Polysaccharides

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3. Pectin

Pectin or pectic substances, also called galacturonans or rhamnogalacturonans in scientific literature, is a collective name for heteropolysaccharides that consist predominantly of partially methylated galacturonic acid residues [18, 74, 85, 96, 104-111]. The name pectin (Greek: *Pectos* = gelled) was coined by BRACONNOT [112] who first described this compound in detail in 1825 and indicated its primary use as a gelling agent. Native pectin plays an important role in the consistency of fruits and vegetables, and in textural changes during ripening and storage [85], [113]. The often desired cloud stability of fruit juices and fruit drinks is lost when enzymes endogenous to the fruit degrade pectin (tomatoes) or cause it to precipitate as calcium pectate after demethylation. On the other hand, enzymes may be added as processing aids to degrade native pectin, for example, to apple juice to facilitate clarification or to the pulp of berries to improve press and color yield [114-116]. Pectin is extracted on an industrial scale from the press residues in apple and citrus juice manufacture and used mainly as gelling and stabilizing agents in the food industry [117].

3.1. Occurrence and Structure

Occurrence. Pectins occur in virtually all higher plants, Zosteraceae seaweed, and certain freshwater algae. Pectins are major structural components of the primary cell wall and the middle lamella of young growing plant tissues (meristimatic and parenchymatic) but do not occur in more mature tissue. The composition of the cell wall therefore is of major importance in the texture of fruit and vegetables [19], [20]. The primary cell wall consists of 90 % polysaccharide and 10 % glycoprotein on a dry matter basis [21]. The polysaccharide composition is 30 – 60 % cellulose, 15 – 45 % pectic substances, and 15 – 25 % hemicellulose [22]. A schematic structure of the plant cell wall is illustrated in Figure 1.

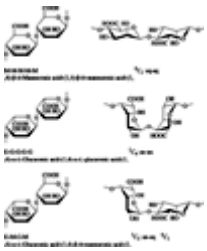

Figure 1. Schematic structure of the plant cell wall [23]
[Full View]

The biosynthesis of pectin takes place in the cell plate during cell division. Pectin is formed as polygalacturonic acid with UDP-D-galacturonic acid — arising from UDP-D-glucose by an epimerase- catalyzed reaction — as the most active glycosyl donor. Immediately after the galacturonan chain has been formed, methoxyl groups are formed with *S*-adenosylmethionine as the methyl group donor [120].

Pectic substances can be partially solubilized from plant tissues without degradation by using weakly acidic, aqueous solvents with or without calcium chelating agents. The pectin fraction that is not extractable with these extractants because of its attachment to other cell wall components by chemical, physical, or mechanical (enmeshment) bonds, is often designated as *protopectin*. Commercial pectin extraction must break down protopectin to a soluble, high molar mass pectin. This is achieved by acid hydrolysis in which, on the one hand, the molar mass of pectin molecules is lowered and, on the other hand, connections to the hemicellulose fractions are split. This transition from protopectin to soluble pectin also occurs during ripening of fruit or cooking of vegetables, resulting in textural changes [85], [113]. Pectin technology is therefore interested in the nature of protopectin and its fixation in the cell walls in an attempt to develop more efficient methods of extraction. FRY [119] has discussed cross-links in cell walls and agents used to cleave the individual bonds. Pure enzymes may also be used for extraction of firmly bound pectin [24], [25].

Structure. Pectin is composed of 1,4-linked α-D-galactopyranosyluronic acid units in the $^4C_1$ conformation, with the glycosidic linkages arranged diaxially (Fig. 2). A proportion of the carboxyl groups is esterified with methanol. Commercial pectins are divided into low-ester and high-ester pectin. In *low-ester pectins (LM-pectins)* less than 50 % of the carboxyl groups are methylated (typical range is from 20 to 40 %) whereas in *high-ester pectins (HM-pectins)* more than 50 % are methylated (typical range is from 55 to 75 %). If less than 10 % of the carboxyl groups are methylated the polysaccharide is called *pectate* or *pectic acid*. Pectins prepared from pears, potatoes, sugar beets, and sunflower heads are acetylated to varying degrees at the secondary hydroxyl groups of the galacturonic acid residues [118].

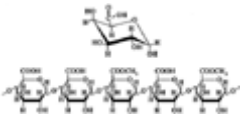

Figure 2. α-D-Galactopyranosyluronic acid in $^4C_1$ conformation (above); fragment of galacturonan chain, 40 % methylated (below)

[Full View]

The heteropolysaccharide nature of pectin derives from the fact that other sugars are incorporated in the pectin molecule. The most common ones being L-rhamnose (Fig. 3), occasionally inserted by α-1,2-linkages in the galacturonan backbone, providing “kinks” in the molecular chain. Other sugars are β-D-xylose, attached as single-unit side chains mainly to O-3 of the galactopyranosyluronic acid residues in the backbone; and D-galactose and L-arabinose, which occur in long side chains, only attached to rhamnopyranosyl residues (for projection formulae, see → Carbohydrates).

