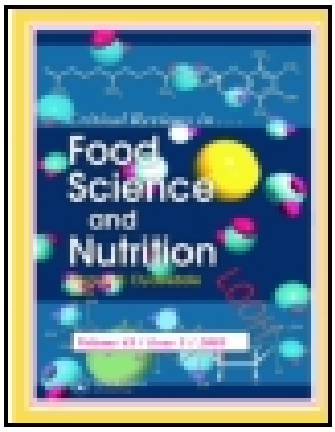

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Pectin Modifications: A Review

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Pectin Modifications: A Review

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ABSTRACT

In recent years, the interest in studying modification of pectin has increased. A number of hydroxyl and carboxyl groups distributed along the backbone as well as a certain amount of neutral sugars presented as side chains make pectin capable of preparing a broad spectrum of derivatives. By forming pectin derivatives, their properties may be modified and some other new functional properties may be created. This article attempts to review the information about various methods used for pectin modification, including substitution (alkylation, amidation, quaternization, thiolation, sulfation, and oxidation, etc), chain elongation (cross-linking and grafting) and depolymerization (chemical, physical and enzymatic degradation). Characteristics and applications of some pectin derivatives are also presented. In addition, the safety and regulatory status of pectin and its derivatives were reviewed.

Keywords: Pectin, Modification, Derivative, Substitution, Chain elongation, Depolymerization

INSTRUCTION

In the past few years, considerable attention has been devoted to the applications of natural plant polysaccharides because of their unique properties (Crini, 2005). A large number of studies showed that natural plant polysaccharides and their derivatives were widely used as a type of desirable biomaterials in many fields, such as drug delivery, immunization, controlled release, as well as in food application (Fox et al., 2011; Ramberg et al., 2010; Vel̆išek and Cejpek, 2005). Pectin is a high-molecular weight, biocompatible, non-toxic and anionic natural polysaccharide extracted from cell walls of higher plants. It mostly consists of three structurally well-characterized polysaccharide motifs: homogalacturonan (HG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II) (Figure 1A) (Ridley et al., 2001; Willats et al., 2001). HG represents the backbone chain of the pectin molecule, containing α-1, 4-linked D-galacturonic acid units. RG I is located in the highly branched area containing a large number of neutral sugars such as arabinose, galactose and mannose as side chains of α-1, 2-linked resides of L-rhamnopyranose. RG II is a branched pectic domain containing an HG backbone, and is a highly conserved but widespread domain.

Pectin itself is known as functional ingredient, gelling/thickening agent and stabilizer in food industry due to its ability to form aqueous gels and has been used in jams and jellies, fruit preparations, fruit drink concentrates, fruit juice, desserts and fermented dairy products (Rao and Silva, 2006). Moreover, characters of excellent gelling properties, good biocompatibility, and non-toxicity, as well as biodegradability entitle pectin to be an attractive novel biopolymer material, which can be employed in pharmaceutical industry, health promotion and cosmetic applications. However, pectin has some inherent drawbacks when used in some specific areas.

For example, gelation of high methoxyl pectin is typically in the presence of high sucrose concentrations (55-75%) (Rao and Silva, 2006), therefore, the resulting products may not be suitable for diabetes patients. The tendency of forming lumps and agglomerations during dissolution imposes a serious obstacle in efficiently dissolving (Kurita et al., 2012). The rapid hydration, swelling and erosion due to their high water solubility reduce the ability to control drug release efficiently in different dosage forms (Bhatia et al., 2010). In order to overcome such drawbacks, controlled physical, chemical and/or enzymatic modifications of pectin structure are necessary.

Pectin is capable of preparing a wide range of derivatives due to its special structure: a number of hydroxyl and carboxyl groups distributed along the backbone as well as a certain amount of neutral sugars presented as side chains. By forming pectin derivatives, properties such as solubility, hydrophobicity, physicochemical and biological characteristics may be modified and some other new functional properties may be created. Thanks to the efforts from many research groups, the modification of pectin has been achieved using techniques such as substitution (amidation, thiolation and sulfation, etc.), chain elongation (cross-linking and grafting) and depolymerization (acid or enzymatic hydrolysis, β-elimination and mechanical degradation). The majority of the previous reviewed works have been focused on native pectin (Thakur et al., 1997; Voragen et al., 2009; Willats et al., 2006), with few literature surveys covering the modifications of pectin, the corresponding physicochemical properties and applications of modified pectin. In this review, we have re-examined the information about various methods used for the modification of pectin. Characteristics and applications of some

pectin derivatives were also presented. In addition, the safety and regulatory status of pectin and its derivatives were reviewed.

SUBSTITUTION

Alkylation

Alkylation of carboxylate group

Alkylating carboxyl group into COO-alkyl ester group was successfully used by researchers to increase the hydrophobicity of pectin (Fischer et al., 1998; Miralles-Houzelle et al., 2001). When one carbon was alkylated to carboxylate group, it is termed as methoxylation. The C-6 of galacturonic acid in pectin backbone often naturally methoxylated is one of the most marked structural characteristics of pectin (Figure 1B). The ratio of methoxylated D-galacturonic acid units to total D-galacturonic acid units is called the degree of methoxylation (DM), which strongly influences the solubility, gel forming ability, conditions required for gelation, gelling temperature and gel properties (BeMiller, 1986). Depending on the DM, pectin is usually classified as high methoxyl pectin (HMP, DM > 50) and low methoxyl pectin (LMP, DM < 50). HMP forms a gel under acidic conditions in the presence of high sugar concentrations (Evageliou et al., 2000), and can be used as gelling agents in fruit-based products, especially in the manufacture of jams and fruit preservatives. While LMP forms a gel by interaction with divalent cations, particularly Ca^{2+}, according to the 'egg box' model (Durand et al., 1990). They are usually used to prepare gels with a reduced level of dissolved solids and are of great interest because of their reduced calorific value.

A wide range of different methods for methoxylation is available. Reaction of tetrabutylammonium (TBA) pectinates with methyl iodide as an electrophile has previously been used by (Renard and Jarvis, 1999a), who reported that methoxylation took place in dimethyl sulfoxide (DMSO), using CH_3I as the reagent, and can be stoichiometric up to a DM > 60 with limited degradation of pectin. However, some other authors reported that it is unable to achieve high DM without extensive depolymerization (Rosenbohm et al., 2003). This difference may be due to different isolation procedures used. The most commonly used method for methoxylation of pectin has been treated with methanol that acidified with either sulfuric acid or hydrochloric acid (Ralet et al., 2001b). In this method, pectin was directly esterificated with methanol in the presence of sulfuric acid or hydrochloric acid as catalyst and the methanol is present in excess to ensure that the equilibrium is in the favor of product formation. However, the reactions have to be carried out at 0-5 °C in order to minimize the cleavage of the glycosidic linkages. This means that very long reaction times, typically in the order of weeks, are required to obtain fully methoxylated material. Whereas, utilization of anhydrous methanol acidized with acetyl chloride at 5 °C was reported to be the most efficient method, proceeding with the least degradation and a reasonable reaction rate (Rosenbohm et al., 2003). The DM of pectin can reach 100% by this preparation.

