CHAPTER 2

LITERATURE REVIEW

2.1 Plant polysaccharides

Polysaccharides are the polymers of monosaccharide linked together by glycosidic bonds. It may be of a linear, branched or occasionally cyclic structure, may contain a small or a large number of residues and may be homogeneous or heterogeneous in monosaccharide types. Polysaccharides are present in the various part of plant including seeds, stems, leaves and tubers. The two major functions of polysaccharides in these biological materials are included the first as structural for rigidity of the tissues and the second as energy sources for developing embryos and metabolic reactions. Most of plant polysaccharides do not occur in pure form but rather as heterogeneous mixtures with other cellular components. Normally, plant polysaccharide is found in 2 forms: starch and non-starch polysaccharides (NSPs).

2.1.1 Starch

Starch is the basic form of storage polysaccharide. It is the major polysaccharides found in cereal grains and functions as an energy source for human and animals. Starch present as a reserve food material in the tubers and seeds of nearly all plants; it is also present in green leaves. In general, starches have the formula $(C_6H_{10}O_5)_n$, where "n" denotes the total number of glucose monomer units. Starch contains 2 types of polymer that are amylose and amylopectin. Amylose consists of long unbranched chains of glucose attached to each other in α-(1,4)

glycosidic linkages. These chains may be range from 3,000 to 500,000 in molecular weight.

Amylopectin, is highly branched with 24-30 residues/branch. The chains have α (1,4) linkages but the branch points consist of β-(1,6) linkages (Figure 1 and 2). Amylopectin forms colloidal or micellar suspensions, and its molecular weight can be as high as 100

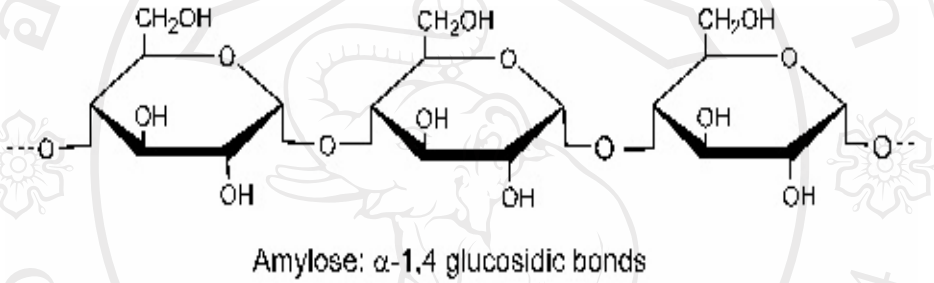


Figure 1 Structure of amylose
(Source: Oregon state university, 2007)

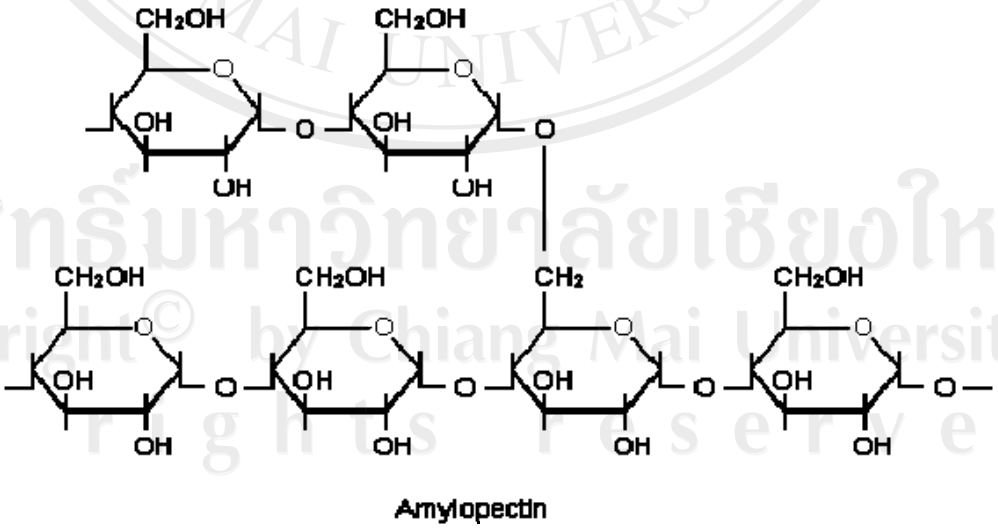


Figure 2 Structures of amylopectin
(Source: Zamora, 2007)

2.1.2 Non-starch polysaccharides (NSPs)

Non-starch polysaccharides (NSPs) are fiber components found in legumes, wholegrain cereals, vegetables and fruits. These polysaccharides inhibit utilization of nutrient in monogastric because lack of enzymes for digestion. NSPs are classified into three main groups including cellulose, hemicellulose and pectin as show in Figure 3 (Bailey, 1973).

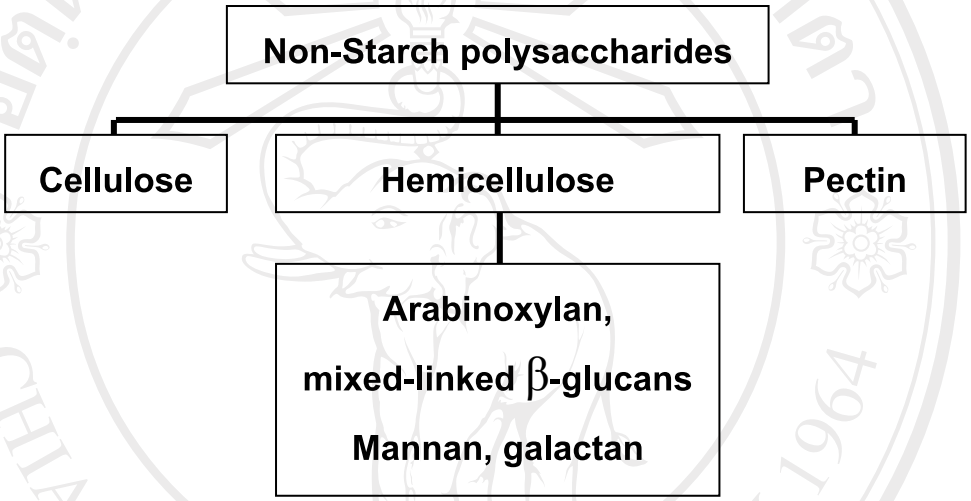


Figure 3 Classification of non-starch polysaccharides
(Source: Bailey, 1973)

Cellulose is the most abundant organic compound in nature, found in cell wall of plant. Cellulose is a linear homopolymer of β-(1,4)-glucose units. It is of high molecular weight and may be consist of up to 7,000-10,000 glucose units.