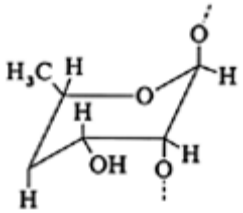

Figure 3. 1,2-Linked L-rhamnopyranosyl unit in $^1C_4$ conformation

[Full View]

The frequency of rhamnose occurrence remains to be established, however it has been suggested that α -rhamnosyl units are concentrated in rhamnose-rich areas. In other words, the soluble pectin is built up of homogalacturonan-dominated areas, so-called *smooth regions* linked to rhamnogalacturonan areas rich in neutral sugars, so-called *hairy regions* (Fig. 4). The neutral sugars account for 10 – 15 % of the weight of the pectin. In the hairy regions, the neutral sugar chain length may be in the range 8 to 20 residues [26], [27]. By degradation with chemical β-elimination and endo-polygalacturonase, it was found that 90 % of the

rhamnose units are found in the hairy region [28], [30].

Figure 4. Schematic representation of pectin backbone, showing the “hairy” regions (rhamnogalacturonan and side-chains) and the “smooth” regions (linear galacturonan) [31] [Full View]

By acid hydrolysis, i.e., splitting the acid labile glycosidic bonds between rhamnose and galacturonic acid, nearly pure homogalacturonic acids with molecular masses in the range 20 000 to 25 000 have been obtained [32], [33]. This corresponds to a chain length of 75 to 100 galacturonic acid residues. For comparison, a molecular mass of 90 000 has been quoted for intact pectin [34], but the molecular mass of pectin is somewhat uncertain (see Section Chemical Analysis).

Commercial pectins may also contain neutral polysaccharides that are not covalently attached to the pectic backbone, such as galactans, arabinans, arabinogalactans, and starch, often referred to as “ballast” compounds. Purified pectins prepared from apple pomace or citrus peel may contain 75 – 90 % anhydrogalacturonic acid on an ash- and moisture-free basis.

3.2. Pectolytic Enzymes

Pectins can be attacked by various enzymes [114], [115], [121]. The significance of native pectic substances in food technology can be evaluated properly only when the activity of these enzymes is taken into account.

Pectinesterase (PE, pectin methylesterase, pectase, pectin demethoxylase, pectin pectylhydrolase, EC 3.1.1.11) splits off the methoxyl groups and converts high-methoxyl pectins to low-methoxyl pectins. The latter are extremely sensitive to complex formation and precipitation with Ca^{2+} ions, particularly when a pectinesterase of plant origin is used. This type of enzyme does not saponify methyl esters in a random fashion as microbial PEs do, but acts along the galacturonan chain, creating blocks of free carboxyl groups. Pectinesterase occurs in many higher plants, particularly tomatoes, citrus, and other fruits; it is also produced by many fungi and bacteria.

Polygalacturonase (PG, pectinase, pectate hydrolase, poly-α-1,4-D-galacturonide glycanohydrolase, EC 3.2.1.15 and 3.2.1.67) preferentially hydrolyzes low-methoxyl pectins or pectic acid because these enzymes can cleave glycosidic linkages only next to free carboxyl groups. PGs can be divided into enzymes that degrade their substrate by an endo attack (splitting randomly in the backbone, *endoPG*), and enzymes that act from the nonreducing end removing mono- or digalacturonic acid (*exoPG*). PGs are produced by fungi and certain bacteria, and also occur in higher plants (tomatoes). The endoPGs, with their strong depolymerizing action, are of particular technological importance.

Pectate lyase (PAL = pectic acid lyase, PATE = pectic acid transeliminase, LMPL = low-methoxyl pectin lyase, poly-α-1,4-D-galacturonide lyase, EC 4.2.2.2 and 4.2.2.9) also splits glycosidic linkages next to free carboxyl groups. In this group of enzymes, endo and exo enzymes also exist. The preferential substrates for *endoPAL* are LM-pectins rather than pectic acid. Pectate lyases have an absolute requirement for Ca^{2+} ions. The glycosidic linkages are split by a *trans*-elimination reaction. PALs are produced predominantly by bacteria and are not important in fruit and vegetable processing because of their high optimum pH (8.5 – 9.5).

Pectin lyase (PL, PTE = pectin transeliminase, pectinase, poly-α-1,4-D-methoxygalacturonide lyase, EC 4.2.2.10) splits glycosidic linkages between methoxylated galacturonide residues by a *trans*-elimination reaction. These enzymes therefore have a preference for HM-pectins. Pectin lyases are produced only by

fungi.

A newer pectin-degrading enzyme acts in cooperation with a pectin acetylesterase only on highly branched regions of pectin to release oligosaccharides consisting of alternating sequences of α-1,2-linked L-rhamnosyl residues and α-1,4-linked D-galacturonosyl residues, with galactosyl residues β-1,4-linked to part of the rhamnosyl units. The nonreducing end is always a rhamnose unit [122], [123].

Commercially available pectinases, used on an industrial scale in fruit and vegetable processing are of fungal origin and generally contain in addition to PE, PG, and PL, proteases and various hemicellulases and cellulases.

3.3. Production [106], [109], [117], [122], [124]

The production of pectin is summarized in Figure 5.