In contrast to methoxylation of pectin, demethoxylation is a reaction which removes the methyl esters from esterified galacturonic acid residues, and converting the C-6 carbon to the carboxylic acid. The demethoxylation is an important and frequently used reaction for modifying the properties of pectin that occurs in the kinds of physiological processes during plant development (growth, abscission and fruit ripening, etc.) or in physicochemical treatments. At

the industrial level, pectins obtained are typically HMP while LMP is usually manufactured from HMP by controlled acid demethoxylation (El-Nawawi and Heikal, 1995), alkali demethoxylation (Renard and Thibault, 1996), or by demethoxylating reaction of pectin methyl esterase (PME) (Kim et al., 2008). Alkali or acidic demethoxylation was normally carried out at low temperatures to avoid the potential depolymerization of pectin, and the enzymatic demethoxylation was believed to a more specific and mild method.

One of the greatest important issues in pectin demethoxylation is the pattern of methoxylation (blockwise or more random) that induced. The resulting methoxylation pattern of pectin may influence the properties of pectin to a large extent, not only by modulating calcium binding properties of pectin (Ralet et al., 2001a), but possibly also by influencing depolymerization rates of pectin (Fraeye et al., 2007). Alkali treatment was reported to result in random de-methoxylation (Hunter and Wicker, 2005). In the case of enzymatic demethoxylation, the mode of action varied with the origin of enzyme. Fungal PMEs generally have non-blockwise action patterns, producing HG with dispersed arrangement of methyl-esters along the HG chain. In contrast, plants PMEs usually have a blockwise action pattern, producing long stretches (or 'blocks') of un-esterified HG (Willats et al., 2006).

Alkylating more carbons to carboxylate group of pectin is also carried out to improve the hydrophobicity of pectin, which is often carried out via reaction of its TBA salt with corresponding alkyl halides (Scheme 1), similar to that used in methoxylation. For example, Miralles-Houzelle et al. (2001) prepared a C_{12}-pectin derivative by reaction of a C_{12} alkyl bromide with the pectin TBA salt in DMSO solution. C_{12}-pectin derivatives prepared in this

method displayed the main characteristic rheological features of classical hydrophobically associating water-soluble polymers.

There are also some other methods can be used to prepare the alkyl esters of pectin or alkyl esters of pectic acid, which was described in the report of Klavons and Bennett (1995): (i) Diazoalkane method. (ii) Triethyl orthoacetate method. (iii) Alkanol/*p*-toluenesulphonic acid method. However, these methods were not frequently used. In addition, great care must be taken when the Diazoalkane method was used, because diazoalkanes in ether are explosive. The resulting products showed enhancements of intrinsic viscosity, binding to bile acids and to isolated soy protein.

Alkylation of hydroxyl groups

Acetylation is one of the most important alkylations of hydroxyl groups in pectin. Pectins extracted from some plants, for instance, from sugar beet (Dea and Madden, 1986), potatoes (Bush and McCann, 1999) and sunflower (Iglesias and Lozano, 2004), may be naturally acetylated at O-2 and/or O-3 of galacturonic acid units (Figure 1B). Such esterification decreased the stability of binding of calcium and pectin (Kohn and Furda, 1968; Kohn and Malovíková, 1978), hampering gelation so much so that complete inhibition of gelation occurs when one out of eight D-galacturonic acid are acetylated at O-2 or O-3 (BeMiller, 1986). This was ascribed to a steric effect of acetyl groups that prevent, to a certain extent, the access of calcium ions to a close proximity of two neighboring carboxyl groups. Renard and Jarvis (1999b) showed that the effect of acetylation might also be attributed to modifications of conformation and complexation. Presence of acetyl groups could lower the strength of binding of the cation to individual

galacturonic acid residues or hinder adoption by the polymer of binding-favorable conformations.

However, acetylation of pectin does have some other potential applications such as being used as stabilizer and emulsifier (Leroux et al., 2003). In addition, the pectin modified with acetylating agent was found to be promising in modifying the release pattern of ibuprofen, a weak acidic drug, throughout the gastrointestinal tract, due to the reduction of polarity and solubility of pectin (Bhatia et al., 2010).

Generally, acetylation was carried out in kinds of solvent-catalyst systems with acetic anhydride. The solvent might be DMSO or formamide, and the catalyst might be pyridine or *N*-methylimidazole. Renard and Jarvis (1999a) investigated three solvent-catalyst systems (DMSO-*N*-methylimidazole, DMSO-pyridine, and formamide-pyridine), and reported that best results were obtained with pyridine in formamide. The environment of homogeneous or heterogeneous may determine the distribution pattern of acetyl. Acetylation of homogalacturonans as their TBA salts (homogeneously dispersed), leading to a homogeneous distribution of acetyl groups (Renard and Jarvis, 1999a), while acetylated in suspension were highly preferentially or exclusively to be diacetyl derivatives (Rexová-Benková et al., 1977).

To endow pectin more potential properties and applications, fatty acid anhydrides were also tentatively alkylated to the hydroxyl group of pectin. Monfregola et al. (2011) performed the esterification of the pectin OH groups by using palmitic acid, oleic acid or linoleic acid. All esterification reactions were carried out by mechanical milling of the pectin with the appropriate fatty acid anhydrides in the presence of a catalytic amount of base K_2CO_3 under a solvent-free

condition (Scheme 2). This chemical modification was considered efficient, fast, economic and eco-friendly, and enlarged the application field of pectins as their water sorption was reduced.

Amidation

The simplest but most widely used amidated pectins are those containing primary amino groups -CO-NH_2. They are important pectin derivatives commercially available in food technology with good gelling properties and reduced sensitivity against Ca-ions and pH. Furthermore, gels from amidated pectin are thermo-reversible. They can be heated and solidify again after cooling, whereas conventional pectin-gels will afterwards remain liquid. Their excellent gelling properties entitle them to be prepared into hydrogel beads, which could be used in delivery of colonic-specific drug such as indomethacin and sulphamethoxazole (Munjeri et al., 1997), and be used to entrap insulin so that it would be oral administrated (Musabayane et al., 2000). The common method of this kind of pectin preparation is ammonolysis of methyl ester groups of HMP with ammonia in anhydrous methanol (R = H, Scheme 3). This method is a type of alkaline demethoxylation by the action of ammonia on the ester groups and some of methyl ester groups are replaced by amino groups (Einhorn-Stoll et al., 2001).

