CHAPTER I

INTRODUCTION

The pectins are found universally in the primary cell walls and intercellular layers in land plants. They are most abundant in soft tissues such as the rinds of citrus fruits, sugar beet pulp and apples, but are present in only small proportions in woody tissues. Pectin is composed preponderantly of α-1,4 linked D-galacturonate units with varying degrees of methylated carboxyl groups. The remainder of pectin is composed of the neutral sugars, L-arabinose, D-galactose, L-rhamnose, D-glucose, D-mannose and D-xylose. The neutral sugars are located in the hairy fragments with the exception of xylose which may exist as short chains attached to the galacturonic backbone. The rhamnose is involved in the pectic backbone and may constitute the point of attachment of the neutral sugar side chains. The other neutral sugars may occur in short side chains but are also found in more or less branched multiple units.

The high proportion of galacturonic acid in most plant pectins confers a highly acidic character to the
polysaccharide and facilitates ionic interactions with calcium. Plants alter this acidic character by addition or removal of methyl esters from the galacturonic acid residues. The pectic substances in the cell wall which tend to occur with a proportion of the acid groups as salts (most frequently calcium) are extracted with ammonium oxalate or reagents such as sodium hexametaphosphate which complex with divalent metal ions. Polysaccharides in which a proportion of the D-galacturonic acid residues are present as methyl esters are designated "pectinic acids" and those devoid of ester groups as "pectic acids". The degree of methyl esterification (DOM) varies between plant tissues and stages of development. One major function of pectin in plants is cohesion via numerous calcium cross-bridges formed between adjacent pectin molecules.\textsuperscript{5}

Glucose is not considered to be a genuine constituent of pectic polysaccharides, but the occurrence of such residues has been reported in a variety of pectins\textsuperscript{6,7} and such units have been interpreted as evidence in support of a covalent linkage between the amyloid (xyloglucans) and pectic materials.\textsuperscript{8} Glucuronic acid residues have been reported in many pectic polysaccharides.
Pectins are extensively used in the food industry as gelling agents in the preparation of food spreads and also as emulsifying agents between oils and water. The ability of pectins to form gels depends on the length of the polygalacturonic acid chains as well as the degree of methoxylation. The longer the chain of the pectin macromolecule and the higher degree of methoxylation, the better will be the gel forming ability of the pectin.\(^9\)

Pectins contribute to many physiological effects such as lowering of cholesterol by binding to bile acids and thus increasing cholesterol catabolism.\(^{10,11}\) In vitro studies on vegetable fiber from carrot, cabbage, broccoli and onion have indicated that binding of bile acids to fiber largely occurs through calcium salt linkages to the calcium pectate residues in cell wall. The binding of certain other dietary anions (fatty acids, phytate, and oxalate) to calcium pectate seems beneficial, since elimination of these substances is very desirable from a physiological standpoint.\(^{12}\)

Recently, in view of their physiological significance, renewed attention has been focused on the pectin polysaccharides of fruits and vegetables. The noteworthy studies in this connection are those carried
out on apple, carrots, onions, dwarf french and runner beans\textsuperscript{13-17}.

Hypolipidemic activity

Atherosclerosis is a common cause of morbidity and mortality in industrialized nations. This disease involves the accumulation of lipids, particularly free and esterified cholesterol, in and between cells within the vascular bed. The involvement of plasma cholesterol metabolism in atherogenesis is supported most convincingly by epidemiological studies. An increased level of plasma cholesterol (particularly LDL cholesterol) is an independent risk factor for this disease. There are also data that HDL "protects" against the deleterious effects of LDL in plasma\textsuperscript{18}.

Several developments have enhanced the emphasis on preventing coronary heart disease (CHD) through control of blood cholesterol. A report prepared for the Federation of American Societies for Experimental Biology (FASEB) describes > 50 studies with soluble fibers and concludes that "soluble fibers such as pectin, guar gum, locust bean gum, oat gum or psyllium muciloid significantly reduce serum total cholesterol and low density lipoprotein
cholesterol levels with little effect on high-density lipoprotein cholesterol levels.\textsuperscript{19}