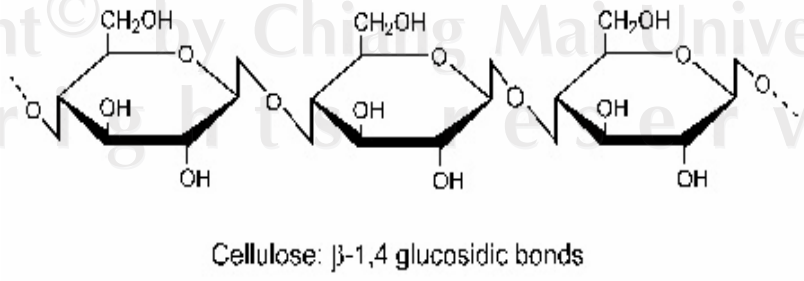


Figure 4 Structures of cellulose
(Source: Oregon state university, 2007)

Hemicelluloses are a group of heteropolysaccharides found in plant cell wall plant the same as cellulose. It consists of sugar units containing 5 or 6 carbons; it is insoluble in water, but soluble in alkali (basic solutions). The sugars, which form a basis for hemicellulose classification, include xylose, mannose, and galactose in the hemicellulose backbone and arabinose, glucuronic acid, and galactose in the hemicellulose side chains. The sugars in the side chains also confer important characteristics on the hemicellulose. For example, hemicelluloses that contain acids in their side chains are slightly charged and water-soluble. Other hemicelluloses are insoluble. The most common hemicelluloses are: xylan, beta-glucan, arabinoxylan, glucomannan, etc. Figure 5 and 6 shows the examples of hemicelluloses structure as β-glucan and xylan.

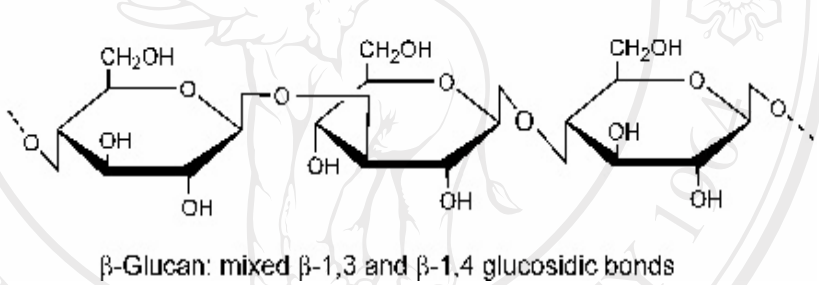


Figure 5 The structure of β-glucan
(Source: Oregon state university, 2007)

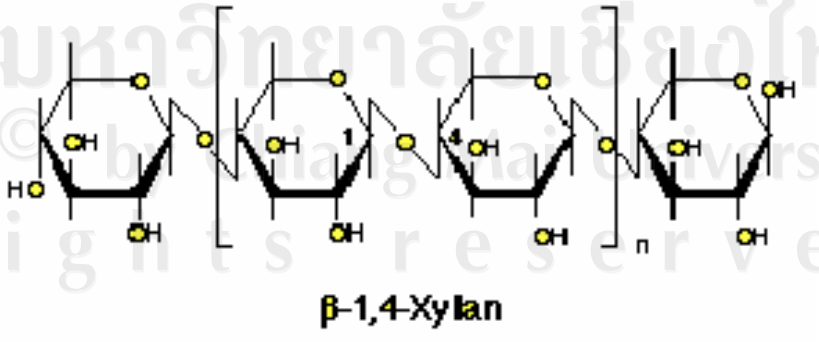


Figure 6 The structure of xylan
(Source: Nakamura, 2005)

2.1.3 Applications of plant polysaccharides (Aspinall, 1982)

Plant polysaccharides are widely used commercially and can be divided into 5 groups according to their utilizing purposes.

1. Food industry, these polysaccharides are used in the food industry as the functional ingredients such as thickeners in jams, sauces), stabilizers in mayonnaise, milk shakes, gelling agents in dessert gels and fabricated food and as emulsifier in salad dressing.

2. Paper industry, polysaccharide has functions as thickeners in paper manufacture.

3. Textiles industry uses polysaccharides as thickening and sizing agent.

4. Energy fuel and alcohols, polysaccharides are being used as raw material to produce alcohol.

5. Pharmaceutical proposes

2.2 Moo-noi

Moo-noi is classified in the *Menispermaceae* family, *Cissampelos* genus and *Pareira* species. There are many common names in Thailand such as Moo-noi, Khruea-ma-noy and also Krung-ka-mao. It is a woody vine climbing plant which the vine is tough and dark green. The leaf have diameter up to 30 cm in length, hairy and cordate shape. The plant is found throughout in the tropical regions of Asia, East Africa, and South America (Smitinand and Larsen, 1991). It is widespread in the northern and northeastern of Thailand.

Moo-noi is commonly referred to as a medicinal herb by indigenous people due to their analgesic properties and they have been used for treating a variety of ailments such as asthma, dysentery, diuretic and traumatic pain (Mukerji and Bhandari, 1959). The extracts from root of this plant exhibited some biological function such as antitumor, antileukemia, diuretic and muscle relaxant (Caceres *et al.,* 1987; Itokawa *et al.,* 1993) and these functionalities have been largely attributed to the presence of a number of alkaloids such aknadinine, chondodendrine, hayatine (Dwuma-badu *et al*., 1975; Manske and Holmes, 1954). In food application, due to the gel formation

property of Moo-noi leaves, it has been utilized in food such as desert and also eaten as fresh vegetable.

Figure 7 *Cissampelos pareira* (Moo-noi)
(Source: Plant genetic conservation project, 1996)

Table 1 Chemical composition of Moo-noi leaves.

Composition (%)	Crude extract	Dialyzed
Moisture	10.57±0.54	8.53±0.39
Ash	9.86±0.01	8.40±0.69
Protein	3.19±0.10	0.29±0.07
Total sugar (as glucose equivalent)	9.06±0.02	8.56±0.01
Uronic acid (as galacturonic acid equivalent)	70.56±0.10	75.93±0.83
Monosaccharide		
Rhamnose	1.07±0.00	1.07±0.00
Arabinose	0.60±0.01	0.51±0.00
Galactose	0.78±0.01	0.64±0.01
Glucose	0.61±0.03	0.32±0.01
Xylose	0.28±0.00	ND
Mannose	0.58±0.02	0.56±0.00
Minerals		
Na	0.16±0.00	0.26±0.00
K	3.90±0.00	1.60±0.00
Ca	1.40±0.00	2.70±0.00
Mg	0.90±0.00	0.71±0.00
Fe (mg/g)	0.03±0.00	0.03±0.00
Zn (mg/g)	0.12±0.00	0.14±0.00

(Source: Singthong *et al.*, 2004)

It is interesting to note that the formation of the gel occurs in a very short period of time after water extraction of the leaf. There are few reports described the chemical component of polysaccharide from Moo-noi leave. Ruangsuriya *et al.* (2004) reported that the gel from Moo-noi leave contained arabinose, glucose and other unknown sugar. Mixing the gel with lemonade can be reduced its rank odor. The taste of the food product derived from gel proved acceptable to a tasting panel. In addition, Singthong *et al.* (2004) have recently proved that the polysaccharide responsible for gelation of Moo-noi extract is a pectin related compounds. The extracted pectin is low methoxyl pectin which consisted mainly of uronic acid (~70-75%) and small amounts of neutral sugars. Furthermore information by Singthong *et al.* (2005) reported that pectin extracted from Moo-noi leaves mainly consisted of galacturonic acid with trace amount of neutral sugars. The dominant structure of Moo-noi was established as a 1, 4-linked α-D-galacturonan by a combination of carboxyl reduction and methylation analysis and further confirm by FT-IR spectroscopy. The degree of etherification of Moo-noi was 41.7 and 33.7% for crude and dialyzed pectins, respectively. Moo-noi has an average molecular weight of 55 kDa, radius of gyration of 15.2 nm and intrinsic viscosity of 2.3 dl/g.

