Technical Article

Pectin Basics

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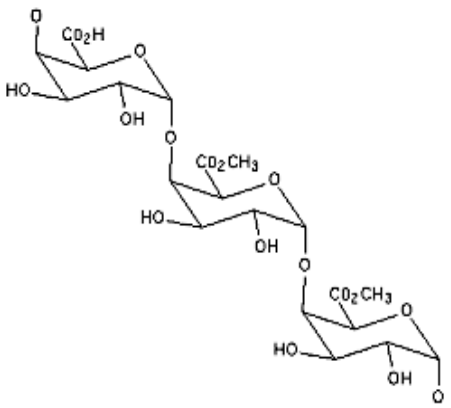
Introduction

Pectins have been utilised for their functionality in foods for many years. Ubiquitous in the conserves and preserves industries, development of pectins has centred on its use to impart texture in high sugar systems. Pectin has been widely studied and published but is difficult to characterise as a model system due to the heterogeneous nature of the polymer. Industrial use has mainly been focussed on tailoring the polymer to specific needs.

Backbone structure

Pectins are a family of complex polysaccharides that contain 1,4-linked α-D-galactosyluronic residues. Three pectic polysaccharides, homogalacturonan, rhamnogalacturonan-I and substituted galacturonans, have been isolated from primary plant cell walls. Homogalacturonan (HG) is a linear chain of 1,4-linked α-D-galactosyluronic residues, in which some of the carboxyl groups are methyl esterified. They may also be O-acetylated at the C-2 and C-3 positions. Homogalacturonans have been isolated from sunflower heads and apple pectin but were obtained by extraction treatments likely to cleave covalent bonds so they may have been released from a heterogeneous pectic polysaccharide.

Pectin is not a homopolysaccharide however and has rhamnopyranosyl residues inserted in the galactosyluronic backbone at 1 to 4% substitution. The other major feature of these rhamnogalacturonan-I (RG-I) chains are large substituted side chains. Between 20 and 80% of the rhamnopyranosyl residues are, depending on plant source and method of isolation, substituted at C-4 with neutral and acidic oligosaccharide side chains. The predominant side chains contain large linear and branched L-arabinofuranosyl and/or D-galactopyranosyl residues and their relative proportion and chain lengths may differ depending on plant source. Other rarer side chains are also present and generally shorter. These are illustrated in illustration 1.



Pectin structure ➔

The final and much more minor component of the backbone is rhamnogalacturonan-II (RG-II). This is not structurally related to RG-I since its backbone is composed of 1,4-linked D-galactosyluronic residues like HG. At approximately 30 glycosyl residues long it has a non-saccharide and an octasaccharide side chain attached to C-2 of some of the backbone residues and two structurally different disaccharides attached to C-3 of the backbone. RG-II is of interest as it occurs in relatively high amounts in wine and other fruit juices and it has been demonstrated that it binds heavy metals and has immunomodulating activities.

It is possible to separate essentially pure galacturonan fractions from other high molecular weight pectin fractions by degrading purified pectins specifically in the galacturonan backbone either chemically or enzymatically. It appears that there is an intramolecular distribution in which the neutral sugars are concentrated in blocks of more highly substituted rhamnogalacturonan regions ('hairy' regions) which are separated in the polymer by D-galactosyluronic-rich regions ('smooth' regions). These smooth regions can be up to 100 units in length.

Functional groups

Pectins also carry non-sugar substituents, essentially methanol, acetic acid, phenolic acids and occasionally amide groups. The esterification of galacturonic acid residues with methanol or acetic acid is a very important structural characteristic of pectic substances. The degree of methylation (DM) is defined as the percentage of carbonyl groups esterified with methanol. If more than 50% of the carboxyl groups are methylated the pectins are called high-methoxy pectins (HM), and less than that degree of methylation are called low methoxy (LM) pectins. This same principal applies to acetylation although the degree of acetylation (DAc) can be larger than 100% as galacturonosyl residues can be acetylated with more than one group per monosaccharide. Acetyl groups are generally present in the 'hairy' rhamnogalacturonan regions and only present in very low amount in homogalacturonan from apple and citrus. They can be present in much higher amounts in homogalacturonan from sugarbeet and potato.