Figure 5. Flow chart of pectin production

[Full View]

Raw materials of importance to pectin manufacturing are currently various kinds of citrus peel, and apple pomace. Lemon and lime are the preferred citrus sources, and more pectin is produced from these than from apple or the less preferred citrus materials, orange and grapefruit. Some of the pectin producing companies which historically developed in connection to apple production now partly or wholly base their production on imported citrus peel. Sugar beet was once used as a pectin source to some extent [35], but the pectin is inferior as a gelling agent compared to citrus or apple pectin. It has been reintroduced, and is currently being marketed as a stabilizer. Numerous other sources like mango [36], pea hulls [37], sunflower heads [38], [39], and pumpkin [40] have been suggested.

Citrus peel and apple pomace are available as byproducts from juice manufacturing. They are usually washed in water and dried before being used for pectin manufacturing, but some citrus material is used in pectin plants neighboring the juice production without previous drying. In either case processing of the raw materials has to commence immediately after juice production in order to prevent microbial degradation. The washing leaches out organic acids, sugar, and pigments and is thus one of the separation processes in the purification of pectin. Most importantly, it prevents discoloration either from browning of pigments or from caramelization during the raw materials drying.

Extraction. The pretreated raw material is extracted in water which has been acidified with e.g., hydrochloric or nitric acid. Typical conditions are: pH 1 to 3, temperature 50 to 90 °C, duration 3 to 12 h. During the extraction, limited depolymerization of the pectin and possibly of other connecting biopolymers takes place, and the pectin dissolves. The low pH further dissociates ionic linkages which hold the pectin in the plant tissue. In addition to hydrolyzing glycosidic bonds, the extraction conditions also hydrolyze ester linkages, more specifically the methyl ester at C-6, and the acetate to which pectin may be esterified by its hydroxyl groups. The extraction process thus causes a reduction in degree of polymerization as well as in degree of methyl and acetate esterification. The pectin yield increases with the acidity, the temperature, and the duration, but the product will lose too much in degree of polymerization if all these parameters are at their maximum. The combination of low pH and low temperature favors hydrolysis of ester linkages over

hydrolysis of glycosidic bonds, and it is thus preferred for production of pectin with a relatively low degree of esterification.

Filtration in one or more stages separates the extract containing the solubilized pectin from the insoluble, but at this stage very soft and fragile, plant tissue. The rather difficult filtration requires reasonably low viscosity, and as a consequence the pectin concentration must be less than 0.6 to 1 %, depending upon the pectin type. Further, the solids must not have been comminuted by excessive mechanical treatment such as vigorous agitation. Water-insoluble materials like wood cellulose or diatomaceous earth may be added in order to improve the porosity and mechanical strength of filter cakes. Amylase may be added to remove starch from apple pectin extracts.

The spent plant raw material is typically used for cattle feed.

Isolation. Following filtration, the extract may optionally be passed through a column with cation-exchange resin and concentrated by evaporation. The pectin is then precipitated by mixing the extract with an appropriate alcohol, e.g., 2-propanol. Finally, the precipitate is separated from the spent alcohol, washed in more alcohol, pressed to drain as much liquid as possible, and then dried and milled. The powder is now ready for standardization, i.e., mixing with other pectin batches and/or sucrose in order to ensure uniformity. The alcohol is recovered by distillation. An alternative to alcohol precipitation is precipitation by adding appropriate metal salts to the extract. Pectin forms insoluble salts with, e.g., Cu^{2+} and Al^{3+}. The Al^{3+} precipitation [125] was previously used industrially. Removal of the metal ions from the precipitated pectin is done by washing in acidified aqueous alcohol.

Modification. Pectin derived from citrus or apple raw material as described above will normally have a degree of esterification between 55 and 75. A lower degree of esterification can be achieved by acidifying the extract and leaving it for some time before precipitation, or by treating precipitated (but not dried) pectin with acid or alkali during suspension in aqueous alcohol. Ammonia may convert methyl-esterified carboxylate groups of a pectin to primary amides [42], [43]. This is done industrially by suspending precipitated pectin in a mixture of alcohol and water with dissolved ammonia [127], [128]. Deesterification takes place concurrently. By choosing proper conditions with respect to ammonia concentration, water activity, and temperature, pectins with various proportions of amidated, methyl esterified and free carboxylate groups can be produced.

Standardization. The properties of botanical raw materials like those used for pectin fluctuate due to, e.g., weather conditions or sorts variation. Pectin as it appears directly from milling contains this variation. In order to maintain a constant quality, the pectin manufacturer may mix different batches. Further, pectin intended for food is typically diluted with sucrose in order to achieve a uniform grade (i.e., “strength,” for a definition, see Section Measurement and Standardization of Gel-Forming Capacity). Pectin without admixed sugar, e.g., for pharmaceutical purposes, is also available from the major manufacturers.

Due to the multitude of ways in which pectins may vary, it is not possible to ensure batch to batch consistency with respect to all possible attributes at the same time. Pectin is normally standardized with respect to a few properties which are measured in defined chemical systems which simulate the applications, e.g., breaking strength of a gel, gelation temperature, etc. The major pectin manufacturers have developed a great number of specialty types which are tailor-made for individual applications and which have each their set of standardization criteria (control methods). In fact, a pectin type is defined by its set of standardization criteria. When using pectin, it is obviously important to choose a type which has been standardized in a way that corresponds reasonably to the intended use.