The isolation and characterization of hydrocolloids from Moo-noi leaves was also studied by Vardhanabhuti and Ikeda (2006). It was found that hydrocolloids from Moo-noi leaves was extracted using cold water and precipitated with alcohol. The hydrocolloid fraction contained 49.7% anhydrouronic acid, and 4.24% methoxyl groups. Degree of esterification of 48.45% was calculated assuming that the methoxyl groups are attached to anhydrouronic acid only. While the study of Arkarapanthu *et al.* (2005) reported that gel extracted from Khruea-ma-noi leaves main polymer is polygalacturonic acid of average molecular weight 741 kDa with 66.3% methylated. Dried powders of extract and purified gels contained 415 and 724 g kg^{-1} of soluble dietary fiber and 7.1 and 13.9 g kg^{-1} of divalent cations, respectively. Factors affecting gelling ability and characteristics include phenolic compounds, oxidizing and reducing agents, pH, divalent cations and temperature. Most gels formed were thermoreversible except at pH 7.

2.3 Pectin

Pectin is a heterogeneous group of acidic structural polysaccharides constituting (in all land plants other than the grasses and their allies) a high proportion of the primary cell wall matrix and of the middle lamella. The most pectin are found in fruit and vegetables and mainly prepared from 'waste' citrus peel (oranges, lemons, grapefruits) and apple pomace.

2.3.1 Structure of pectin

Pectin is a complex polysaccharide consisting mainly of esterified D-galacturonic acid resides in the α-(1-4) chain. The acid groups along the chain are largely esterifed with methoxy groups in the natural product. There can also be acetyl groups present on the free hydroxy groups. The galacturonic acid main chain also has the occasional rhamnose group present which disrupts the chain helix formation. The molecular weight of pectin ranges from 50,000 to 150,000 Daltons.

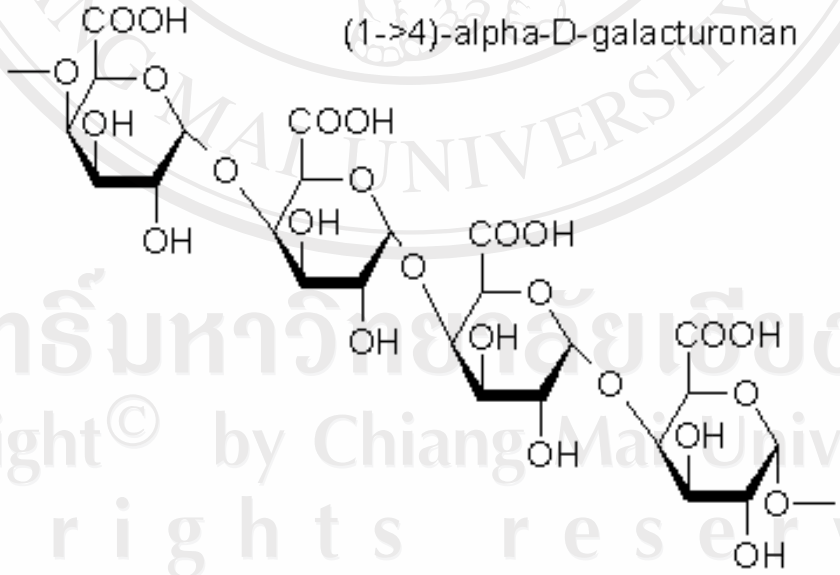


Figure 8 Structure of pectin
(Source: Steve's place, 2007)

Pectin is also known to contain other neutral sugars which are present in side chains. The most common side chain sugars are xylose, galactose and arabinose. The sidechains tend to occur in groups and have led to the description of the pectin molecule as having hairy and smooth regions. They are characterized by a high content of α-D-GalA residues. Three major domains of pectin are recognized: homogalacturonans and rhamnogalacturonans I and II (RG-I and –II).

2.3.1.1 Homogalacturonan

Homogalacturonan (polygalacturonic acid) is a linear polysaccharide of repeating of repeating (1-4)-linked α-D-GalA residues. The homogalacturonan chain seems to consist of blocks of methyl-esterified (neutral) GalA residues alternating with blocks of non-esterified (negatively charged) GalA residues. The charged blocks can be cross-linked by Ca^{2+} ions. Some GalA residues are also O-acetylated: this does not affect the charge of the molecule, but increases its hydrophobicity.

For analysis, homogalacturonans can be de-exterified within the cell wall by treatment with mild alkali and then degraded by treatment with fungal endo-PG (see below). The homogalacturonan is thereby degraded to yield small, water-soluble oligogalacturonides (e.g. DP 2-4) and free GalA; as a side-effect of this, "intact" RG-I and RG-II are solubilised from the cell wall. It therefore appears likely that the three domains of pectin are linked together, possibly as contiguous beads on a string.

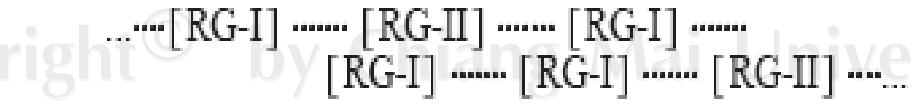


Figure 9 Structures of homogalacturonan
(Source: Dumville and Fry, 2000)

2.3.1.2 Rhamnogalacturonan I

Rhamnogalacturonan I (DP~1000) has as its backbone repeat-unit: -α-D-GalpA-(1-2) - α-L-Rhap-(1-4)-. As in homogalacturonan, some of the GalA residues in RG-I are O-acetylated (Komalavilas and Mort, 1986). Onto the 2-position of about half the Rha resiues, a neutral side-chain is attached. There are many different side-chains; they are rich in (1-4)-linked β-D-galactan and (1-5)-linked α-L-arabinan, the latter being often but not always attached via the former:

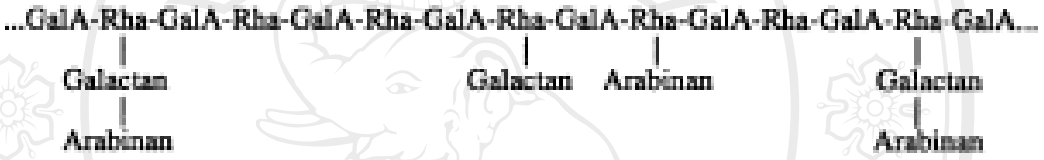


Figure 10 Structures of Rhamnogalacturonan I
(Source: Dumville and Fry, 2000)

2.3.1.3 Rhamnogalacturonan II

Rhamnogalacturonan II (DP~60) is an exceedingly complex structure. It has not been reported to occur in free form in plants, but has been found in red wine (Pellerin *et al.,* 1996), suggesting that it can be released by the action of yeast enzymes. RG-II has a backbone rich in GalA, to which several different side-chains with unusual structures are attached. Structure of homogalacturonan, and rhamnogalacturonans I and II (RG-I and –II) show in Figure11 (A).