HMPs can also react with hydroxylamine, where hydroxylamine was inserted into carboxyl groups in pectin polymer chains to form hydroxamic acid, and amide groups are generated with a general structure of -CO-NHR (R = OH, Scheme 3). Rha et al. (2011) synthesized a pectin hydroxamic acid derivative by hydroxamation. First, pectin was treated with alkaline hydroxylamine (2 M, pH 12.0) for 4-48 h, then the pH was adjusted to 6.5 using HCl, after that the pectin derivatives were precipitated by 2-propanol. The resulting pectin derivatives

were shown to have enhanced anti-radical activities against DPPH in a dose-dependent way. Moreover, the scavenging effect of the pectin derivative was about 3-fold higher than that of the native one. Pectin from orange (*Citrus unshiu*) peels modified in the same way showed enhanced water solubility (Bae et al., 2011). However, attention should also be paid to some undesirable side reactions such as demethoxylation at alkine pH and a decrease of the molecular weight by depolymerization.

Introducing primary amines instead of ammonia in the reaction with HMPs can lead to another kind of amidated pectins which contain secondary amide groups -CO-NHR (R = carbon radicals, Scheme 3). This method can be used to link various carbon radicals or functions to pectin macromolecules (Sinitsya et al., 2000). Typically, these kinds of pectin derivatives were prepared by treatment of HMPs with primary amines in methanol, but can also be prepared in the presence of water and protease using amines or amino acids (Cheng et al., 2000). The functional groups attached to pectin macromolecule influence the physical and chemical properties of pectin derivatives and their possible application. For instance, the amidated pectin with aliphatic or aromatic groups is more hydrophobic than the original pectin, whereas amidated pectins with an amine radical containing polar or charged groups can still have hydrophilic properties (Sinitsya et al., 2000). In addition, if the amine radical carries cationic groups, the resulting derivatives will have properties of polyampholytes, while introduction of double carbon-carbon bonds or aromatic rings may lead to new pectin derivatives following by electrophilic addition or substitution. *N*-octadecylpectinamide prepared based on this principle could be used as a hydrophobic sorbent or bioavailable surfactant (Synytsya et al., 2004), while pectin octylamide,

pectin dodecylamide and pectin octadecylamide showed to be promising for oil-in-water emulsions or for particle stabilization (Zouambia et al., 2009).

Amidation of pectin can also be prepared through reacting with amine acids. Kurita et al. (2012) modified citrus pectin with glycine, glycine methyl ester or glycylglycine through precipitation of pectin by acetone and drying. The water-soluble ability of pectin was significantly improved, and the problem of forming lumps and agglomerations during dissolution was solved by glycylglycine-modifying. The resulting glycylglycine-modified pectin can readily dissolve into water at pH range from acidic to neural, even at high ionic strength. However, only glycine methyl ester-modified pectin was proved to be successfully amidated, the reaction mode between glycylglycine and pectin need to be further investigated.

Quaternization

It is reported that quaternization is an efficient means of imparting new functional properties to polysaccharides (Geresh et al., 2000), and is a method that could turn ionic hydrocolloids into their cationic derivative. Fan et al. (2012a) prepared a quaternized pectin by reacting pectin with 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTAC) in presence of sodium hydroxide (Scheme 4). The resulting quaternized pectin has an excellent moisture absorption and moisture retention abilities, as well as a pronounced antimicrobial effect. This kind of pectin derivative may be further used in pharmaceutical, packaging, preservatives and cosmetic fields. Besides pectin, Pectin-NH_2 prepared by modifying the galacturonic acids carboxyl groups with ethylenediamine could also be further quaternized with CHPTAC to generate a pectin-NH_2-Q (Q = $[N^+(CH_3)_3]$), which is an efficient DNA carrier, and a

promising non-viral carrier for targeted gene delivery to cancer cells (Katav et al., 2008). In both studies, sodium hydroxide was used. Under the alkaline conditions, epoxide is produced in situ from CHPTAC, quaternized pectins are then formed through reaction between the pectin and the epoxide. In addition, demethoxylation occurs in alkalic pH may favor the reactions.

Šimkovic et al. (2009) also modified pectin with trimethylammonium-2-hydroxypropyl- (TMAHP) or 2-hydroxypropylsulfonate- (HPS) groups during the extraction of pectin from sugar beet pulp. The advantage of this method is that by introducing ion-exchanging groups, more material could be solubilized and simultaneously the solubilized product is quaternized, which results in new properties. These authors also proved that, the TMAHP-group was mostly attached to C-2 or C-3 positions of arabinose units while the HPS was mainly connected to the C-5 position of arabinose or the C-6 position of galactose. However, since acidic conditions could be more sensitive for pectin extraction, the authors further quaternized sugar beet pulp with glycidyltrimethylammonium chloride under acidic conditions (Šimkovic et al., 2010), and showed that the arabinan structure is modified with the quaternary group at terminal C-5 of arabinofuranose unit.

Thiolation

Research in thiolation of pectin has blossomed quickly. It is an extremely active subject of study due to an interest in development of ‘second generation’ of mucoadhesives. Natural polysaccharides belong to the ‘first generation’ of mucoadhesive polymers, which can non-covalently interact with the mucus layer (Smart, 2005), but this mucoadhesive property is weak. The mucoadhesive properties of natural polymers can be improved by their thiolation. The

thiomers are capable of forming covalent disulfide bonds with the cysteine-rich subdomains of mucus layer on the mucosa (Bernkop-Schnürch, 2005).

Thiol containing ligands can be introduced into pectin chain by formation of either amide or ester bonds. Sharma and Ahuja (2011) synthesized a thiolated pectin by esterification of pectin with thioglycolic acid in the presence of hydrochloric acid (Scheme 5A). The resulting thilolated pectin showed an improved mucoadhesive property while did not affect the release profile of drug. After that, the author prepared thiolated pectin nanoparticles using magnesium chloride as an ionic cross-linker and timolol maleate as a model drug (Sharma et al., 2012). The thiolated pectin nanoparticle provided significantly higher *ex vivo* corneal permeation of timolol maleate, across the excised goat cornea than the conventional formulation which bases on aqueous solution, indicating that thiolated pectin is promising mucoadhesive polymer for ocular delivery of timolol maleate.

Thilolated pectin can also be prepared by formation of amide bonds using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride as an activator. Majzoob et al. (2006) synthesized a thiolated pectin by formation of amide bonds between the primary amino groups of cysteine and carboxylic acid groups of the pectin (Scheme 5B). This new polymer showed biodegradability profile, higher permeation enhancement for sodium fluorescein, increased *in vitro* adhesion duration, improved cohesive properties and no severe toxicity in caco-2 cells. Perera et al. (2010a; 2010b) also modified pectin by immobilization of a hydrophobic thiol-bearing ligand, 4-aminothiophenole, to the backbone of pectin under the same principle (Scheme 5B). This modification leads to alter swelling and disintegration behavior and allows cross-linking of pectin by oxidation of thiol groups to disulfide bonds, hence, it leads to improved

stability of drug delivery system along the upper gastrointestinal tract and to sustained drug release.