Methyl esterification is common in native pectins but acetylation is rarer in natural extracts. Overall the degree of substitution is known as the degree of esterification (DE). For native apple pectins a random distribution of the methyl esters groups over the galacturonan backbone was found. For commercially extracted pectins with tailored DM the distribution depends on the raw material and the extraction and de-esterification conditions. The overall polysaccharide is therefore ionic but with large neutral regions giving interesting functionalities.

Commercial pectins can also be amidated. The amidation improves the gelling ability of low methoxy pectins in that they need less calcium to gel and are less prone to precipitate at high calcium levels.

Table 1: Composition of a number of pectin sources

Component	Sugarbeet pulp (% w/w)	Apple pomace (% w/w)	Citrus peels (% w/w)	Pea hulls (% w/w)
Rhamnose and Fucose	1.1	1.5	1.3	0.9
Arabinose	17.3	8	6.4	4.2
Xylose	1.5	5.5	2.4	14.6
Mannose	1.5	1.8	2.2	1
Galactose	4.3	5	3.2	1.2
Glc	21.7	27.9	19.6	45.1
Galacturonic acid	18.9	25.2	26	12.7
Methanol	2.3	2.2	-	0.5
Ethanol	3.6	2	-	1
Proteins	8	5.7	-	3.8
Lignin	1.8	-	-	-
Ash	8.4	2	-	1.7

Sources and extraction

Pectin is found in most plants, but is most concentrated in citrus fruits (oranges, lemons, grapefruits) and apples. Pectin obtained from citrus peels is referred to as citrus pectin.

The extraction of pectins can lead to large variations in the chemical structure of the final material. Pectins are industrially extracted from citrus peels and apple pomace by hot acidified water. Extraction conditions of pH 1.5 to 3.0 and temperatures of 60-100 C for 0.5 to 6 hours are varied to give a material that has the desired gelling capacity and degree of methylation. The separation of the viscous material from the swollen and partially disintegrated plant material remains a problem. Grinding and washing with ethanol are used but this can lead to co-precipitation with intracellular proteins, starches and nucleic acids.

Another method by which this contamination may be avoided is by wet ball milling at low temperature. Enzymatic degradation of the pectin is avoided by addition of a surfactant like sodium dodecyl sulphate (SDS). Sodium deoxycholate (SDC) is used dilute to remove pigments and lipids and a treatment with 90% dimethyl sulphoxide (DMSO) will remove the bulk of the starch.

The advantage of alcohol treatment is that the resulting preparation is in a very suitable form for further modification to high methoxylated (HM) pectins using acid treatment or to low methoxy pectin (LM) by treatment with ammonia. The disadvantage of alcoholic treatment could be the possibility of reinforcing hydrogen bonding between cell wall constituents and effecting the extraction of the pectins. The extraction method may therefore be optimised for the type of pectin required, be it modified or native.

Pectins can also be extracted using enzymes. Scientific studies have all extracted pectins using galacturonase enzymes. This results in short but branched segments. In order to extract unaltered pectins arabinase and galactanase could be used to avoid degradation.

Gelation of pectins

Low methoxy pectin (LM)

LM pectins can gel in the presence of divalent cations, usually calcium. In these systems gelation is due to the formation of intermolecular junction zones between homogalacturonic smooth regions of different chains. The structure of such a junction zone is generally ascribed to the so called 'egg box' binding process. Initial strong association of two polymers into a dimer is followed by the formation of weak interdimer aggregation, mainly governed by electrostatic interactions.

The gel forming ability of LM pectins increases with decreasing degree of methylation.

