in Asia for promoting gastrointestinal and urinary tract health. Of these, I. Helenium and I. Britannica are used in China to control diarrhea and dysentary as well as balance spleen, stomach, and large intestinal problems (Hsu, 1986). Inula racemosa is also used in the Kashmir as a diuretic (Kurup et al 1979). Campbell et al (1997) suggest that gastrointestinal health can be improved by feeding oligosaccharides because of their effect on short chain fatty acid production, lowering pH, and increasing *Bifidobacteria.* However, since oligosaccharides reach the large intestine largely intact, the possibilities exists that gastrointestinal discomfort will be experienced and might be a factor with which to contend (Rumessen et al 1990). Some human studies have shown that intestinal discomfort, particularly flatulence, is present (Davidson and Maki, 1999, Pedersen et al 1997) while others show no gastrointestinal side effects up to 20 g/day of short-chain FOS, especially when dosages are increased gradually (Molis et al. 1996). However, longer more polydispersed, inulin molecules (DP 2 to 60; avg. 10 units) have been shown to be

more well tolerated, with only mild discomfort at consumption levels of about 40 g-day (Kleeseen et al 1997).

Because inulin and FOS have somewhat laxative effects, they might be helpful in reducing constipation with only mild discomfort (Hidaka et al 1991, Kleessen et al 1997, Tominaga 1999). It has been proposed that irritable bowel syndrome is a common gastrointestinal disorder that also may be aided by supplementation with inulin due to its effects on *Bifidobacteria* numbers. Further, Scheppach (1994; 1997, 2001) has suggested that butyrate, one of inulin's fermentation products, could potentially be an important variable in ulcerative colitis and malabsorption disorders. Hunter and others (1999) found that supplementing 6 g of oligofructose (2 g three times daily) was insufficient for achieving differences in fecal weight, pH, whole-gut transit time, and fasting breath hydrogen concentrations in individuals who were suffering from irritable bowel syndrome. As long chain inulin molecules are generally fermented more slowly they are capable of reaching later stages of the colon, likely providing more effective means of meeting a desired response with less undesirable responses such as diarrhea, pain or flatulence.

Among the diseases associated with older people, gastrointestinal disorders have become priority areas for clinicians and researchers. The process of ageing is associated with physiological and histological changes in the gastrointestinal tract, which may have functional implications in terms of digestion and absorption and in some cases, pathological damage to the mucosa. Recent studies suggest that, in older subjects (over 65 years), disorders of the GI tract are the third most prevalent cause of visits to GPs (Destro et al 2003).

Currently, there are a number of studies being conducted on the fecal flora during ageing using more sophisticated and accurate molecular methods of analysis (Blaut et al 2002). Sequence analysis of over 280 clones from a single elderly person's fecal sample showed that the flora was even more diverse than that of a young adult. Furthermore, the proportion of unknown molecular species was much higher among the clones derived from the older subjects, and 22% of the flora comprised species outside the major groups found in younger adults, namely *Bacteroides/Prevotella,*

*Clostridium coccoides,* and *Clostridium leptum* groups (Blaut et al 2002). A somewhat more extensive study of the fecal microflora of humans in different age groups was conducted by Hopkins et al (2001), who compared, using conventional microbiological and molecular methods, children (16 months-7 years), adults (21-34 years), and healthy elderly subjects (67-88 years). Although total bacterial counts were similar in all three age groups, bacterial composition varied considerably. Most notably, *Bifidobacterial* numbers were significantly lower in older people; in three of the four subjects, numbers of *Bifidobacteria* were undetectable or very low. However, in the final elderly subject, very high numbers (approximately $10^{10}$/g feces) were detected. Data from 16S rRNA analyses confirmed the results obtained by conventional bacteriology.

Hopkins et al (2001), reported a further study in which the fecal microfloras of healthy young adults (19-35 years), healthy elderly (67-75), and hospitalized antibiotic-treated elderly subjects (73-101 years) were compared. In this study, only conventional microbiological methods were employed (Woodmansey et al 2004). The results showed again that total anaerobe numbers remained relatively constant with age, although, as before, individual bacterial genera changed markedly. Reductions in both numbers and species diversity of bacteroides and *Bifidobacteria* in both the health and hospitalized elderly groups were seen. In particular, *Bifidobacterial* populations showed marked variations in the dominant species, with *Bifidobacterium angu-latum* and *B. adolescentis* being isolated from older people and *B. longum, B. catenulatum, B. bourn,* and *B. infantis* being detected only in the healthy young subjects. Other differences in the intestinal ecosystem in elderly subjects were observed, with alterations in the dominant clostridial species in combination with greater numbers of facultative anaerobes. It is not clear why this study showed decreased species diversity in the fecal flora of older subjects, although it may be related to the methodology used (conventional microbiology rather than molecular methods) or to the study being conducted in a different location to the Hopkins et al (2001) investigation.