Sulfation

Sulfation, in which hydroxyl groups in polymer structure are replaced with sulfate groups, is interesting due to its significant effects on the physiological functions of polysaccharides such as anticoagulant, antithrombotic, contraceptive, antioxidant, anti-inflammatory, antitumor, antimicrobial and anti-HIV infection activities (Martinichen-Herrero et al., 2005; Wang et al., 2008). The effects of introduction of sulfate groups into pectin macromolecules on the improved antimicrobial, anticoagulant and antithrombotic activities have been recently investigated (Fan et al., 2012b; Maas et al., 2012).

Sulfation of pectin is commonly performed using sulfuric acid, chlorosulfonic acid, monomethyl sulfate, sulfotrioxide, sulfuryl chloride or sulfamic acid in the presence of formamide, DMSO, trimethylamine or pyridine. The chlorosulfonic acid was thought to be superior to the other two sulfating agents, pyridine sulfotrioxide and pyridine monomethyl sulfate, when considering the sulfation degree, destruction degree and yield of sulfated derivatives (Vityazev et al., 2010). However, the chlorosulfonic acid method also results in hydrolytic or degradation of the polysaccharide chain during the reaction as well as serious pollution problems due to these agents used, and the degree of substitution value of 0.15 was very low in Bae et al. (2009)'s report. Therefore, some other sulfating agents were developed to sulfate the pectin. For example, Fan et al. (2012b) synthesized apple pectin sulfates in aqueous

solution with a new mild sulfating agent, trisulfonated sodium amine [$N(SO_3Na)_3$]. This method was reported to be a simple and effective method.

One of the most marked and well reported characteristics of sulfated pectin is its anticoagulant activity and antithrombotic effects. Maas et al. (2012) indicated that replacement of carboxyl group with sulfate group in citrus pectin increased anticoagulant and antithrombotic effects, without affecting the hemorrhagic effect. Bae et al. (2009) demonstrated that the sulfated pectin exhibited anticoagulant activity, which was favorably compared to that of heparin, a highly sulfated glycosaminoglycan and an anticoagulant drug. The specific anticoagulant activity of sulfated pectins depends on plant species, pectin monosaccharide composition, degree of sulfation (Vityazev et al., 2010) and their molecular weight (Cipriani et al., 2008). However, the mechanism of anticoagulant of sulfated pectins is still not clear and need to be further investigated. Cipriani et al. (2008) reported that the sulfated pectins directly inhibited α-thrombin and factor Xa by a mechanism independent of antithrombin (AT) and/or heparin co-factor (HCII), which is different from that of heparin. While Maas et al. (2012) reported that sulfated pectins inhibited thrombin by a mechanism dependent on AT and HCII like that of heparin. In addition to anticoagulant and antithrombotic activities, this pectin derivative also exhibited improved antimicrobial effects against *Bacillus cereus* and *Vibrio fischeri* (Bae et al., 2009). The other potential physiological properties such as antioxidant, anti-inflammatory and antitumor activities may be further exploited.

Oxidation

In recent years, oxidation of pectin has received much attention because oxidized pectins present more groups that are reactive and a faster degradation when these ones are used in supports for drug controlled delivery (Takei et al., 2010).

Oxidation reactions on -OH groups at C-2 and C-3 positions of the galacturonic acid units of pectin can be performed with sodium periodate, which leads, by rupture of carbon-carbon bond, to formation of two aldehyde groups in each oxidized monomeric unit (Abdel-Hamid et al., 2003). This pattern of oxidization distinguishes from that observed for $NaVO_3$ that oxidizes the terminal unit of the polymer chain (Gessa et al., 1983), and Cr^{VI} as well as Cr^{IV} and Cr^{V} selectively oxidize the free carboxylic groups of galacturonic acid units through C-C bond break of the –(H)C(OR)-CO_2H moieties (Bellú et al., 2008). It should note that periodate oxidation also results in degradation of polysaccharides due to the cleavage of internal glycosidic linkages, and enhanced the biodegradability of the polysaccharides. The cleavage of diol groups in monosaccharide units by periodate induced larger rotational freedom and formed new reactive groups along the backbone. The aldehyde groups can be utilized both for immobilization of a drug and for hydrogel formation with cross-linkers.

Other Substitutions

There are also some other kinds of substitutions that may be used to modify the structure and properties of pectin. For example, Maior et al. (2008) reported chemical modification of low methoxyl pectin by reaction with glycidyl methacrylate to give a material with low hydrosolubility (Scheme 6). Benzyl bromide can also be esterified on carboxylate groups of pectin in the DMSO/TBAI/catalyst system like in Scheme 1 (Morris et al., 2002). The advantage

of this modification is the UV-tagging of molecular chains and, thus, it is possible to use UV-based analyses to characterize the structural and molecular properties of the derivatives. In addition, the hydroxyl groups of pectin can be esterified with *p*-carboxybenzyl bromide in aqueous alkali (Morris et al., 2002). The resulting *p*-carboxybenzyl derivative is water-soluble, tensioactive and is also an UV-tagging molecule chain.

Moreover, polygalacturonic acid can also be sulfonated with 3-chloro-2-hydroxy-1-propanesulfonate. However, this reaction was inefficient, the excess of 3-chloro-2-hydroxy-1-propanesulfonate and NaOH is a need, and cannot be taken place under ambient pressure (Šimkovic et al., 2008).

CHAIN ELONGATION

Cross-linking

Typically, a pectin gel is formed when portions of HG are cross-linked to form a three dimensional crystalline network in which water and solutes are trapped (Willats et al., 2006), such as Ca^{2+} cross-linking for LMP, hydrophobic interactions between methyl groups and hydrogen bonding for HMP (Rao and Silva, 2006). There are also many kinds of cross-links between pectic substances, such as borate-diol ester cross-linking of RG II (Matoh and Kobayashi, 1998) and uronyl ester cross-linking between HG and other cell wall polysaccharides (Vincken et al., 2003). For sugar-beet pectins, the presence of ferulic acid in the molecule allows gel formation through oxidative cross-linking of the ferulic acid groups. Oxidative cross-linking of sugar beet pectin is frequently achieved with ammonium persulfate or peroxide/peroxidase (Thibault, 1986; Oosterveld et al., 2000). This cross-linking could lead to new applications of

sugar-beet pectins with good swelling ability and rheological property. More recently, other promising approaches to obtain pectin hydrogels were developed by using kinds of cross-linking agents.

Epichlorohydrin was used to cross-link pectins (Semdé et al., 2003). The methoxyl groups of pectin were first hydrolyzed to obtain sodium pectate, which was then cross-linked in alkaline media with increasing amounts of epichlorohydrin (Figure 2A). The chemical cross-linking of pectin with epichlorohydrin allowed us to prepare new hydrophilic pectin derivatives that are much less soluble in water. The epichlorohydrin cross-linked pectins can be potentially used, under the form of matrix tables, for the specific delivery of drugs to the colon. In the colon, they will be degraded by bacterial enzymes, resulting in a huge release of the incorporated drug.