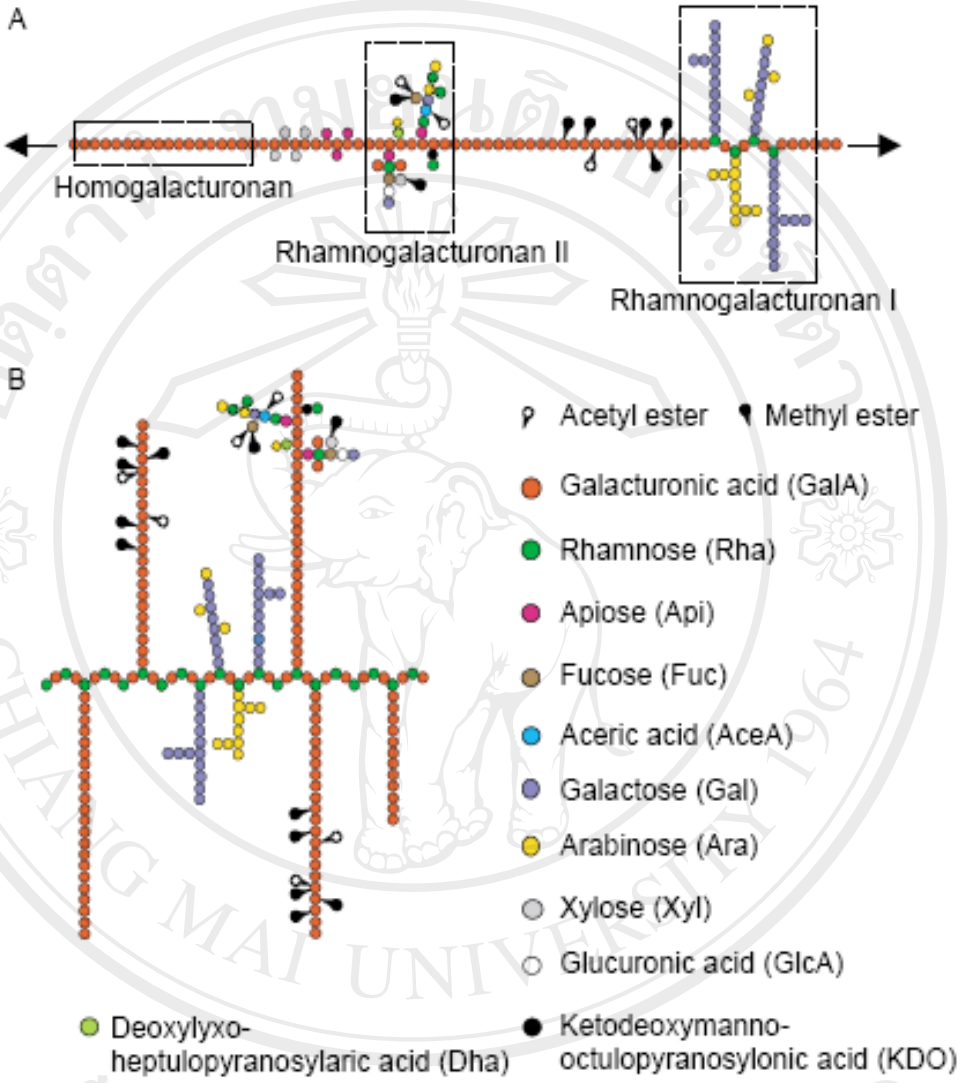


Figure 11 Structure of homogalacturonan, and rhamnogalacturonans I and II (RG-I and –II) (A) and strutures of pectin contain other sugars.(B)
(Source: William *et al*., 2006)

The polygalacturonic acid is partly esterified with methyl groups and the free acid groups may be partly or fully neutralized with sodium, potassium or ammonium ions. The ratio of esterified galacturonic acid groups to total galacturonic acid groups - termed the degree of esterification (DE) - has vital influence on the properties of

pectin, especially the solubility and the gel forming characteristics. The highest DE that can be achieved by extraction of natural raw material is approximate 75%. Pectins with DE from 20-70% are produced by controlled de-esterification in the manufacturing process. The DE of 50% divides commercial pectins into high ester (HM) and low ester (LM) pectin. These two groups of pectin gel by different mechanisms.

HM-pectin requires a minimum amount of soluble solids and a pH within a pretty narrow range, around 3.0, in order to form gels. LM-pectins require the presence of a controlled amount of calcium or other divalent cations for gelation and do not require sugar and/or acid. Degree of esterification of HM-pectins controls their relative speed of gelation as reflected by the designations ‘slow set' and ‘rapid set' high ester pectin. Degree of esterification of LM-pectins controls their calcium reactivity. Some types of LM-pectins also contain amide groups, which strongly affects the calcium reactivity.

2.3.2 Properties of pectins

Pectin gelation characteristics can be divided into two main types: High methoxy gelation and low methoxy gelation.

Gelation of high methoxy pectin usually takes plave at a pH of below 3.5 and total solids content of above 55%. This is the typical gel formed during jam making. High methoxy pectins are characterised by their setting time and the gel strength. Setting time is usually categorised as rapid set, medium set and slow set. High methoxy pectins gel slower as more of the methoxy groups are removed during processing. The pectin garde is often expressed as the number of units of sugar that a unit of pectin can gel.

Low methoxy pectin is gelled with calcium ions and hence is not dependant on the presence of acid or high solids content. The less ester groups present the more sensitive the pectin becomes to pectin and hence a rapid set, low methoxy pectin as

the lowest level of esterification. Amidation can interfere with the gelation causing the gelation to be delayed. Another useful property of amidated pectins is the ability of the gel to reheal after shearing.

Table 2 Property of high methoxy pectin and low methoxy pectin

Types	Methylation level	Amidation level	Common Description
High Methoxy	74-77	0	Ultra Rapid set
High Methoxy	71-74	0	Rapid set
High Methoxy	66-69	0	Medium Rapid set
High Methoxy	58-65	0	Slow set
Low Methoxy	40	0	Slow set
Low Methoxy	30	0	Rapid set
Amidated	35	15	Slow set
Amidated	30	20	Rapid set

(Source: CyberColloids, 2005)

2.3.3 Applications of pectin (CP Kelco company, 2006)

Food applications

Pectin is first and foremost a gelling agent used to impart a gelled texture to foods, mainly fruit based foods. The gelling ability is further utilized where stabilization of multiphase foods is required, either in the final product or at an intermediate stage in the process. The thickening effect of pectin is utilized mainly where food regulations prevent the use of cheaper gums or where the "all natural" image of a product is essential.

Jams and Jellies

HM-pectin requires 55-85% sugar and pH 2.5-3.8 in order to gel. These requirements limit the possible uses of HM-pectin as a gelling agent to sweetened fruit products and about 80% of the world production of HM-pectin is used in the manufacture of jams and jellies, the pectin being added to make up for "deficiency of natural pectins. The role of pectin is to impart a texture to the jam or jelly that allows transportation without changes, that gives a good flavor release and that minimizes syneresis during manufacture of a jam the pectin must ensure a uniform distribution of fruit particles in the continuous jelly phase from the moment the mechanical stirring ceases, i.e. the pectin must set quickly after the filling operation. The use concentrations for pectin vary from 0.1-0.4% in jams and jellies. Pectin gelation can be obtained in a cold process by mixing a pectin-sugar-syrup with soluble solids 60-65% and pH 3.8-4.2 with fruit acid solution to achieve pH 3.0. This process is used in Scandinavia by the bakers to make jelly-covered fruit tarts. A variation of the technique is mixing a pectin solution with pH 2.9 and soluble solids 25% with a liquid sugar to obtain soluble solids 53%.