3.4. Properties

A range of parameters — intrinsic and extrinsic — are important for the performance of pectin which is in

most cases used to impart certain rheological properties, e.g., by forming a gel. The *intrinsic parameters* determine the nature of the gel and may include molecular mass, degree of esterification (DE), degree of acetylation (DA), neutral sugar content, and composition. The *extrinsic parameters* which determine the gelation process may include pectin concentration, pH, ionic strength, water activity, and temperature.

The most important properties of pectin preparations depend on their molecular mass and DE and DA. The proportion and nature of neutral sugars in the side chains as well as in the "ballast" are also of significance. Pectin with a high degree of polymerization is more viscous in solution compared to a pectin with a lower degree of polymerization. Further, the gel strength will typically increase with the degree of polymerization, i.e., less pectin is needed with a high molecular mass pectin [44-46]. The DE strongly influences the functional properties of pectin with the two main groups, HM- and LM-pectin being influenced differently. At the typical conditions in HM-pectin applications, high DE means high gelling temperature whereas at typical use conditions for LM-pectins, low DE means high gelling temperature. No experimental evidence is available to demonstrate the influence of the hairy regions (see Structure.) on the functionality of pectin. It could be speculated that by being bulky and providing kinks in the molecular chain, these parts will prevent molecules from aligning throughout their entire length. This may contribute to preventing precipitation and reducing potential syneresis (spontaneous exudation of solvent from the gel).

Chemical Reactions. By treating pectin with ammonia under alkaline conditions in alcohol suspensions, ca. 20 % of the methyl ester groups are converted to acid amide groups, and amidated pectins are obtained [129]. Amidated pectins have a higher calcium reactivity than LM-pectins, and gels can be obtained with very few Ca^{2+} ions [134]. Carboxyl groups in pectin can be esterified easily with methanol [126], glycol, and glycerol but poorly with ethanol [135]. By using polyols, cross-linked, insoluble systems are obtained. Insoluble pectates can also be prepared by cross-linking with epichlorohydrin; these pectates have ion-exchange properties with a certain selectivity for calcium and heavy-metal ions. They are successfully used for the isolation of pectolytic enzymes [136], [137]. Pectins are readily degraded by oxidants [138] except for chlorite and chlorine dioxide, which can selectively oxidize aldehyde groups at the reducing chain end [139].

3.4.1. Physical Properties

Pectin is water-soluble, exhibiting an increased solubility with increasing DE and decreasing degree of polymerization. In order to ensure complete dissolution of pectin, it is necessary that it is properly dispersed without lumping. Once formed, lumps are extremely difficult to dissolve. Pectin, like any other gum or gelling agent, will not dissolve in media where gelling conditions exist. In order to add pectin to complex formulations such as food systems three alternative procedures are recommended: (1) dissolve the pectin in pure water and add the solution; (2) dry blend the pectin with five parts of sugar and add the mixture; (3) disperse the pectin in a liquid in which it is not soluble and add the dispersion.

Aqueous dilute pectin solutions, i.e., with a pectin content below 0.5 % are almost Newtonian whereas more concentrated pectin solutions exhibit pseudoplasticity, i.e., shear-thinning behavior. From dilute viscosity data, the intrinsic viscosity, $[\eta]$ (dL/g) may be determined. $[\eta]$ indicates the hydrodynamic volume of a polymer molecule and depends primarily on the molecular mass, however viscosity is also influenced in a complex manner by many other factors such as the DE, pH (dissociation), and ionic strength. $[\eta]$-values for pectins typically lie in the range of 1.0 to 6.0 dL/g [47-51]. The molecular mass of pectin is often estimated by intrinsic viscosity methods using the Mark – Houwink relationship $[\eta] = KM^{\alpha}$ (see Section Analysis). Originally an α-value of 1.34 [52] was suggested corresponding to a rigid rod-like molecular structure, however more recent findings estimate α to be in the range 0.7 – 0.8 indicating a random coil structure [47], [49], [53], [54].

Pectin is insoluble in most organic solvents such as alcohols and acetone. Pectin can also be precipitated from aqueous solutions by quaternary detergents, water-soluble cationic polymers including proteins, and

multivalent cations. LM-pectins can be precipitated by calcium ions; pectates by alkali cations and by acid.

Pectin is a polycarboxylic acid. Dissolved pectin is negatively charged at neutral pH and approaches zero charge at low pH. Since pectin is a polyprotic acid it is not possible to determine an exact value of the apparent dissociation constant, pK_a. Rather, pK_a is different for varying carbohydrate concentrations and for varying degree of dissociation, α. The negative charge density, in turn, is dependent on DE which implies that pK_a-values are increasing with increasing content of unesterified galacturonic acid units. Typically, pK_a-values at 50 % dissociation, i.e., $\alpha = 0.5$, lie in the range 3.5 – 4.5 [55-59]. The usual dependence of pK_a with polymer concentration and ionic strength is observed, i.e., pK_a is lowered with increasing concentration and ionic strength.