Although epichlorohydrin has a good cross-linking performance, it has great toxicity and easily leads to side reactions. Therefore, some alternative cross linkers such as ethylene glycol diglycidyl ether and glutaraldehyde (Yoshimura et al., 2005), adipic acid (Li et al., 2007) and trisodium trimetaphosphate (STMP) (Souto-Maior et al., 2010) have been introduced. For example, Li et al. (2007) cross-linked saponified pectin using adipic acid (Figure 2B). The modified pectin had a rough, porous phase covered with carboxyl groups, and can be used for removal of heavy metal such as Pb^{2+}, Cu^{2+}, and Zn^{2+} from wastewater due to their high adsorption capacity. While Souto-Maior et al. (2010) chemically modified pectin through reaction with STMP (Figure 2C), which is an effective cross-linking agent used in the food industry and does not show any toxicity in humans. The reaction mechanism for polysaccharide cross-linked by STMP involves two steps: opening of the STMP cycle by an alcohol moiety followed by reaction with a second alcohol moiety leading to the cross-linking of

polysaccharides. This reticulation reaction promotes a decrease in polysaccharide solubility as the number of hydroxyls free to interact with water is reduced.

For some modified pectins we had stated above can be further cross-linked. For example, the thiolation allows cross-linking of pectin by oxidation of thiol groups to disulfide bonds (Perera et al., 2010a; Perera et al., 2010b). The amidated pectin can be cross-linked with glutaraldehyde reagent to prepare hydrogels (Mishra et al., 2008). The alkyl esters prepared with alkyl or alkylaryl halides can also be further cross-linked by cross-linkers, including 1, 6-dibromohexane, 1, 3-diiodopropane and 1, 10-diiododecane (Matricardi et al., 1995). In addition, pectin can be cross-linked by adipic dihydrazide after it was oxidized. Takei et al. (2010) developed in situ gellable hydrogels by cross-linking periodate oxidized citrus pectin using adipic dihydrazide, This hydrogel was used to localized anticancer drug (Doxorubicin) delivery, and have a potential to prevent progression of primary cancer by the released the Doxorubicin and generation of metastatic cancer by the released oxidized pectin.

Grafting

The synthesis of pectin graft copolymers is one of the key ways for modifying physicochemical properties of pectin. This is usually achieved by modifying the pectin molecules through creation of branches (grafts) of polymers and by doing so imparting specific properties onto the pectin substrate. The graft copolymerization of many monomers onto pectin has been carried out by different methods based on various initiators used, and the resulting copolymer was further cross-linked to form hydrogel, which has many applications especially in the drug delivery system.

Among the various chemical initiation methods, the formation of free radicals on the pectin molecules by direct oxidation with Ce^{IV} ions has gained considerable importance, due to its ease of application and its high grafting efficiency. In this technique, the Ce^{IV} ions produce free radicals on the pectin backbone in the presence of acid (e.g. HNO_3). When pectin is oxidized by a salt such as a cerium ammonium nitrate, free radicals are formed on the pectin by a single electron transfer process. This Ce^{IV} ion technique has been used to graft poly (*N*-vinylpyrrolidone) (Fares et al., 2010) and poly (*N*-isopropylacrylamide) (Assaf et al., 2011) into pectin under N_2 atmosphere. The resulting grafted polymers can be further cross-linked by *N*, *N*'-methylenebisacrylamide to form a hydrogel, which can control release of theophylline, an effective drug used in the treatment of nocturnal asthma and pulmonary disease. Sutar et al. (2008) also investigated another Ce^{IV} agent, ceric ammonium sulphate, which was used to graft acrylamide into pectin molecules. The resulting copolymer was cross-linked by glutaraldehyde for controlling release of salicylic acid, an antipyretic drug.

Different free radical initiators, such as hydrogen peroxide (Cathala et al., 2001), ammonium persulfate (Pourjavadi and Barzegar, 2009) and ammonium persulphate-ferrous ammonium persulphate (Chauhan et al., 2007) can also be used. Pourjavadi and Barzegar (2009) modified pectin by grafting acrylic acid-co-acrylamide in the presence of ammonium persulfate as free radical initiator. These pectic derivatives can be further cross-linked by *N*, *N*'-

methylenebisacrylamide to form an intelligent pH and thermosensitive superabsorbent hydrogel, which could successfully deliver a drug to the intestine without losing the drug in the stomach, and could be a potential candidate for an orally administrated drug delivery system. Chauhan et al. (2007) also grafted acrylamide, *N*-isopropylacrylamide, or 2-acrylamide-2-methyl-1propane sulfonic acid onto pectin with an ammonium persulphate-ferrous ammonium persulphate redox initiator system. The resulting hydrogel with good biodegradability can be used in absorption of some common metal ions pollutants found in soil, industrial and mining water.

In addition, based on the review about graft copolymerization of cellulose (Mishra et al., 2007), there are a lot of initiators (azobisisobutyronitrile, benzoyl peroxide, Fe^{2+}-H_2O_2 system, etc.) and technologies (γ-radiation grafting, ultraviolet-radiation induced grafting, plasma grafting, etc.) may also be available for pectin grafting, which need to be further investigated.

DEPOLYMERIZATION

Researches about depolymerization of pectin are important and have always been a hot topic due to several reasons. First, although pectin is safe for human consumption and has been used successfully for many years in food and pharmaceutical industries, one of the greatest difficulties in their optimal utilization is the tendency to undergo degradation caused by different kinds of physicochemical treatments. Second, degradation of pectin into smaller oligosaccharides is necessary for revealing its structure, as the pectin molecule is too large and heterogeneous to analyze as a whole (Coenen et al., 2008). Finally, depolymerization of pectin was an important method to prepare pectic oligosaccharides. The oligosaccharides have been shown in many applications, such as repressor of liver lipid accumulation (Yamaguchi et al., 1994), antioxidant

and cancer cell proliferation inhibiter (Byun et al., 2006), anti-metastatic agent (Maxwell et al., 2012), angiogenesis inhibitor (Xu et al., 2011), antibacterial agent (Li et al., 1997) and prebiotic (Olano-Martin et al., 2002).