LM pectins with a blockwise distribution of free carboxyl groups are very sensitive to low calcium levels. The presence of acetyl groups prevents gel formation with calcium ions but gives the pectin emulsion stabilising properties.

High methoxy pectin (HM)

HM pectins have the ability to form gels with sugar and acid, so-called low water activity gels or sugar-acid-pectin gels. Such a gel is considered a 2-dimensional network of pectin molecules in which the solvent (water) with the co-solutes sugar and acid are immobilised. This results in a system resisting deformation and showing a stress-strain relationship for small deformation. The build up of the 3-d network is based on the formation of junction zones in which there are chain associations stabilised by hydrogen bonding between undissociated carboxyl and secondary alcohol groups and by hydrophobic interaction between methyl esters.

The gelation mechanism of pectins is mainly governed by their degree of esterification (DE). For the low methoxy pectins, denoted LMP (DE < 50%), gelation results from specific non-covalent ionic interactions between blocks of galacturonic acid residues of the pectin backbone and with divalent ions such as calcium. The affinity of pectin chains towards calcium is known to increase with decreasing degree of esterification or ionic strength, and with increasing polymer concentration. Besides the influence of the charge density of the polygalacturonate chain, the distribution pattern of free and esterified carboxyl

groups has an important effect on the strength of calcium binding.

Molecular weight of pectins can be expected to vary with plant source, raw material and extraction conditions but molecular weight determination is a challenge because of the extra problems of heterogeneity and aggregation which can obscure data gathering.

Gelling properties and applications

Low methoxy pectin gels

Calcium induced gelation is predominant in low methoxy pectin gels. Gelation is due to the formation of intermolecular junction zones between the 'smooth' HG regions of separate polymers. The nature of the interaction, although known to be electrostatic to some extent, is still debated. Gel forming ability decreases with degree of methoxylation and some blockwise distributions of carboxyl groups are very sensitive to calcium presence. The effect of calcium is decreased by the acetylation of the pectin. Amidation, conversely, improves the gelling ability of LM pectins and are less prone to precipitation by high calcium levels.

The modification of the hydrogen bonding nature of the polymer by the addition of amide or acetyl moieties indicates that the 'egg-box' mechanism of gelation may not apply for all situations of calcium induced low methoxy pectin gelation.

High methoxy pectin gels

Jams and preserves are of course the main use of industrially extracted pectins. High dissolved sugar and acid conditions ensure that chain-chain interactions dominate over chain-solvent interactions. Most chain-chain interactions in these systems are not based on electrostatic interactions and so the other hydrophobic and hydrogen bonding effects exert most influence. High sugar conditions create low water activities which can be mimicked by other solutes with the same resulting gels. This is reflected in the subdivision of HM pectins into rapid-set pectins of DM~77 to slow-set with DM~60. The larger hydrophobic element in HM pectins allows for rapid arrest of the systems.

HM Pectin	Ultra Rapid Set	Rapid Set	Medium Set	Slow Set
DM (%)	74-77	71-74	66-69	58-65
Setting time (min)	1 - 3	3 - 7	15 - 25	30-120
pH	3.1 - 3.4	3.0 - 3.3	2.8 - 3.1	2.6 - 2.9
Application	Jams with whole fruits	'Classical Jams'	Acid jams and jellies	Acid to very acid and jellies

Many factors influence the conditions of gel formation and the gel strength achieved. The major role is played by the pectin molecules, their chain length, and the chemical nature of the junction zones. Under equal conditions gel strengths increase with the molecular weight of the pectin used, and any treatment depolymerising the pectin chains is reflected in weaker gels. Rupture strength depends on the number of junction zones per single long chain molecule whereas for rigidity the number of junction zones per unit gel volume plays a greater role. Acetyl groups prevent gelation and the DM within the group of high methoxy pectins determines the setting temperature of a gel.