3.4.2. Gel Properties

Pectin is used mainly as a gelling agent in industry; therefore its gelling properties are most important [4], [6], [106], [117]. The gel formation mechanism of pectin is similar to that of other gelling polysaccharides: Some regions of the polymer molecules associate in junction zones to form a three-dimensional network, which traps the solvent with cosolutes; free stretches of the molecules provide elasticity to the gel obtained [111], [140], [141] (Fig. 6). Irregularities in the pectin molecule, such as the distribution pattern of methyl ester or *O*-acetyl groups, rhamnosyl residues in the backbone, or the presence of side chains, limit the length of the junction zones and give shape to the free stretches of the macromolecules [142].

Figure 6. Network of a gel

[Full View]

A comprehensive, coherent description of pectin gelation does not exist. Conventionally, a distinction is made between HM- and LM-pectin gelation, however, in reality this paradigm is too simple. It applies that different gelation mechanisms may act simultaneously. This is e.g., illustrated by the fact that gelation of LM-pectin, which is normally claimed to gel in the presence of certain metal ions, is further favored by a decrease in pH. If the gelation was solely determined by the formation of calcium-bridges between molecules, the opposite effect would be expected, an increase in pH leading to an increase in gel strength.

In order to adapt the conventional and still widely accepted theories for pectin gelation, the gelation phenomenon will, however, be treated as two distinct mechanisms, ie HM- and LM-pectin gelation. Gels used for jams and jellies are typically formed with HM-pectin at an acidic pH and require the presence of a high concentration of sugar. LM-pectin is typically used for yogurt fruit preparations; these gels can be formed without sugar over a wide pH range, however, the presence of a divalent cation is necessary. In most cases the cation, i.e., calcium, is inherently present in the fruit material. LM-pectin gels can be remelted whereas usually it is not possible to melt an HM-pectin gel, i.e., with HM-pectin gel preparations, the difference between the apparent temperatures of setting and melting is so large that the gels are said to be thermo-irreversible. Further, LM-pectin gels solidify almost immediately after gelling conditions have been introduced, while an HM-pectin gel will build up over time.

HM-pectins form so-called *low-water-activity* or *pectin – sugar – acid gels* and are used in jam, jelly, and marmalade production. The basic galacturonan chain (smooth regions) of the pectin molecule apparently contains blocks with conformational regularity to provide opportunities to build up junction zones. The homogalacturonan part of the molecule is configured as helices with three anhydrogalacturonic acid units per turn, with the methyl ester groups protruding from the helix. According to OAKENFULL and SCOTT [143], junction zones are stabilized by different forces between pectin chain molecules: hydrogen bonds between

undissociated carboxyl and secondary alcohol groups, and hydrophobic interactions between methoxyl groups (Fig. 7). Both types of forces are fortified by sucrose; the low pH suppresses the dissociation of carboxyl groups. Sugar reduces the water activity of the system and thereby influences hydrogen bonding by decreasing polymer – water interactions and increasing polymer – polymer interactions. To a certain extent, sugar and acid are interchangeable: at lower sugar concentration, lower pH is required, but at higher sugar concentration, higher pH values are possible and necessary to avoid setting during the boil. The lower limit for the sugar concentration is 55 %. At this concentration, the pH should not be higher than 2.8. At a sugar concentration of 80 % (jellies), the mass will also gel at pH 3.5. This means that within the gelling range, at the same sugar concentration, more acid will give a stronger gel and, at the same pH value, this is achieved by adding more sugar. Addition of urea to a gel cancels out hydrogen bonds and results in a weaker gel with a lower setting temperature [144].

Figure 7. A) Junction zone in high ester pectin gel by hydrogen bonds (dotted lines) and hydrophobic interactions (filled circles); B) Junction zone in low ester pectin gel by dimerization of polygalacturonate (polyguluronate blocks) induced by their strong binding power for Ca^{2+} ions which fill the oxygen-lined cavities between the polysaccharide chains [110]

[Full View]

In general, pectin – sugar – acid gels are prepared by a boiling process followed by cooling. At a certain temperature the system sets to a gel. The food technologist is interested in the rate of setting. At the same rate of cooling the rate of setting determines the setting temperature or the setting time. The parameters that contribute to stronger gels also accelerate the setting rate. Based on the pH limits of gelling as well as on the setting rate, HM-pectins can be subdivided into rapid-set (DM > 70 %) and slow-set (DM 60 – 65 %) pectins. At the same sugar concentration, rapid-set pectins have a higher pH limit for gelling because these very highly methyl esterifiedpectins have few carboxyl groups that must be protonated. A fully methyl esterified pectin gels with sugar alone and does not need acid. The concentration of pectin in the gel influences the rheological properties of the gel, not the gelling rate.