Chemical depolymerization

Pectins are most stable in aqueous solutions around pH 3.5 (Schols and Voragen, 2002). At lower or higher pH, the removal of methoxyl, acetyl and neutral sugar groups, as well as the cleavage of the polymer backbone may occur. Pectin may undergo either acid or base catalyzed depolymerization. First and most important problems in the use and study of pectins is their susceptibility to the base-catalyzed splitting of chains through the β-elimination reaction (Scheme 7) even when pectin is heated at neutral or weakly acidic conditions (Keijbets, 1974; Renard and Thibault, 1996). The cleavage only takes place at a glycosidic linkage next to an esterified galacturonic acid, as a result, pectin with a high DM is more subject to β-elimination than pectin with a low DM, and inhibited by saponification. In alkaline conditions, any increase in temperature increases the rate of β-elimination more than that of demethoxylation, while an increase of pH increases demethoxylation than β-elimination (Kirtchev et al., 1989). β-elimination is also stimulated in the presence of cations, whereby the extent of the cleavage increases roughly with salt concentration and with valence of the ions. Sajjaanantakul et al. (1993b) reported that the β-eliminative degradation of pectin increased as cation concentration in the pectin solution was raised. At the same level of pectin methoxyl content, divalent cations promoted more depolymerization during the heating of pectin than monovalent cations did. The

enhancement effect of cations increased in the order $Zn^{2+} > Ca^{2+} > Cd^{2+} \approx Sr^{2+} > Mg^{2+} \approx Na^{+} \approx K^{+} > NH_4^{+}$ (Sajjaanantakul et al., 1993a).

The second mechanism leading to pectin chemical degradation is acid hydrolysis (pH < 3.0), whereby in acidic conditions, pectin with low DM hydrolyses faster (Krall and McFeeters, 1998). It has been shown by Thibault et al. (1993) that mild acid hydrolysis (0.1 M HCl, 80 ^{o}C) releases different sugar residues present in pectic polysaccharides at very different rates, with galactose and particularly arabinose linkages being most labile and galacturonic acid being the most resistant. At lower temperatures, the hydrolysis of the methoxyl, acetyl groups and neutral side chains is predominant. Elevated temperatures accelerate degradation rates, and cleavage of the glycosidic bonds of the galacturonan backbone progressively increases. The selective degradation of pectins by acid hydrolysis is an important method for revealing the structure of pectin. Round et al. (2010) used a mild acid hydrolysis and atomic force microscopy observed that homogalacturonan exist in the pectin as a side chain with a minimum mean size some three times larger than that previously reported (Thibault et al., 1993; Yapo et al., 2007). If homogalacturonan as a side chain of rhamnogalacturonan I is still a hot debate in pectin area.

Pectin degraded through hydroxyl radical-mediated scission of the polymer was thought to predominate in post harvest fruit softening (Fry et al., 2002). Elboutachfaiti et al. (2008) reported depolymerization of commercial polygalacturonic acid by free radical with copper as a metallic catalyst. The free OH radical was generated by a non-enzymatic “Fenton reaction” between Cu^{+} and H_2O_2. This method was proved to be a good tool to generate a large amount of small oligogalacturonides quickly. Moreover, this chemical method appears as very efficient to cleave glycosidic linkages while keeping the basic structure without drawbacks (sample coloration,

monosaccharides and salts implying post-degradation purifications, etc.) that generally observed with other harsh processes (high temperature and pressure, etc.).

Physical (mechanical) degradation

Some physical treatments such as ultrasonication, high pressure treatment, radiation and photolysis can also induce the degradation of pectin, but the results of degradation varied according to physical methods used.

Seshadri et al. (2003) found that rheological properties of pectin could be negatively influenced by ultrasonication. As sonication time and intensity increased, more time was needed to form gel and the gel strength was reduced. On the other hand, the optical properties of pectin gels were improved. These results were ascribed to the ultrasonic processing reduced the average molecular weight of pectin due to cavitation (Leighton, 1995). Tiwari et al. (2010) also found a significant reduction in apparent viscosity and a trend towards Newtonian flow behavior for pectin solutions. These phenomenons were ascribed to cleavage of pectin due to cavitations, increase of temperature and OH radicals.

In the cases of high pressure treatment, hydrostatic and dynamic high pressure treatments have different effects. High hydrostatic pressure treatment did not cause degradation of the main chain (covalent bonds) of pectic substance (Kato et al., 1997). In addition, when combining high temperature with high pressure, β-elimination that predominates in high temperature was retarded or even stopped, whereas demethoxylation was stimulated (De Roeck et al., 2009). These results are very promising in the texture preservation of high-pressure sterilized fruits and vegetables, as β-elimination is accepted to be one of the main causes of thermal softening and

low methoxylated pectin can enhance tissue strength by forming cross-links with calcium ions present. However, in the case of dynamic high pressure homogenization, pectin was degraded. Corredig and Wicker (2001) reported that homogenization at 124 MPa had a significant effect on molecular weight distribution and average molecular weight of high methoxyl pectin. Dynamic high pressure microfluidization (DHPM), an emerging dynamic high pressure homogenization technology was also reported to induce a serious degradation of pectin (Chen et al., 2012). Apparent viscosity, average molecular weight and particle size of pectin decreased, whereas the amount of reducing sugars increased with increasing DHPM pressure. The author also proved that DHPM had no effect on the primary structure of pectin, and the degradation may derive from the rupture of glycosidic bond of pectin. In addition, neither β-elimination nor demethoxylation occurred with DHPM. More attention should be paid to the degradation of pectin, when food that contains pectin being treated at low pH, because low pH favored pectin degradation in this process.

For radiation, it was reported to be a useful tool for degradation of biological polymers and it is often viewed as being the last process after packaging to control pathogenic and spoilage organisms (Harish Prashanth and Tharanathan, 2007). It is well known that degradation of polysaccharides by ionizing radiation is due to the free radical induced scission of the glycosidic bonds (Makuuchi, 2010). Zegota (1999) reported that the radiation degradation of pectin was enhanced in the presence of dissolved oxygen, and discussed that pectin degradation based on the rearrangement of radicals may be localized at C(5)-carbon atom in the pyranose ring of galacturonic acid residue. Several authors reported that the irradiation of pectin in aqueous solutions caused a dramatic decrease of molecular weight and viscosity (Ayyad et al., 1990;

Sjöberg, 1987). However, Dogan et al. (2007) stated that the rheological characteristics of pectin were not considerably affected by gamma irradiation and pectin can be irradiated for preservation purposes. The differences between these studies may be due to the different pectins used, but more investigations about irradiation on pectin may be needed.

Recently, an environmentally friendly procedure, a photochemical reaction relying on a titanium oxide catalyst (TiO_2) has been applied to depolymerization of pectin (Burana-osot et al., 2010). In a period of 6 h at pH 7, the UV/TiO_2 photolytic process decreased the average molecular weight of pectin from 400 to 200 kDa. The advantage of this technology is that the intact structure of pectin did not change, and the molecular size of the depolymerized product could be controlled by the exposure time to the UV light and pH. Therefore, it is a promising technique that might be useful for preparation of pectin oligosaccharides as an ingredient in food and pharmaceutical products.

In addition, mechanical treatments such as grinding (Dongowski et al., 1997), extrusion (Hwang et al., 1998) and dehydration (Plat et al., 1991) can also lead to degradation of pectin, however, they are not frequently used.