Fruit preparations for yogurt

Low ester pectins are often used in fruit preparations for yogurt to create a soft, partly thixotropic gel texture, sufficiently firm to ensure uniform fruit distribution but still allowing the fruit preparation to be easily stirred into the yogurt. The pectin may further-especially when combined with other plant gums - reduce color migration into the yogurt phase of the final product.

Fruit drink concentrates

Gelation of pectin may be used as a means of stabilizing a multiphase system if gelling conditions can be achieved at some stage in the process. Gelation provides the yield value which is required to obtain permanent stabilization of emulsions, suspensions and foams. HM-pectin is used in fruit drink concentrates, stabilizing any oil emulsions and fruit particle suspensions. In this application the gelation is apparent in the end product only as a thickening effect, as the coherent gel texture has

been broken mechanically to obtain a smooth flow. Extensive homogenization must not be used, as sufficient yield value must still be present to ensure stabilization.

Fruit juice

The viscosity or mouth feel creating properties of HM-pectin find use in recombined juice products to restore the mouth feel of the juice to that of the fresh juice. Pectin is further used to provide a natural mouth feel in instant fruit drink powders.

Fruit/milk desserts

The calcium response of LM-pectin may be utilized to obtain an instant gelation when adding calcium ions (milk) to a syrup containing LM-pectin. A canned fruit preparation containing 2% LM-pectin in a fruit syrup with 25-30% soluble solids and pH 4.0 is mixed with an equal amount of cold milk to quickly make a fruit flavored semi-gelled milk dessert. LM-pectin has excellent stability at the conditions of fruit preparation manufacture, i.e. pH 4.0 and suitable pasteurization conditions. The LM-pectin solution remains fluid at room temperature as calcium content is insufficient to cause gelation. When the fruit preparation is mixed with milk, sufficient calcium is available to gel the LM-pectin.

Fermented and directly acidified dairy products

The ‘protective colloid' effect of HM-pectin is utilized to stabilize sour milk products either cultured or produced by direct acidification (fruit juice-milk combinations). The pectin reacts with the casein, prevents the aggregation of casein particles at pH below the isoelectric pH (4.6) and allows pasteurization of the sour milk products to extend their shelf life. The texture of yogurt may be improved by small amounts of LM-pectin which is added before the yogurt milk is heated. The LM-pectin does not prevent syneresis.

Gelled milk products

LM-pectin is suited as a gelling agent in milk desserts, but less economical in use than carrageenan, which gels milk a much lower use concentrations. LM-pectin

may, however, be preferred as gelling agent for sour milk puddings or milk desserts combined with fruit. Unlike carrageenan, LM-pectin does not co-precipitate with casein at reduced pH-values and thus ensures a reasonable shelf life of the product.

Confectionery products

High ester pectin is mainly used within the confectionery industry for making fruit jellies and jelly centers, flavored with natural fruit constituents and/or synthetic flavors. In combination with whipping agents it is further used as a texturizer for aerated fruit flavored products. Low ester pectin not requiring addition of acid for gel formation is used for jellies and centers in which the low pH-range necessary for HM-pectin gelation is not acceptable for flavor reasons (e.g. peppermint or cinnamon flavored jellies).

At low concentrations, LM-pectin may further impart a thixotropic texture to confectionery fillings. At higher concentrations a cold gelation can be obtained if calcium ions are allowed to diffuse into the filling.

Compared to other gelling agents commonly used for confectionery products, pectin requires strict observance of the recipe and production parameters, but offers the advantage of a very fine texture and mouth feel, excellent flavor release and compatibility with modern continuous processing due to a fast and controllable gelation.

Pharmaceutical applications

The ability of pectin to add viscosity and stabilize emulsions and suspensions is utilized in a number of liquid pharmaceutical preparations. Pectin is further reported to possess a number of valuable biological effects-the most well-known being an anti-diarrhea effect. Anti-diarrhea suspensions, powders or tablets often contain a mixture of kaolin, pectin and antibiotic.

Pectin is extensively used as a component in the adhesive part of ostomy rings. In this application the water binding effect and the ability to adhere to moist surfaces are utilized. Pectin is further non-irritating in contact with the skin, and certain bactericidal and wound healing effects have even been reported

2.3.4 Pectin and health of human

The health effects of pectin are receiving increasing interest. It is generally accepted that a high fiber diet is beneficial to health and pectin is an important soluble fiber component of fruits and vegetables. There is clear evidence that pectin can lower cholesterol levels, serum glucose levels and may also have anti-cancer activities (Yamada, 1996; Behall and Reiser, 1986). Pectin and pectic oligosaccharide have been shown to induce apoptosis in human colonic adenocarenoma cell (Olano-Martin *et al*., 2003a). Most studies have involved relatively crude pectin preparations containing a large number of different structural domains. It has therefore been impossible to causally relate specific health related activities to defined molecular structures. Progress is being made and most evidence indicates that the complex side chains of pectin are important with regard to anti-cancer activities and other bioactive properties (Yamada *et al*., 2003). So far pectin producers have been hesitant about promoting the potential neutraceutical effects of their products. However, if convincing evidence of the health activities of defined pectic domains is demonstrated then this may change.

Pectin is considered a soluble dietary fiber and exerts physiological effects on the gastrointestinal tract such as delayed gastric emptying (Schwartz *et al.* 1982; Flourine *et al.* 1985), reduced transit time and reduced glucose absorption(Spiller *et al.* 1980). These effects are mainly due to its gel forming and water holding capacity (Roberfroid, 1993). Other actions that have been attributed to the ingestion of pectin are interaction with medicines and interaction with the intestinal metabolism of ions (Seyrig *et al*. 1983).

2.4 Pectic oligosaccharides (POS)

Pectic oligosaccharides (POS), produced by microbial enzymes, are well-known oligosaccharides. Olano-Martin *et al.,* (2001) reported that POS were manufactured from commercial pectin in an enzyme membrane reactor. The prebiotic properties of POS was also evaluated (Olano-Martin *et al*., 2002). These POS had a low prebiotic

potential compared to fructo-oligosaccharides (FOS), although they were more selectively fermented than were the parent pectins. Pectic oligosaccharides also protected colonocytes against *Escherchia coli* verocytotoxins (Olano-Martin *et al.,* 2003b) and stimulated apoptosis in human colonic adenocaricnoma cell (Olano-Martin *et al*., 2003a).

2.4.1 POS as prebiotics

Hotchkiss (2003) showed that *In vitro* tests by pectin acts as a prebiotic, preventing pathogens from binding to the intestine and increasing the growth of probiotic bacteria in the large intestine. Probiotic bacteria are thought to stimulate gut health.

Olano-Martin *et al.* (2002) concluded that pectic oligosaccharides (POS) 1 and 2 [derived from low methlyated pectin (LMP) and high methylated pectin (HMP), respectively] gave a better prebiotic effect than LMP and HMP. With the exception of POS2 at 8 h fermentation, POS 1 and 2 had higher prebiotic index (PI) scores than LMP and HMP at all time points.

Manderson *et al.* (2005) reported that POS can be used effectively as a prebiotic because of the increase in bifidobacteria and *Eubacterium rectale* munbers with the subsequent increase in butyrate concentrations, giving an added possible health benefit.