LM-pectins form so-called *calcium pectate gels.* Theories about the chemical structure of calcium pectate gel junction zones were first developed in comparison with alginate gels (see Chapter Alginates). In calcium alginate gels, the junction zones are formed by α-1,4-L-polyguluronate blocks in which the diaxial configuration of the glycosidic linkages leads to a buckled ribbon with limited flexibility and a strong binding power for Ca^{2+} ions, which induce dimerization of alginate chains by filling the oxygen-lined cavities between them. This has evoked the picture of an eggbox, and the expression eggbox-type junction zones has become universally accepted [111], [141], [145], [146] (Fig. 7). In comparing the primary structures of poly-L-guluronate and poly-D-galacturonate (pectin), they are seen to be stereochemically analogous mirror images of each other, except at C-3. The two-fold helix, however, has not been observed with X-ray diffraction techniques, but molecular modeling calculations indicate that it can exist [60]. Circular dichroism data [61] suggest that a conformational change takes place when dissolved or Ca^{2+}-gelled pectate is dried and it is suggested that a transition takes place from a 2_1 ribbon-like to a 3_1 helical symmetry. Similar to the low-water-activity gels, junction zones in calcium pectate gels are terminated by rhamnosyl residues in the backbone, side chains attached to the backbone, or acetyl groups. The presence of some methoxyl groups does not inhibit formation of eggbox-type junction zones. With LM-pectins, acids or sugars are not so important. Gels of acidic fruit juices with LM-pectins can be made by addition of a calcium salt (low-sugar jams) or of milk with its neutral pH and calcium ions (desserts). The amount of calcium necessary for gelation depends on the degree of esterification, the way the LM-pectin has been prepared, and the types and amounts of other ingredients. Coagulation as a result of the addition of calcium salts must be

absolutely avoided. Slow availability of Ca^{2+} for the pectin molecules is a prerequisite for obtaining a gel network. This can be accomplished in various ways: (1) an insoluble calcium salt (phosphate, citrate, tartrate) may be used resulting in a slow exchange of Ca^{2+} ions with the LM-pectin and formation of a gel; (2) use of calcium chelating agents such as diphosphates help to retard the availability of Ca^{2+} ions. The fact that calcium pectate gels and precipitates are often thermoreversible (i.e., they are soluble under conditions of gel formation at high temperature) can also be used to advantage. Soluble calcium salts such as calcium lactate or calcium chloride can therefore be added at boiling temperature, and gelling occurs on cooling. LM-pectins can be solubilized in milk by heating; the calcium caseinate of the milk provides the calcium necessary for the system to gel on cooling. Addition of sugars to such gels gives stronger gels; however, at higher concentrations the risk of coagulation increases. A solution to this problem is offered by *amidated pectins*. Gel formation with amidated pectins is also explained by chain associations via eggbox junction zones. Eggbox junction zones can accommodate amide groups, which, however, provide less drive for Ca^{2+} ion binding [146], [147]. On the one hand, they need fewer Ca^{2+} ions for gelation, and on the other hand in the presence of excess Ca^{2+} ions they are not as sensitive to coagulation [148]. In the United States, amidated LM-pectin is used for all applications of LM-pectins.

3.4.3. Stability and Chemical Reactions

Stability of pectin molecules in aqueous solution depends upon the temperature and the pH. Pectin has, in contrast to most other hydrocolloids, optimal stability at pH 3.5 to 4. Figure 8 shows the stability of pectin and some other thickening agents at various pH values, expressed as residual viscosity of buffered solutions after heating for 10 min at 90 °C [131]. As is evident from the figure, highly esterified pectin is vulnerable to high pH. Even at pH 5, depolymerization is considerable, in particular at elevated temperatures [133]. Consequently, it is difficult to raise the pH of pectin solutions without causing a decline in the average degree of polymerization, because when trying to mix an alkaline solution into a pectin solution, too high pH cannot be avoided locally. It is recommended to ensure good agitation and low temperature, and to avoid the use of hydroxides.

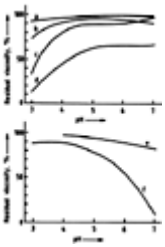

Figure 8. Stability of some polysaccharides at various pHs [131]: Residual viscosity after 10 min incubation at 90 °C

a) Carboxymethylcellulose – methyl cellulose; b) Locust bean gum; c) Agar; d) Carrageenan; e) Pectate; f) Pectin
[Full View]

Low pH hydrolyzes ester bonds causing a decline in DE as well as in the content of *O*-acetyl groups, and it causes a decline in degree of polymerization by hydrolysis of glycosidic bonds [64], in particular at rhamnose insertions in the molecular backbone [65]. Very high acid concentration may degrade galacturonic acid to CO_2, furfural, reductic acid (2,3-dihydroxy-2-cyclopenten-1-one; $C_5H_6O_3$) and alginetin. Carbon dioxide production which is quantitative by boiling in 12 mol/L HCl has in the past been used for the quantitative determination of pectin [85], [132].

High pH depolymerization is due to β-elimination [66] (Fig. 9), it requires the presence of a methyl ester group at the anhydrogalacturonic acid residue which has its 4-C attached to the bond being split. Since the presence of methyl esters is required for β-elimination, vulnerability to this degradation mechanism is related to the DE. High pH further reduces DE (whereby the β-elimination becomes incomplete) as well as the content of *O*-acetyl groups.