Enzymatic degradation

Although various degradation products of pectin could be produced by a variety of methods, enzymatic methods are gaining importance because they allow regioselective depolymerization under mild conditions. Many detailed reviews have been dedicated to pectin degrading enzymes (Pedrolli et al., 2009; Tucker and Seymour, 2002; Yadav et al., 2009) and therefore, only general summary of pectic enzymes are briefly discussed here. Given the

complex structure of pectin, it is perhaps not surprising that there is a wide range of enzymes capable of degrading this polymer. Pectinesterase, polygalacturonase, pectate lyase, pectin acetylesterase, β-galactosidase and arabinosidase are the major enzymes that involved in the degradation of pectin. These enzymes fall generally into two groups. In the first group, there are enzymes responsible for the depolymerization of the HG backbone ("smooth region") of the pectin. These include pectate lyase, polygalacturonase, pectin acetyl esterase and PME. The second group of enzyme activities is towards the degradation of "hairy region" composed of RG and side chains. These include rhamnogalacturonase, rhamnogalacturonanan lyase, rhamnogalacturonanan rhamnohydrolase, and rhamnogalacturonanan galactohydrolase. These enzymes have rarely been studied and need extensive studies on their structures and functions. There are some other accessory enzymes involved in degradation of side chains of pectins such as β-galactosidases and α-arabinosidases.

The complexity of pectin sometimes hampers enzymatic degradation. As a consequence, a lot of substitutions and structural organizations require treatment with several enzymes simultaneously, and several pectin-degrading enzymes have been demonstrated to act synergistically. For example, Bonnin et al. (2002) used a rhamnogalacturonanase associated or not with pectin methylesterases and side chain degrading enzymes (galactanase and arabinanase) to degrade pectin from sugar beet, lime and apple, and found that when rhamnogalacturonase was used in combination with arabinanase and galactanase, the hydrolysis was more efficient than with PMEs from *Aspergillus aculeatus*.

When pectin structures are not degradable by the available enzymes, a combination of chemical and enzymatic approaches can be applied. For instance, in the structural

characterization of enzyme resistant highly branched RG I structures, partial side chain removal by chemical treatments enables subsequent enzymatic breakdown (Azadi et al., 1995; Mutter et al., 1998).

SAFETY AND REGULATORY STATUS OF PECTIN AND ITS DERIVATIVES

Commercial pectin is regulated as a food additive in most countries and is not considered a problem from a toxicological point of view. Both pectin and amidated pectin have been generally recognized as safe by the United States Food and Drug Administration (FDA, 2012), European Commission (EC, 2010), and Food and Agriculture Organization of the United Nations (FAO, 2012). However, the applications of many other pectin derivatives as food ingredients still are in their infancy, because new food additives that are intentionally added to food must be shown to be safe under their intended conditions of use.

Nowadays, regulatory authorities strictly control the approval of food additives. However, physical and enzymatic modification of food hydrocolloids is allowed (Seisun, 2009). Kinds of techniques such as homogenization, microwave, ultrasonication and pectinases treatment are frequently used in food processing. These techniques either have no effect on the basic structure of pectin or are used to reduce the size of pectin molecule as we discussed above. Therefore, pectin derivatives obtained by these modifications are widely accepted as being similar to unmodified pectins for use in foods. Physically modified pectin, sold under the name Slendid™ by CP Kelco Division of JM Huber, is an example (Seisun, 2009). In addition, pectin depolymerized by altering pH can also be ranked to be safe. For example, modified citrus pectin (MCP) is produced from citrus pectin via pH and temperature modification that breaks pectin

into shorter, non-branched, galactose-rich carbohydrate chains (Wai et al., 2010). This MCP has anti-metastatic properties (Maxwell et al., 2012), and the use of this product was approved by the U.S. Food and Drug Administration (FDA, 2012).

For derivatives from chemical modification through reactions such as methoxylation/de-methoxylation, acetylation/de-acetylation and amidation/de-amidation, they are also considered to be safe. Because the group $–COOCH_3$, $-COCH_3$, $-CONH_2$ can be naturally presented in pectin molecule, and these reactions only change the degree of substitution, while the basic structure of pectin was not changed. Amidated pectin, approved by the FDA, EC and FOA, is an example. However, it should be noted that some toxic agents such as *N*-methylimidazole and pyridine are sometimes used in the preparation of these derivatives. These agents may still exist in the products even though a dialysis is always included to be a necessary step of the preparation. The residual toxic agents may lead to unsafe of products, therefore, a evaluation of the presence of contaminants is necessary before the derivatives are used in food.

In the case of other chemical modifications that changed the basic structure of pectin, the resulting derivatives should be presumed to be unsafe for their intend uses in food unless and until they are proven “safe” on the basis of scientific data and information. Till now, the information about the safety of these pectin derivations is scarce, substantial research efforts will still be necessary to obtain substantial scientific evidence and to get an approval from the appropriate government bodies before the derivatives are introduced into food area.

CONCLUSIONS

Because of considerable interest in the use of pectin derivatives in many fields, an overview is given on modifications of pectin (including substitutions, chain elongations and depolymerizations), the corresponding characteristics and applications of modified pectin. Although there are extensive works have been carried out on modification of pectin, there are still many other potential pathways that have yet to be investigated. The further development in the modification technology will certainly result in more research on modifications and on the use of modified pectin.

Currently, pectin is mostly modified in order to obtain some specific properties, however, the modified pectin may have many other functions, which need to be further exploited. In addition, more efforts should be taken to promote the applications of pectin derivatives, leading them to be commercial, industries and many other fields available.

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Figure 1 Schematic representation of (A) the structure of pectin and (B) the structure of HG (Ridley et al., 2001).