Mandalari *et al*. (2007) was studied in vitro evaluation of the prebiotic activity of a pectic oligosaccharide-rich extract enzymatically derived from bergamot peel by using pure and mixed cultures of human faecal bacteria. This was compared to the prebiotic effect of FOS. It was found that addition of the POS resulted in a high increase in the number of bifidobacteria and lactobacilli, whereas the clostridial population decreased

2.5 Prebiotic

2.5.1 Definition of prebiotic

The term “prebiotic” was introduced by Gibson and Roberfroid in 1995 who exchanged “pro” for “pre,” which means “before” or “for.” They defined prebiotic as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health”. This definition is more or less overlap with the definition of dietary fiber, with the exception of its selectivity for certain species.

2.5.2 Properties of prebiotic substances

The properties of prebiotics, which was classified by Gibson and Roberfroid (1995) as following.

1. It must be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract.
2. It must consequently, be able to alter the colonic flora in favor of a healthier composition.
3. It must be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated.
4. It must induce luminal or systemic effects that are beneficial to the host health.

As with most functional foods or ingredients (Diplock *et al*., 1999), the final demonstration must be carried out *in vivo*, either humans or in domestic animals or pets through appropriate clinical feeding trials. The methodologies used should be validated and supported by sound scientific approaches. Although each of these criteria is equally important, the third, concerning the selective stimulation of growth and/or activity of one or a limited number of bacteria, remains the most important and

probably the most difficult to fulfill. This leads to a consideration of fermentable substrates in the human diet, availability for the microflora and selective metabolism.

2.5.3 Structure-function relationships

The prebiotic properties of carbohydrates are likely to be influenced by the following factors:

Monosaccharide composition

Recognized prebiotics are built primarily from glucose, galactose, xylose and fructose. The prebiotic potential of oligosaccharides composed of other monosaccharide is not known at the present time.

Glycosidic linkage

The linkage between the monosaccharide residues is a crucial factor in determining both selectivity of fermentation and digestibility in the small intestine. Fermentation of FOS prebiotics is selective because of a cell-associated β-fructofuranosidase in the bifidobaccteria.

Molecular weight

Polysaccharides are generally not prebiotic in their metabolism but oligosaccharides are (Wang and Gibson, 1993). Inulin has the highest molecular weight, but most of the carbohydrate in inulin has a degree of polymerization less than 25, with an average of about DP 14. The effect of molecular weight on prebiotics can be seen from the fact that xylan is not selective whereas xylo-oligosaccharides are thought to be. Similar effects occur with pectin (Olano-Martin *et al*., 2000).

2.5.4 Fiber fermentation by gut bacteria

Foods rich in dietary fiber include vegetables, fruit, cereal grains and legumes. Dietary fibers display different degrees of solubility. Some such as pectin, hemicellulose, guar gum and inulin are readily soluble in water (Woods *et al.*, 2001). This leads to the formation of gels in the gastrointestinal tract. This aids their

fermentability by the gut microflora by virtue of an increased surface area available for enzymatic attack. The relative fermentability of defferent fibres is dependant on a number of physiochemical properties. Fiber particle size and degree of solubility have a considerable effect on the susceptibility of fibers to bacterial fermentation in that they govern the surface area exposed to bacterial degradation.

The fermentation of dietary fiber in the colon has a number of attributes. The main product of polysaccharide fermentation in the colon is bacterial biomass, which not only increases stool bulk but gives rise to increased numbers or metabolic activity of the main saccharolytic bacterial species. Increased stool bulk contributes towards reduced colonic transit times which is seen as beneficial not only for the relief and prevention of constipation, but in reducing the impact of detrimental microflora associated characteristics such as toxic nitrogenous compounds, H_2S, and the production of carcinogenic or gentoxic compounds. Dietary fibre contributes towards stool bulking through increased bacterial biomass and more directly through the sheer volume of fiber compounds such as the lignins and residual cellulose with escape bacterial fermentation.

Bacterial fermentation also results in a lowering of colonic pH. Lower pH values impede the growth of certain pathogenic bacterial species while encouraging the growth of the bifidobacteria and lactic acid microflora. A low colonic pH may also aid in the excretion of carcinogens, which bind to dietary fiber in the colon (Rowland, 1995).

In particular, a confirmation on the selectivity of fermentation is not apparent, especially using high fidelity molecular based procedures for determining flora changes as a response to the fermentation. As such, their capacity to stimulate probiotics in the gut is unproven. More research is required, however promise does exist with derivatives of common fibers. For example, one of the best characterized fibres is pectin which is not selectively metabolized by the gut flora. Pectic derived oligosaccharides do however show good promise. This effect may be explained by the observation that probiotics like the bifidobacteria prefer to utilize carbohydrates

of oligosaccharide size rater than higher molecular weight polymers. The latter do not generally have the selective metabolism required of efficacious probiotics.

Among the food ingredients, nondigestible carbohydrates (oligo- and polysaccharides), some peptides and proteins, and certain lipids (both ethers and esters) are candidate prebiotics. The selectivity was show, for example Bifidobacterium, which may be promoted by the ingestion of fructooligosaccharide, inulin, trans-galactooligosaccharide and soy bean oligosaccharide.

2.5.5 Oligosaccharides as prebiotic

The following describes the various oligosaccharides which are classified as prebiotics.

Fructo-oligosaccharides (FOS) typically refer to short-chain oligosaccharides comprised of D-fructose and D-glucose, containing from three to five monosaccharide units. FOS, also called neosugar and short-chain FOS, are produced on a commercial scale from sucrose using a fungal fructosyltransferase enzyme. FOS is resistant to digestion in the upper gastrointestinal tract. They act to stimulate the growth of *Bifidobacterium* species in the large intestine. FOS is marketed in the United States in combination with probiotic bacteria and in some functional food products.

Inulins refer to a group of naturally-occurring fructose-containing oligosaccharides. Inulins belong to a class of carbohydrates known as fructans. They are derived from the roots of chicory *(Cichorium intybus)* and Jerusalem artichokes. Inulins are mainly comprised of fructose units and typically have a terminal glucose. The bond between fructose units in inulins is a beta-(2-1) glycosidic linkage. The average degreee of polymerization of inulins marketed as nutritional supplements is 10 to 12. Inulins stimulate the growth of *Bifidobacterium* species in the large intestine.

Lactulose is a semisynthetic disaccharide comprised of the sugars D-lactose and D-fructose. The sugars are joined by a beta-glycosidic linkage, making it resistant to hydrolysis by human digestive enzymes. Lactulose is, however, fermented by a limited number of colonic bacteria. This can lead to changes in the colonic ecosystem in favor of bacteria, such as lactobacilli and bifidobacteria, which may confer some health benefits. Lactulose is a prescription drug in the United States for the treatment of constipation and hepatic encephalopathy. It is marketed in Japan for use as a dietary supplement and in functional foods. Its use in the United States as a prebiotic substance is still experimental.

Soy oligosaccharides refer to oligosaccharides found in soybeans and also in other beans and peas. The two principal soy oligosaccharides are the trisaccharide raffinose and the tetrasaccharide stachyose. Raffinose is comprised of one molecule each of D-galactose, D-glucose and D-fructose. Stachyose is comprised of two molecules of D-galactose, one molecule of D-glucose and one molecule of D-fructose. Soy oligosaccharides act to stimulate the growth of *Bifidobacterium* species in the large intestine. They are marketed in Japan as dietary supplements and in functional foods. They are being developed in the United States for similar uses.