Generally reported mechanisms by which soluble dietary fibers lower blood cholesterol include the following: 1) decrease in cholesterol absorption and enhanced excretion of fecal sterols and 2) suppression of endogenous cholesterol synthesis via short-chain fatty acids (SCFA) produced by colonic bacterial fermentation of soluble fibers.\textsuperscript{20} Several possible mechanisms whereby the effective dietary fibers may influence lipid absorption include disruption of micellar solubilities of lipids, altered diffusibility of bulk and monomolecular forms of lipids, reduced transport into epithelial cells, and decreased exocytosis of lipids into lymph.\textsuperscript{21}

The effect of pectins on lipid metabolism has been studied extensively in humans and experimental animals. Borgmann and Wardlaw\textsuperscript{22} reported lowering of serum cholesterol in rabbits fed beef tallow by including pectin. Miettinen and Tarpila\textsuperscript{23} studied the effect of pectin on serum cholesterol, fecal bile acids and biliary lipids in normolipidemic and hyperlipidemic individuals. Administration of pectin had no effect on serum triglycerides in both normolipidemic and hyperlipidemic
subjects but did cause a significant decrease in total and unesterified serum cholesterol in hypercholesterolemic subjects in particular.

Bobek et al\textsuperscript{24} studied the effect of citrus pectin on diet induced hypercholesterolemia in guinea pigs. They found that vitamin C, pectin and a combination of the two substances prevented effectively further progress of hypercholesterolemia, induced by the administration of a high fat diet.

**Hypoglycemic activity**

Diabetes mellitus is a complex disease that can no longer be considered a single disease entity. It is a major contributor to chronic ill health and early death in all industrial societies and can be expected to become (and in some cases already is) a major health problem in developing countries. Atleast two major subdivisions are now recognised by WHO, insulin-dependent diabetes mellitus (Type 1 Diabetes or IDDM) and non-insulin-dependent diabetes mellitus (Type 2 Diabetes or NIDDM)\textsuperscript{25}. Absolute or near absolute insulin deficiency is the prime aetiologial factor in type 1 diabetes. In contrast, the metabolic derangements in type 2 diabetes are more complex. Abnormalities of pancreatic cell function are
traditionally recognised and may have a strong genetic-hereditary background\textsuperscript{26}. Early in the natural history of type 2 diabetes, however, insulin responses are often equal to or greater than those found in subjects with normal glucose tolerance. These data have therefore raised the possibility that the tissues of patients with type 2 diabetes do not respond normally to insulin; that is they are insulin resistant.\textsuperscript{27} Over the last decade, this concept has received increasing support and insulin resistance is now recognised as a characteristic feature of type 2 diabetes.\textsuperscript{26,27} Thus, both impaired cell function and insulin resistance are features of type 2 diabetes.

Although a hereditary influence is beyond dispute, NIDDM is probably not caused by defects at a single locus. NIDDM is usually not clinically apparent until adulthood, although manifestations of the disease may be detectable much earlier. The following observations suggest a complex aetiology based on genetic and non-genetic factors: (1) many patients who develop NIDDM are also obese; (2) the disease often goes into remission after severe, prolonged caloric restriction; (3) the incidence of NIDDM increases with increasing age; and (4) environmental agents can cause β-cell damage.
Increased glycosylation and consequent accumulation of advanced glycosylation end products in the tissue appear to be a biochemical link between chronic hyperglycemia and pathophysiology of diabetes complications. It is also well established that a constellation of traditional cardiovascular risk factors, including hypertension and dyslipidemia (high TG and low HDL cholesterol), also proceed the onset of type 2 diabetes. Numerous reports have demonstrated that poor glycemic control is associated with elevated plasma cholesterol levels in both diabetic humans and diabetic animal models.

The object of diabetes treatment is to restore normal carbohydrate, protein and lipid metabolism. The cornerstone of this treatment has been diet since the end of the 18th century, but true antidiabetic therapy started only with the identification and purification of insulin. The hypoglycemic effect of guanidines was discovered in 1919, leading to their oral therapeutic use, but they were withdrawn in 1932 due to their hepatotoxic effects. The related biguanides appeared in the 1950s but have since diminished in importance so that metformin is practically the only representative still used today. Work in the 1940s and 1950s led to the discovery and development of
hypoglycemic sulfonylureas (SU), a therapeutic class unique for its specificity and safety. These products were found to stimulate insulin secretion by the endocrine pancreas.\textsuperscript{31}