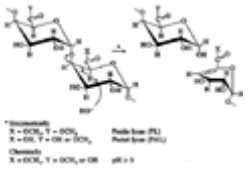

Figure 9. β-Eliminative depolymerization of a galacturonan chain by pectin lyase (PL) or pectate lyase (PAL) or chemically at pH ≥ 5

[Full View]

Derivatization. The low-pH hydrolysis of natural ester linkages may be reversed under conditions of low water activity, e.g., in mixtures of methanol and concentrated sulfuric acid [67], [68], [126], or, for introduction of *O*-acetyl groups, mixtures of concentrated sulfuric acid and acetic anhydride [69]. Further, the carboxyl group may readily be esterified with glycol or glycerol but poorly with ethanol [135]. By using polyols, cross-linked, insoluble systems are obtained.

At high pH, ammonia may convert methyl esterified carboxylate groups to amides [42], [129]. This is used industrially since amidated pectins are of commercial importance.

Insoluble pectates can be prepared by cross-linking with epichlorohydrin; these pectates have ion-exchange properties with a certain selectivity for calcium and heavy-metal ions. They are successfully used for the isolation of pectolytic enzymes [136], [137].

Pectins are readily degraded by oxidants [138] except for chlorite and chlorine dioxide which can selectively oxidize aldehyde groups at the reducing chain end [139].

3.5. Analysis

3.5.1. Measurement and Standardization of Gel-Forming Capacity

HM-pectins are generally standardized to uniform strength at specified constant conditions. Expressing the sugar binding capacity of the pectin, the USA – SAG method suggested by the IFT Committee for Pectin Standardization, has been universally accepted for grading HM-pectins [149]. A standard gel is prepared in conical test glasses with the following conditions: soluble solids 65 %, pH 2.20 – 2.40, gel strength 23.5 %, SAG measured with a *ridgelimeter* [106], [153]. After 24 h at 25 ± 3 °C the gel is deposited on a glass plate, and the sagging of the gel under its own weight is measured after 2 min. From the SAG value and the pectin quantity, the grade can be calculated. Most commercial HM-pectins are standardized to 150 grade USA – SAG. (A gel strength of 150 grade SAG implies that 1 part of pectin is able to transform 150 parts of sucrose into a jelly with above standard properties.)

LM-pectin may be standardized by closely analogous procedures, however, no universally accepted method exists. With LM-pectins it is difficult to set up a single universal test because the conditions under which LM-pectins are used may differ widely with respect to soluble solids, calcium content, and pH (see also Standardization.).

3.5.2. Chemical Analysis [70]

In addition to gel-forming ability, the analysis of pectin preparations is concerned particularly with the *degree of of esterification, DE*. This is determined by converting the pectin to its acid form by passing it over an ion-exchange resin or washing it in an alcohol suspension, first with hydrochloric acid – alcohol and then with neutral alcohol. The acid and saponification equivalent is determined by titration, and from these values the anhydrogalacturonic acid content and the DE (in percent) can be calculated [63]. This principle for determination of DE has been adopted by the major legislative bodies [71-73]. Presence of *O*-acetyl groups in the pectin will result in an overestimation of DE as well as the anhydrogalacturonic acid content, therefore, it should be evaluated in a separate aliquot of the preparation. The pectin can also be precipitated

with copper ions before and after saponification to determine the copper in the well-washed precipitate. From the amount of copper ions bound to the original pectin and the saponified pectin the anhydrogalacturonic acid and the DM can then be calculated without the interference of acetyl groups [151], [152]. Various methods exist for the separate determination of methyl ester and acetyl groups. Methanol is released on saponification of methyl ester and can be determined by GLC either directly [153] or in the headspace of a closed vial after conversion of the methanol to volatile methyl nitrite [154], [155]. *Acetyl groups* can be conveniently determined by an enzymatic spectrophotometric method (supplier Boehringer), by GLC [156], or by distillation and titration after alkaline saponification [157]. A convenient new method is to saponify the pectin preparation in alkaline alcohol, which is then analyzed by HPLC for methanol and acetic acid [158]. Methods measuring the relative content of carboxylate groups to total material, either using size exclusion chromatography with combined detection by conductivity and refractive index [75], or using capillary electrophoresis [76], have also been published. The DE can be inferred from calibration curves if it can be assumed that the anhydrogalacturonic acid content is the same in the samples compared. Previously, the analytical methods used by legislative bodies did not comprise a correction for *O*-acetyl groups since the content of those is small in citrus pectin and modest in apple pectin, but it has now been included in the latest version of the FAO/WHO specification for pectin [71].

In commerical pectins, up to 25 % of the carboxyl groups may be amidated. The *degree of amidation* is calculated from the amount of ammonia released on alkaline distillation [63], [159]. Amidated pectic acids undergo β-elimination reactions, whereas pectic acid does not, which permits quantitative analysis of mixtures of amidated and nonamidated pectins [160]. The anhydrogalacturonide content of pectins can also be determined from aqueous solutions by colorimetric methods with carbazole [161], the more specific *m*-hydroxydiphenyl [162], or sulfamate – *m*-hydroxydiphenyl [163]. Sometimes corrections must be made for interfering compounds (neutral sugars, amide groups, azide). The colorimetric methods can be automated easily and used for routine analyses of large series of samples [130].