Pectin interactions with other polymers

Interactions between alginates and pectins

Mixtures of pectins with other polysaccharides such as alginate has found that good gels are formed from high methoxy pectin and guluronic rich alginates. A pH above 4 also hinders the gel formation. This finding, with the added evidence of low methoxy pectin gelation with alginate at very low pH, indicates that the chains must be sufficiently charge neutralised before interaction can occur, and that esterification is required only to reduce electrostatic repulsion. These mixed systems work well with cold setting conditions.

Interactions between pectins and proteins

Understanding interactions between pectins and proteins is thought to be central to developing satisfactory food texture. Mixtures of proteins and polysaccharides are prone to incompatibility or undesirable complex formation. However, due to the number of interactions possible with pectins, there are many opportunities to explore different systems. One such system sees LM pectin interacting with poly-L-lysine. In pectins with a DE of 36%, strong gels crosslinked by physical bonds with the protein were obtained at pH's close to neutrality. These clear, elastic gels had controlled increase in gel strength with added crosslinker up to an optimum. Excess protein caused increased opacity and eventual network collapse. Poly-L-lysine also serves to control network swelling. As with some hydrocolloids, chain length of the protein was found to have an optimum value, which may correspond to the different regions of the pectin molecule.

Interactions with other polymers

Interactions with other hydrocolloids has been studied in depth recently. Gel formation of LM pectins with guar, locust bean gum, oxidised starch, potato maltodextrin and gum Arabic have shown there to be specific interactions between polysaccharide complexes. Complex formation between gum Arabic and LM pectin (DM 31) was found to be enhanced when there was specific spatial compatibility between the HG areas of the pectin and the gum Arabic fibrils. These interactions were found to be non-ionic and were more likely a hydrophobic association and stabilisation which resulted in differing gel properties. Branched hydrocolloids caused faster destabilisation of calcium induced LM pectin than linear polymers. This takes into account the interactions of branched regions in pectins with other branched regions, interactions that are highly hydrophobic and non-ionic.

Nutritional aspects of pectins

Source of dietary fibre

Pectin is sourced from plant cell walls and is analysed as a soluble and insoluble fraction as galacturonic acid after hydrolysis. The fruits and vegetables which are especially rich in pectins have dietary fibre contents in the range of 1-2%. In order to increase societal intake in fibre it is therefore preferable to add products concentrated in fibres. Pectin fibres exhibit higher hydration properties than other fibres and this property is exploited in its use as a structural component in foods, for example in bakery products. Studies have shown that substitution of flour with citrus fibres, apple flakes and concentrates in bakery and confectionery products had a positive sensory effect. Pectin's adsorbent and bulk-forming properties have promoted its use in some multi-ingredient anti constipation and anti-diarrhoeal preparations.

Mineral binding

Another functionality of dietary fibre is in mineral and ion absorption and exchange. Pectin has the ability to associate ions due to a high content of negative charges and calcium binding is an example of binding strength and specificity. Pectin rich fibres can behave as weak cation exchange resins and are reversible depending on pH conditions.

Prebiotic effect

Following ingestion of pectin, very little of it gets digested in the small intestine. Some fermentation of pectin takes place in the large intestine via the action of bacteria. Pectin substituents (homogalacturonans) are fermented in the colon with the formation of short chain fatty acids. It has been shown that non-methyl-esterified pectins were more rapidly fermented than methyl-esterified pectins. The final products of fermentation of pectin are the short-chain fatty acids, acetate, propionate and butyrate, as well as hydrogen and carbon dioxide. The short-chain fatty acids that escape colonic metabolism are transported via the portal circulation to the liver where they undergo metabolism. The short-chain fatty acids that are not metabolised in the liver enter the systemic circulation and are distributed to the various tissues of the body. Acetate appears to be the principal short-chain fatty acid to reach the systemic circulation from the liver. Pectins are therefore beginning to gain interest as prebiotics. Studies on the metabolising of pectin chains has shown that many bacteria can degrade certain regions of the polymers, generally the HG regions. This use of a plentiful polysaccharide in maintaining and encouraging digestive flora is of advantage in assessing pectin uses in the future.