Ratzman and Thoelk\textsuperscript{32} studied the data collected in the Berlin diabetes register on the prevalence of diabetes and trends in the prescription of oral antidiabetic agents between 1961 and 1989. Their studies show that from 1969 onward, the percentage of carbutamide decreased drastically and was no longer prescribed after 1973. In 1989, the prescription rate of tolbutamide was only 10% of all oral antidiabetics, while 88% of the patient group received glibenclamide. Recent recommendations suggest the use of the combination of insulin treatment with oral antidiabetic agents in NIDDM, especially in secondary failures and insulin resistance with exogenous insulin. Feldman and Strom\textsuperscript{33} have discussed the need to expand the applications of research on the use of oral antidiabetic agents, including assessment of patterns of morbidity across geographic boundaries and over time.

A number of reports are now available on the hypoglycemic activity of pectins.\textsuperscript{34-39} But there is lack of information in this area regarding the pectins from some common vegetables and fruits in India.
Lipid Peroxidation

Maintenance of normal cell functions in the presence of oxygen largely depends on the efficacy of the tissue protection against free radical mediated oxidative stress. Reduction of a molecule of oxygen to water requires four electrons, and in a sequential process, several intermediates will be encountered. These are the superoxide anion radical ($\dot{O}_2^-$), hydrogen peroxide ($H_2O_2$), and the hydroxyl radical ($OH^-$).

\[
O_2 + e^- \rightarrow \dot{O}_2^- + 2H^+ \rightarrow H_2O_2 + e^- + H^+ \rightarrow OH^+ + e^- + H^+ \rightarrow H_2O
\]

These intermediates being highly reactive and toxic, pose a threat to the integrity of the cell.

Lipid peroxidation is a reaction between polyunsaturated fatty acids (PUFA) and oxygen which is initiated by radical intermediates and active oxygen species produced by normal metabolic reactions or during metabolism of chemicals. The $\dot{O}_2^-$ is generated within aerobic biological systems during both enzymatic and non-enzymatic oxidations. The various reactions known to produce substantial amounts of $\dot{O}_2^-$, are autooxidation of hydroquinones, leukoflavins, catecholamine, thiols, hemoglobin and myoglobin.
A number of enzymes such as xanthine oxidase, aldehyde oxidase, dihydro-orotic acid dehydrogenase and several flavin dehydrogenases are shown to produce \( \text{O}_2^- \). Fluxes of \( \text{O}_2^- \) generated enzymatically or photochemically, have been shown to induce lipid peroxidation, damage membranes and kill cells.\(^{47-49}\) Both \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) have been shown to promote the peroxidation of the polyunsaturated phospholipids that make up the biological membranes.

Malondialdehyde (MDA), which may exist in a free form or as complex with various tissue constituents, is formed during the peroxidation of unsaturated fatty acids, and as a by-product of prostaglandin biosynthesis. MDA is formed during the last stages of the breakdown of endoperoxides formed during intramolecular rearrangements in the structure of polyunsaturated fatty acids.\(^{50}\) These rearrangements are necessary to stabilize the free radical formed on the diphatic chain of the fatty acid. In this situation, although fatty acids containing two double bonds, as linoleic acid, may form MDA during peroxidation, fatty acids bearing three or more double bonds are the main source of MDA in biological samples.\(^{51}\)

Tissue antioxidants are essential in preventing the cellular damage caused by free radicals and free
radical-mediated lipid peroxidation. Cellular damage caused by those oxidants has been associated with the aging process as well as the development of a number of chronic diseases including cancer, rheumatoid arthritis, coronary vascular disease and atherosclerosis\textsuperscript{52}.