Transgalacto-oligosaccharides (TOS) are a mixture of oligosaccharides consisting of D-glucose and D-galactose. TOS are produced from D-lactose via the action of the enzyme beta-galactosidase obtained from *Aspergillus oryzae*. TOS are resistant to digestion in the upper gastrointestinal tract and stimulate the growth of bifidobacteria in the large intestine. TOS are marketed in Japan and Europe as dietary supplements and are used in functional foods. They are being developed for similar use in the United States.

Xylo-oligosaccharides are comprised of oligosaccharides containing beta (1→4) linked xylose residues. The degree of polymerization of xylo-oligosaccharides is from two to four. Xylo-oligosaccharides are obtained by enzymatic hydrolysis of the polysaccharide xylan. They are marketed in Japan as prebiotics and are being developed for similar use in the United States.

2.5.6 Effects of prebiotics on health

Prebiotics have positive effects on several biomarkers related to health benefits and they may hence play a role in reducing the risk of colon cancer, inflammatory bowel disease, gastrointestinal infections and in sustaining bone health. The metabolism and associated health benefits was shown in Figure12.

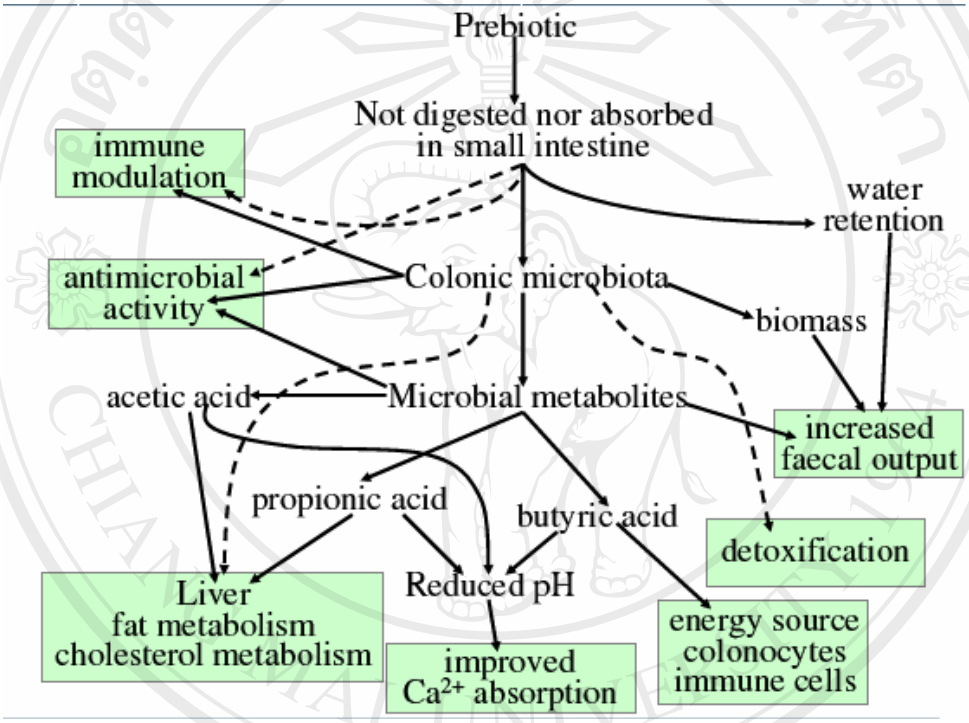


Figure 12 The metabolism and associated health benefits
(Source: Ouwehand *et al*., 2006)

Many reports had presented the effects of prebiotics on host health in several aspects. The effective daily doses of pure oligosaccharide are 3.0 g for fructoologosaccharides, 2.0-2.5 g for galactooligosaccharides, 2.0 g for soybean oligosaccharides, and 0.7 g for xylooligosaccharide (Mazza, 1998). Some of beneficial effects of prebiotics were shown as below.

1. Improved intestinal microflora is well known property of prebitics and fitted to prebiotics definition. Many human and animal studies have shown that

addition of fructooligosaccharide to diet stimulates the proliferation of *Bifidobacterium* spp. and other useful bacteria, while suppressing growth of harmful bacteria, such as Clostridium spp. (Kullen *et al.,* 1998; Bouhnik *et al.,* 1999; Cambell *et al.,* 1996).

2. Effects on pathogens: good evidence for the success of prebiotics lies in their ability to improve resistance to pathogens by increasing bifidobacteria and lactobacilli. Lactic-acid-excreting microorganisms are known for their inhibitory properties (Fuller, 1997). In humans, viruses, protozoa, fungi and bacteria can all cause acute gastroenteritis. Metabolic end-products, such as acids excreted by these microorganisms, may lower the gut pH to levels below those at which pathogens are able effectively to compete. Also, many lactobacilli and bifidobacterial species are able to excrete natural antibiotics, which can have a broad spectrum of activity. For the bifidobacteria, some species are able to exert antimicrobial effects on various Gram-positive and Gram-negative intestinal pathogens (Mackey and Gibson, 1997). A recent study in mice has shown that FOS and inulin protected against enteric and systemic pathogens and tumor inducers (Buddington *et al*., 2002). This includes the verocytotoxin strain of *Escherichia coli* O 157:H7 and campylobacters.

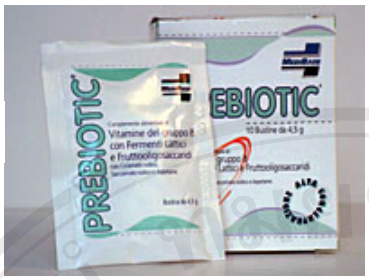
3. FOS and other oligosaccharides are noncariogenic sucrose substitutes. These sweeteners are not utilized by dental cariogenic bacteria, such as *Streptococcus* mutants, that normally from acids and insoluble B-glucan lead to formation of dental caries (Mazza, 1998).

4. Daily consumption of 3-10 g of oligosaccharides produces and anticonstipation effect within a week. This effect has been attributed to increase levels of short chain fatty acid (SCFA) and increased intestinal peristalsis produced by increased population of *Bifidobacterium* spp. in the intestine. However, consumption of large amount of NDOs causes diarrhea, abdominal distention and flatulence (Mazza, 1998). The suitable FOS dose for man and women are 0.3 and 0.4 g/kg body weight per day, respectively. Corresponding nondiarrhea-causing doses for soybean oligosaccharides are 0.64 g/kg for men and 0.96 g/kg for women.

5. Blood lipids can be reduced by prebiotics and prevent of coronary heart disease. The study on 12 hypercholesterolemia men, who daily consumed 20 g of inulin composed in vanilla ice cream for 3 weeks, the significantly reduction of serum triglycerides and cholesterol was observed (Causey *et al.,* 2000). The amount of fructans as FOS or inulin used in the human studies varies between 9 g and 20 g, however this amount is small compared to that which is used in animal studies (50-200 g per kg of rat feed), which is equivalent to a dose in humans of approximately 50-80 g of FOS or inulin per day (Lovegrove and Jackson,2000)

6. Immunological effects: lactic acid bacteria are thought to stimulate both non-specific host defence mechanisms and certain types of cell involved in the specific immune response. The result is often increased phagocytic activity and/or elevated immunological molecules such as secretory lgA, which may affect pathogens such as salmonellae and rotavirus. Most attention in this respect has been diverted towards the intake of probiotics (lactic acid bacteria) (Schiffrin *et al*., 1995) and interactions between cell wall components and immune cells. As prebiotics serve a similar end point to lactic acid bacteria (i.e. improved composition of the gut microflora) similar effects may occur through their intake. A recent study in animal has shown that prebiotics had an effect on immune functions (Swanson, 2002).