Cholesterol regulation

The mechanism of the possible hypocholesterolemic activity of pectin is not well understood. It appears that the viscosity of pectin is related to its possible hypocholesterolemic activity. Pectin preparations with high viscosity appear to be more effective in lowering cholesterol than are pectin preparations with lower viscosity. High-viscosity pectin is thought to lower cholesterol levels by raising the excretion of fecal bile acids and neutral sterols. High-viscosity pectin may interfere with the formation of micelles and/or lower the diffusion rate of bile acid and cholesterol-containing micelles through the bolus, consequently diminishing the uptake of cholesterol and bile acids. Numerous studies have demonstrated that pectin has favourable effects on lipids. In a small early study, administration of 15 grams of pectin daily for three weeks resulted in a mean 13% reduction in plasma cholesterol levels. There was no effect on plasma triglyceride concentrations. Subsequently, giving 40 to 50 grams of pectin daily significantly lowered cholesterol levels in both normolipidemic and hyperlipidemic subjects. In another study, a pectin-supplemented diet (without other dietary or lifestyle changes), significantly reduced plasma cholesterol in volunteers evaluated to be at medium to high risk for coronary heart disease due to hypercholesterolemia. This was a double-blind, placebo-controlled trial. Treatment continued for 16 weeks. The pectin was credited with decreasing plasma cholesterol 7.6% and LDL-cholesterol 10.8%.

Anti-cancer action

Modified citrus pectin, when administered orally to rats, was found to inhibit spontaneous prostate carcinoma metastasis. It had no effect on the growth of the primary tumour. Injected modified citrus pectin was found to inhibit metastasis of melanoma cells in mice. The mechanism of these anticarcinogenic effects is not clear.

Galectins comprise a family of galactoside-binding mammalian lectins. Lectins themselves comprise a group of hemagglutinating proteins found in plant seeds, which bind the branching carbohydrate molecules of glycoproteins and glycolipids on cell surfaces, resulting in agglutination or proliferation, among other things. Galectins are proteins that can bind to carbohydrates via carbohydrate recognition domains (CRDs). At present, the galactin family includes 10 members. Apparently, galectins are secreted from cells via nonclassical secretory pathways. Galectin-3, one of the members of the family, is thought to be involved in mitosis and proliferation. On the cell surface, galectin-3 mediates cell-cell adhesion and cell-matrix interaction via binding to its complementary glycoconjugates, such as laminin and fibronectin, and thereby is thought to play an important role in the pathogenesis of cancer metastasis.

Some metastic events may involve cellular interactions that are mediated by cell surface components, including galectins. The galactose-containing carbohydrate side chains of modified citrus pectin may interfere with these cellular interactions by competing with the natural ligands of the galectins and by doing so, inhibit the metastatic process. It is thought that galectins may play a role in human prostate cancer, and in particular, human prostate cancer metastasis.

Strengths and weaknesses

Strengths

Increasing knowledge of the interaction of pectins with other hydrocolloids is opening opportunity for its use as a more tailored polymer than previous uses. This combined with a 'natural' perception and new information about its prebiotic and metabolic activities may allow its uses to broaden from the bulk polymer to finer, triggered responses.

Weaknesses

Difficulty in extraction and characterisation of the polymer combined with numerous sources of differing compositions makes R&D difficult as any development may find variation with a different pectin source.

Conclusion

An understanding of pectin gelation in terms of the overall molecule as opposed solely to the associating areas may allow the expansion of pectin uses beyond their quite macroscopic behaviours to a more functionalised approach. The inherent properties of pectins have not been fully explored and can, when researched, show some novel and timely applicability.

References and recommended reading

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