Superoxide dismutase, glutathione peroxidase (GSHPx) and catalase act synergistically to detoxify the products of oxygen toxicity. Superoxide dismutase (SOD) catalyzes the disproportionation of two molecules of superoxide to form molecular oxygen and hydrogen peroxide as follows:

\[ \text{O}_2^- + \text{O}_2^- + 2\text{H}^+ \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2 \]

The enzyme GSHPx catalyzes the reduction of hydroperoxides of fatty acid (ROOH) and other compounds to their corresponding alcohols, ROH\textsuperscript{53}. It is non-specific with respect to substrate but has a specific requirement for glutathione as hydrogen donor.

\[ \text{ROOH} + 2\text{GSH} \xrightarrow{\text{GSHPx}} \text{ROH} + \text{G-S-S-G} + \text{H}_2\text{O} \]

The studies of McCay et al\textsuperscript{54} indicated that GSHPx does not reduce lipid peroxides in biological membranes to lipid alcohols but prevents lipid peroxidation by removing \( \text{H}_2\text{O}_2 \) before it can react with \( \text{O}_2^- \) to form \( \text{OH}' \).
Catalase is reported as a less effective scavenger of $\text{H}_2\text{O}_2$ at low concentration whereas GSHPx is suggested to be more efficient scavenger of $\text{H}_2\text{O}_2$.

Glutathione reductase regenerates GSH from GSSG formed in the GSHPx catalysed reaction,

$$\text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{glutathione reductase}} 2 \text{GSH} + \text{NADP}^+$$

and thus maintaining the high ratio of reduced to oxidised glutathione intracellularly.

In the pattern of antioxidant defense, some biological compounds like carotenoids, vitamins E, A, C and thiols play a predominant role. Vitamin E or $\alpha$-tocopherol is a lipid-soluble, chain breaking antioxidant capable of scavenging oxygen-centered free radicals.\(^5\) In fact, it has been found experimentally that $\alpha$-tocopherol suppresses the oxidative damage of the membranes more efficiently than water-soluble chain-breaking anti-oxidants such as vitamin C, which scavenge aqueous radicals but cannot scavenge chain carrying radicals within the membranes.\(^6\)

Thiol groups act as intracellular antioxidants by scavenging free radicals and through enzymatic reactions. Glutathione is the most important cellular thiol, acting
as a substrate for several transferases, peroxidases and other enzymes that prevent or mitigate the deleterious effects of oxygen free radicals.\textsuperscript{57} The protection of biological membranes against lipid peroxidation is an interesting aspect of its function, since this water-soluble thiol prevents damage in a lipid environment. Thiols may also react with tocopheryl radicals to regenerate tocopherol and, conversely, tocopherols can also repair thyl radicals.\textsuperscript{58} However, very little information is available on the antiperoxidative effects of pectins.

Membrane fluidity changes

The wide variety of experiments applying different techniques, including spin labeling, on artificial and biological membranes, and the concordance of the findings concerning the lipid phases in both types of membranes led to the concept of a membrane structure whereby hydrophobic membrane proteins are embedded in a continuous bilayer of phospholipids.\textsuperscript{59} In the lipid phase, particularly in the liquid crystalline state above the transition temperature, the molecules are endowed with a high degree of motion: rotational, translational (or lateral diffusion), and even (although slower) transverse (or flip-flop) motion.
It is common practice to characterize membranes in terms of an ill-defined parameter known as fluidity. Membrane fluidity is the reciprocal of membrane viscosity, which in turn is inversely proportional to rotational and lateral diffusion rates of membrane components. The fluidity of membranes depends on the nature of acyl chain region comprising the hydrophobic domain of most membrane lipids. Most lipid species in isolation can undergo a transition from a very viscous gel (frozen) state to the fluid (melted) liquid-crystalline state as the temperature is increased. This transition has been studied intensively, since the local fluidity, as dictated by the gel or liquid-crystalline nature of membrane lipids, may regulate membrane mediated processes. However, at physiological temperatures most, and often all, membrane lipids are fluid.

The long axes of rigid rodlike molecules in liquid crystals fluctuate over a range of angles determined largely by the available space. These fluctuations affect macroscopic and microscopic physical properties of the liquid crystals, the measurement of which, mainly by spectroscopy, has allowed quantitative estimates to be made of the degree of angular disorder of the molecules. The results are usually expressed in terms of order
parameter(s). Order parameters are related to the mean angular deviation of the spin-labelled fatty acid from its average orientation in the bilayer. High values of order parameters are characteristic of relatively solid lipid and low values characterise very mobile lipids.\textsuperscript{60}