With the above-mentioned methods, only the galacturonide residues in the backbone can be analyzed. The *neutral sugars* can be analyzed conveniently by gas chromatography after acid hydrolysis and conversion to volatile derivatives. By a preceding precipitation of pectins with copper ions the neutral sugars covalently attached to the galacturonan can be analyzed specifically. If starch is present it can be removed by enzymatic degradation [164].

To analyze the *pectin of plant material* the so-called alcohol-insoluble residue is usually prepared first by washing the plant material with refluxing alcohol. This inactivates endogenous enzymes and removes alcohol-soluble constituents. The pectin content is then determined in extracts of the alcohol-insoluble residue. The total pectin is determined in an alkaline extract or in the combined extracts of enzymatic and acid extraction. Another approach is the gradual extraction first with cold or hot water (HM-pectin), oxalate, ethylenediaminetetraacetic acid (EDTA), or cyclohexanediamine tetraacetate (CyDTA) (LM-pectin), and then acid or alkali (protopectin) [165], [166]. The total pectin content and the average degree of methoxylation can also be determined in the alcohol-insoluble residue when this is converted to the acid form before and after saponification. When treated with alcoholic calcium acetate solutions, the free carboxyl groups of pectin set free an equivalent amount of acetic acid that can be determined by titration. An alternative is determination of the bound copper ions from copper solutions [151].

Molecular mass of pectin may be determined with viscosimetry [44], [77], membrane osmometry [78], size exclusion chromatography [47], [48], [54], light scattering [54], [77], [79], [80], ultracentrifugation [34], and analysis of reducing end-groups [80]. Quoted results vary, partly because the intermolecular distribution of molecular mass is broad, partly because pectin molecules aggregate and may contain slight amounts of insoluble material, and, of course, partly because samples are different. As an example, a weight average molecular mass of 90 000 ± 10 000 was reported by HARDING et al. [34]. Quoted values for the Mark – Houwink exponent, relating intrinsic viscosity to molecular mass, are generally in the vicinity of 0.8

[47], [49], [53], [54], suggesting a random coil molecular shape. Integrated systems combining high performance size exclusion chromatography, viscosity detection and light scattering detection are commercially available, including software with which data for molecular mass and Mark – Houwink parameters can be extracted.

3.6. Pharmaceutical and Nutritional Characteristics [167]

Pectin is not significantly degraded in the upper digestive tract of humans, and it can be recovered almost intact from the small intestine [81]. In the cecum and colon, it is fermented by microorganisms mainly to short-chain fatty acids, as can be concluded from in vitro studies [82], rat studies [83] and studies comparing the degradation patterns of humans and rats [84]. Since pectin is a dietary fiber, much attention has been paid to the possible health benefits of pectin which are: (1) reduced glycemic response [86-88], (2) prolonged gastric residence time [89-91], (3) reduced serum cholesterol level [86], and (4) effect against diarrhoea [92]. Pectic polysaccharides from certain botanical sources like ginseng root (*Panax ginseng*) [93], eel grass (*Zosteraceae*), and *Bupleurum falcatum* [94], [95] have shown healing effect on gastric and duodenal ulcers. Most studies, e.g., [97-99], conclude that pectin, in spite of its metal-binding ability, apparently does not inhibit the uptake of minerals from the diet. All of the above effects must be thought of as general tendencies in a vast amount of published findings which are not all mutually consistent. Some discrepancies may be explicable because it is attempted to generalize results achieved with different systems (in vitro, animal, or human) and with different pectins or pectin-rich plant materials. Publications often fail to specify important details about the pectin being used for the study, such as botanical origin, DE, etc.

3.7. Application in the Food Industry [168]

Indigenous manufacture of jams and marmalades, before commercial stabilizers were available, involved the use of pectin-rich fruit and in situ extraction of the pectin during prolonged cooking. Partly owing to this tradition, but mostly due to its superior stability and gelling ability at relatively low pH, pectin is the dominating gelling agent in modern production of jams as well as other products which are gelled, acidulous, and sweet. Examples are jelly fruits, and fruit preparations for industrial production of fruit-containing yogurt (Table 2 groups 1 and 2). Commercial pectins for these applications are tailor-made to yield specific gelation temperatures or gelling rates under specified conditions, and to exhibit specific functionalities such as heat reversibility, heat resistance, firmly gelled textures, pumpable semi-gelled textures, etc. *HM pectin* is used as a stabilizer in yogurt beverages and beverages in which milk proteins are heat-treated at relatively low pH during the production [100-102]. LM pectin finds use for thickening spoonable yogurt (Table 2 group 6). Particles of calcium pectinate are used as a substitute for fat in low-calorie foods [103] (Table 2 group 12). Other applications from Table 2 include groups 4, 8, 9, 10, and 11.

3.8. Market

Annual pectin production is estimated at 25 000 t (80 % citrus pectin), sold mostly in standardized form. Pectin production takes place in Brazil, Denmark, France, Germany, Mexico, Switzerland, and United Kingdom. Smaller amounts are produced in Eastern Europe and the former Soviet Union. Average prices on the world market are $10 – 11 per kilogram HM-pectin and $ 12 – 13 per kilogram LM-pectin.

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