It has been suggested that the decline in fecal *Bifidobacteria* numbers with age plays a role in the increased risk of infections and some chronic degenerative diseases in

older people. For example, there is evidence that *Bifidobacteria* exert inhibitory effects on potential pathogens such as *Clostridium difficile* and may be involved in colonization resistance and immune function (Yamazaki et al 1985, Gibson and Wang 1994). There are also studies that demonstrate reduced pre-cancerous lesions and tumors in the colon of laboratory animals given strains of *Bifidobacteria* (Reddy and Rivenson 1993; Rowland et al 1998). An implication of this theory is that it should be possible to restore at least in part the original balance of the microflora by supplementing the diet with probiotic *Bifidobacteria* or bifidogenic products, i.e prebiotics such as nondigestible oligosaccharides (NDOs), which selectively stimulate the growth of *Bifidobacteria* in the gut.

There is extensive evidence that NDOs modulate the composition of the gut microflora in adults. This has been observed in a large number of dietary intervention trials (Roberfroid 1993). There is evidence from some studies that the stimulatory effects of prebiotics on *Bifidobacteria* numbers in the gut are more apparent when the initial levels are low (Tuohy et al 2001), suggesting that prebiotics would be particularly effective in older people.

Bowel dysfunction is a major problem for older people, with constipation being one of their commonest complaints. A recent systematic review of American studies revealed that four out of six found an increase in constipation with age (Higgins and Johanson 2004), although a cross-sectional survey in Spain found no relationship (Garrigues et al 2004).

The pathophysiology underlying constipation in older people is complex and is thought to include alterations in neural innervation, smooth muscle activity, and neuroendrocrine function, resulting in changes in colonic transit time/difficulty in defecation, and changes in rectal sensation (Potter 2003).

Constipation has also been reported as an adverse side effect in the use of a number of drugs, in particular, opioids, diuretics, antidepressants, antihistamines, antispasmodics, anticonvulsants and aluminum antacids (Talley et al 2003). The complex and varied etiology of constipation in older people suggests that nutritional

solutions may be too simplistic an approach, however, dietary remedies represent a less invasive strategy than enemas and laxatives, with minimal side effects, and there is some evidence that dietary fiber, nondigestible oligosaccharides and probiotics may be effective. A randomized placebo controlled trial in elderly hospitalized patients given a 150 mL portion of yoghurt containing lactitol, guar gum and wheat bran twice daily reported a significant increase in fecal output compared with control yoghurt without fiber (Rajala et al 1988). It is clear that the type of nondigestible carbohydrate selected can have a major impact on the extent of laxation.

In studies of the effects of carbohydrates on fecal bulking in healthy subjects, it has been shown that wheat bran (insoluble fiber) increases stool weight by 5 g/g carbohydrate consumed (Cummings et al 1992), whereas soluble fiber in the form of pectin and guar gum has relatively minor effects (1-2 g increase in fecal weight/g carbohydrate (Cummings et al 1976). Resistant starch and nondigestible oligosaccharides induce increases in stool weight of 1.5-2.2 g/g carbohydrate (Cummings et al 1992, Gibson et al 1995, Heijnen et al 1998).

It is not clear whether the changes in microflora apparent in older people (noted above) are causally related to constipation, but it is known that changes in intestinal flora can alter intestinal motility (Huseby et al 2001), and the short-chain fatty acids produced by bacteria in the gut can influence transit time (Scheppach 1994). A potential approach to relieving constipation is, therefore, to increase the numbers of *Bifidobacteria* using probiotics or prebiotics.

A number of clinical trials have been conducted with conventional and probiotic-enriched yogurts and fermented milks in elderly subjects with constipation. These have been reviewed in detail by Pathmakanthan et al (2000). Of the seven studies, five showed significant laxative effects and one showed a significant improvement in transit time. In general, other similar studies in younger subjects supported these results.

There are reports that other prebiotics such as fructooligosaccharides, galactooligosaccharides, and inulin may also exert mild laxative effects although in most studies to date the effects do not reach statistical significance (Macfarlane et al 2006). For example, Kleesen et al (1997) found some subject-to-subject variation in their study comparing lactose and inulin given to 15 and 10 elderly subjects (respectively) in dosages of 20 g increasing to 40 g/day for a total of 19 days, but inulin had more effective laxative action. Further research needs to be carried out to more fully elucidate the health implications of inulin consumption in older adults.

In light of the literature reviewed, the present study was undertaken with the following scope of investigation.