(A)



(B)

(C)

Figure 13 Some prebiotic products.

(A) Prebiotic (Medibase, 2007)

(B) Inuflora (Wobenzymcatalog, 2007)

(C) Optiflora prebiotic complex (Shaklee Corporation, 2007)

2.6 Probiotic

2.6.1 Definition of probiotic

Schrezenmer and Vrese (2001) reviewed the meaning of probiotics from many reports as follow:

The term of "probiotic" means "for life", is derived from the Greek language. It was firstly used by Lilly and Stillwell in 1965 to describe substances secreted by one microorganism which stimulates the growth of another and this was contrasted with the term "antibiotic". It may be because of this positive and general claim of definition, the term "probitic" was subsequently applied to other subjects and gained a more general meaning.

In 1971 Sperti applied this term to tissue extracts that stimulate microbial growth. Parker was the first to use the term probiotics in the sense that it is used today. He defines probiotics as “organisms and substances, which contribute to intestinal microbial balance”. Retaining the word “substances” in Parker’s definition of probiotics resulted in a wide meaning that included antibiotics.

In 1989 Fuller attempted to improve Parker’s definition of probiotics with the following distinction: “A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. Thus, Fuller’s definition of probiotics was involved animals.

In 1992 Havenaar *et al.* gave definition of probiotics with respect to host and habitat of microflora as follows: “A viable mono- or mixed culture of microorganisms which applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora”.

In 1996 Salminen gave the last definition of probiotics as “a live microbial culture or cultured dairy product, which beneficially influences the health and nutrition of host”. However, all definitions mention that probiotics give benefits for health.

Over the years, many species of microorganisms have been used. They consist not only of lactic acid bacteria (lactobacilli, streptococci, enterococci, lactococci, bifidobacteria), but also *Bacillus* spp. and fungi such as *Saccharomyces* spp. and *Aspergillus* spp. (Rastall *et al.,* 2000).

(A) (B) (C)

Figure 14 Some probiotic products

(A) iFora-Caps (Sedona labs ™, 2007)

(B) Probiotic (Total health centre, 2007)

(C) Probiotic Gold (Healthwize, 2007)

2.6.2 Gut microbiota-an unexplored ecosystem

The intestine's normal microbiota is as yet an unexplored organ of host defence. Although bacteria are distributed throughout the intestine, the major concentration of microbes and metabolic activity is found in the large intestine. The mouth harbours a complex microbota consisting of faclutaive and strict anaerobes including streptococci, Bacteroides, lactobacilli and yeasts. The upper bowel is sparsely populated, and from the ileum on bacterial concentrations gradually increases, reaching 1011-1012 colony-forming units (CFU)/g in the colon (Figure15). Up to 500 species of bacteria may be present in the adult human large intestine; it has been estimated that bacteria account for 35-50% of the volume of the contents of the human colon.

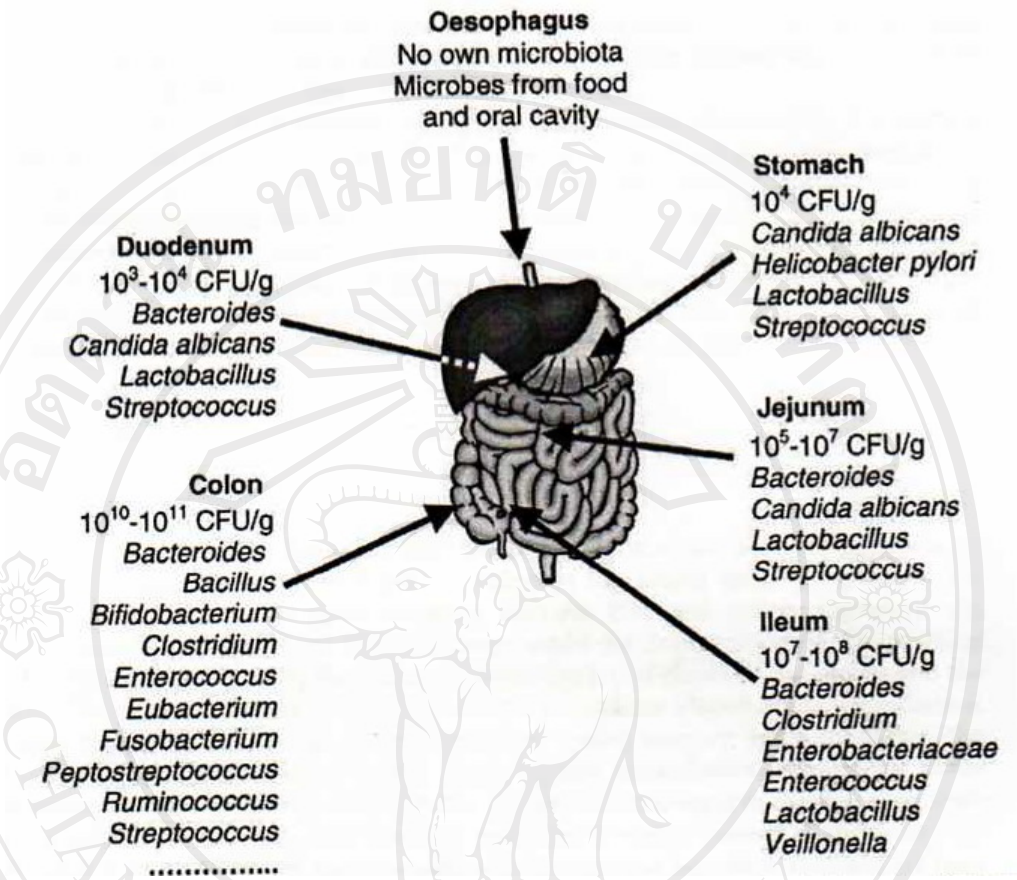


Figure 15 The numerically dominant microbial genera in the adult human gastrointestinal tract
(Source: Isolauri *et al.*, 2004)

2.6.3 A unifying hypothesis for health effects

The health effects attributed to the use of probiotics are numerous. The following outcomes are well documented: 1) lower frequency and duration of diarrhea associated with antibiotics (*Clostridium difficile*), rotavirus infection, chemotherapy, and to a lesser extent, traveler's diarrhea; 2) stimulation of humoral and cellular immunity; and 3) decrease in unfavorable metabolites, eg, ammonium and procancerogenic enzymes in the colon. There is some evidence of health effects through the use of probiotics for the following.

1) reduction of *helicobacter pylori* infection;

2) reduction of allergic symptoms;
3) relief from constipation;
4) relief from irritable bowel syndrome;
5) beneficial effects on mineral metabolism, particularly bone density and stability;
6) cancer prevention; and
7) reduction of cholesterol and triacylglycerol plasma concentrations (weak evidence).

These munerous effects can hardly be explained by a unifying gypothesis that is based on a single quality or mechanism and remains valid for all microorganisms exertion one or the other effect mentioned above.