The ability of lipids to adopt different macroscopic structures on hydration has stimulated studies aimed at understanding the physical properties of lipids which dictate these preferences. Cullis et al\textsuperscript{61} have given support to a simplistic hypotheses that a generalized shape property of lipids determines the phase structure adopted. This concept is illustrated in Figure 1, where bilayer phase lipids are proposed to exhibit cylindrical geometry compatible with that organization, while hexagonal $H_{11}$ phase lipids have a cone shape where the acyl chains subtend a larger cross-sectional area than the bipolar head group region. Detergent-type lipids which form micellar structures are suggested to have reversed geometry corresponding to an inverted cone shape. A striking observation supporting the shape concept is that lipid mixtures containing detergents (inverted cone shape) and unsaturated phosphatidylethanolamines (cone shape) can adopt bilayer structure, which may be attributed to shape complementarity.
<table>
<thead>
<tr>
<th>LIPID</th>
<th>PHASE</th>
<th>MOLECULAR SHAPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>LYSOPHOSPHOLIPIDS DETERGENTS</td>
<td>MICELLAR</td>
<td>INVERTED CONE</td>
</tr>
<tr>
<td>PHOSPHATIDYLCHOLINE</td>
<td></td>
<td>CYLINDRICAL</td>
</tr>
<tr>
<td>SPHINGOMYELIN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHATIDYL SERINE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHATIDYL INOSITOL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHATIDYL GLYCEROL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHATIDIC ACID</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CARDIOLIPIN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DIGALACTOSYLDIGLYCERIDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHATIDYLETHANOLAMINE (UNSATURATED)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CARDIOLIPIN - Ca^{2+}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHATIDIC ACID - Ca^{2+}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pH &lt; 6.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHATIDIC ACID</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pH &lt; 3.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHOSPHATIDYL SERINE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(pH &lt; 4.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MONOGALACTOSYLDIGLYCERIDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PHASE PROPERTIES</td>
<td>MOLECULAR SHAPE</td>
<td></td>
</tr>
<tr>
<td>HEXAGONAL (H_{11})</td>
<td>CONE</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Polymorphic phases and corresponding dynamic molecular shapes of lipids
Under physiological conditions unsaturated and saturated diacyl phosphatidylcholines (PC's) adopt a bilayer phase because of their "cylindrical shape". On the other hand, diunsaturated phosphatidylethanolamines (PE), because of their "cone shape", prefer a hexagonal $H_{II}$ phase, while saturated and unsaturated lyso 'PC's, having an inverted cone or "wedge shape" prefer micellar arrangement.

The number of acyl chain, composition of head group and unsaturation of the acyl chains impart a specific molecular shape to a lipid, and this shape dictates the phase preference upon hydration. It is generally accepted that long-chain (16 carbon atoms) diacyl PC's are cylindrical in shape and form bilayers upon hydration, and indeed, 1,2-dipalmitoyl PC (di $C_{16}$PC) is a cylindrical shaped molecule and forms bilayers. Decreasing the acyl chain length in both chains makes the cylinder behave more like an inverted cone or "wedge". This is found to be true experimentally because upon hydration di$C_{16}$PC forms bilayers, while di C-diC and di $C_{8}$PC form only micelles.62

The widely accepted membrane structure with its essence on the possibility of phase-transition between a
fluid and a mosaic configuration faces hurdles while trying to explain the physico-chemistry of this phenomenon in live systems. It would be overwhelmingly interesting to state that the cells could modulate their own limiting membranes. Exploration in this line would prove exciting and would contribute to a better understanding of membrane-related events. Membrane fluidity changes of the intestinal mucosa by administration of pectin were carried out using little gourd pectin.

Apart from the fairly large reports on the effect of various pectins like citrus pectin, apple pectin etc., very little information is available on the biochemical and physiological properties of pectins from many of the common vegetables and fruits like *Abelmoschus esculentus* (ladies finger), *Coccinia indica* (little gourd), *Carica papaya* (papaya) and *Anacardium occidentale* (cashew apple). So in this study, we concentrated our attention mainly on the physiological and clinical significances of the above mentioned pectins giving more emphasis to hypolipidemic and hypoglycemic activity. Further studies were conducted on little gourd pectin which was found to be the most beneficial one after screening experiments. Different parts of this plant have been used in the
traditional medicine. Detailed studies on *Coccinia indica* pectin involves the biochemical activities of various enzymes in relation to the prevention or inhibition of atherosclerosis and diabetes. The possible membrane modifying properties of pectin in the mucosal layer of small intestine were also studied.