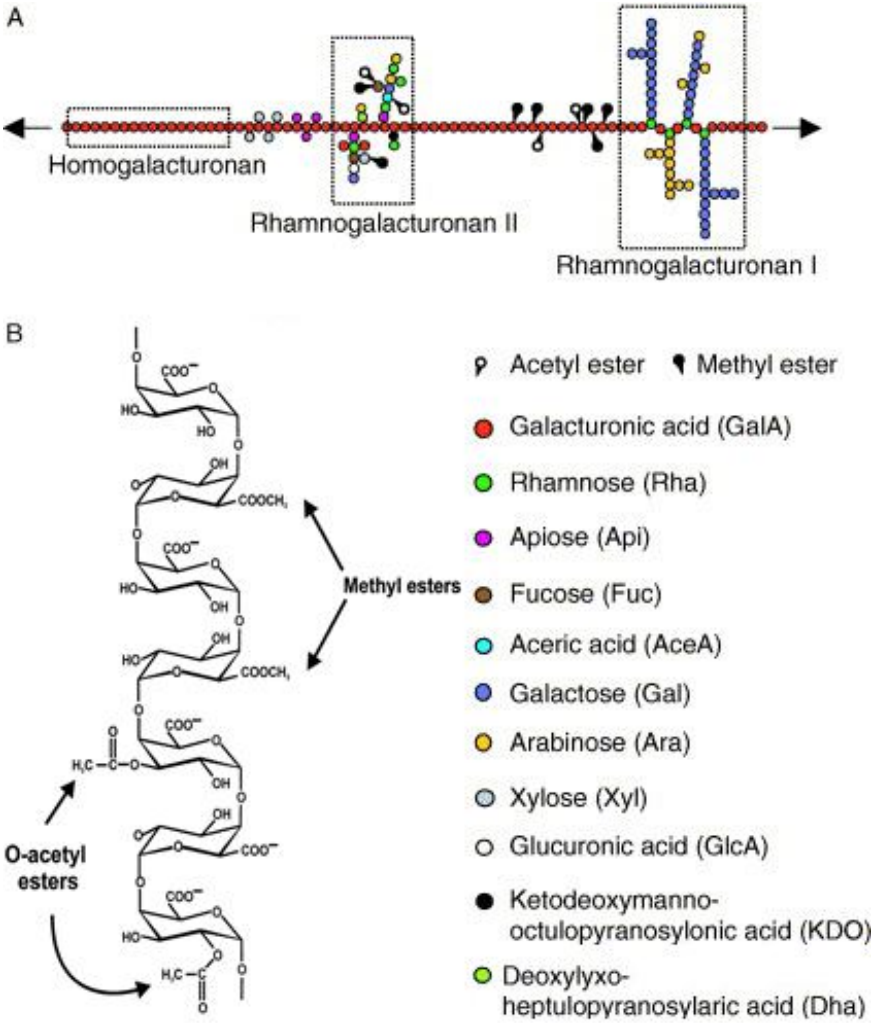
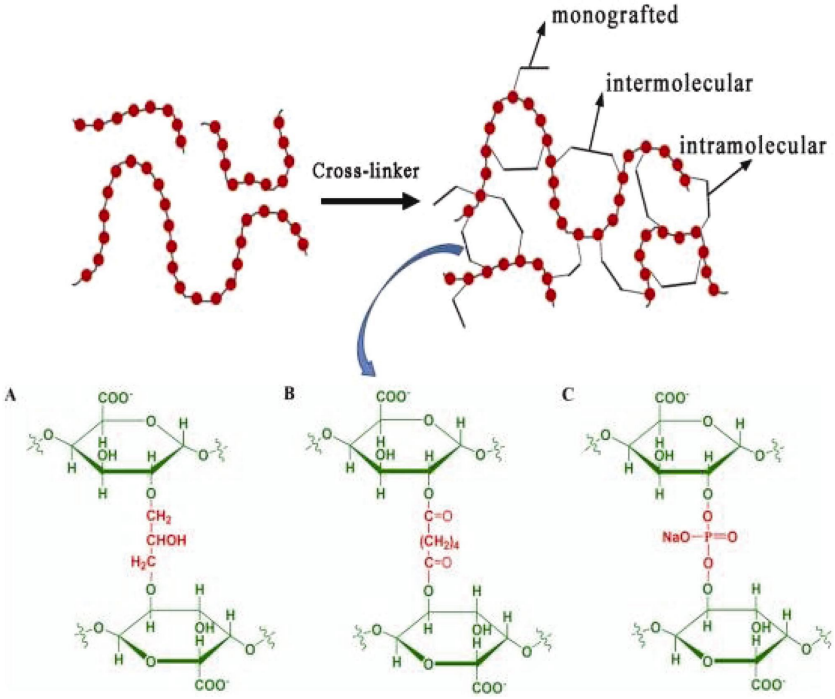
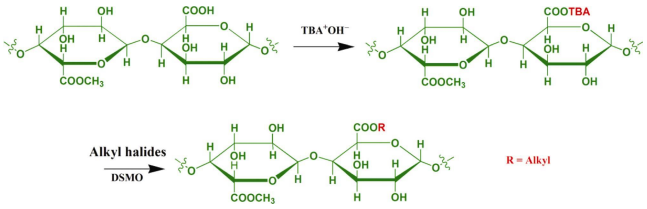


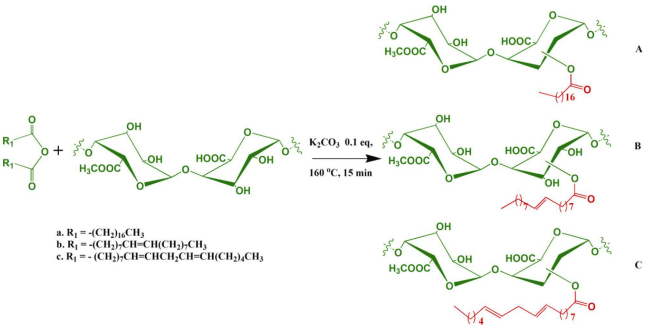
Figure 2 Schematic of the crosslinking of pectin by epichlorohydrin (A), adipic acid (B) and trisodium trimetaphosphate (C), showing three possibility of linking: intermolecular, intramolecular and monografted.



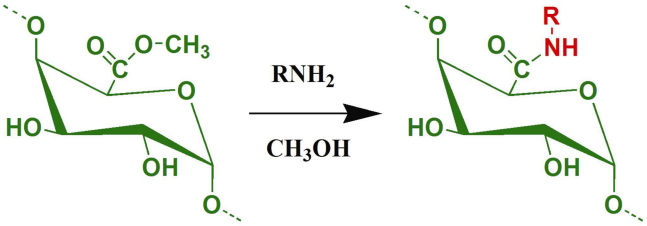
Scheme 1 Alkylation of pectin with alkyl halide.



Scheme 2 Esterification of pectin OH groups by using palmitic acid, oleic acid or linoleic acid (Monfregola, et al., 2011).

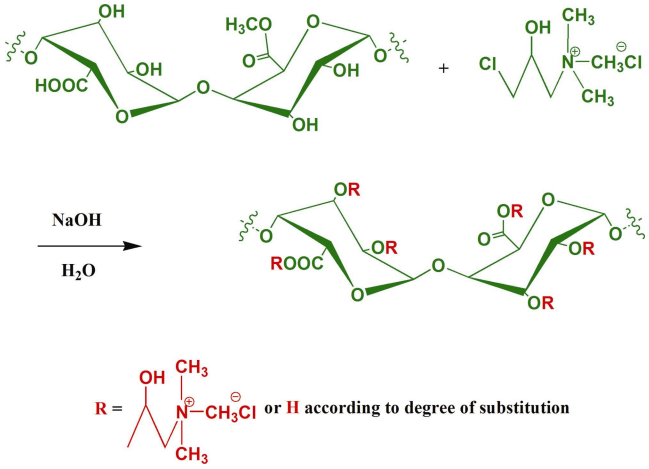


Scheme 3 Amidation of pectin.

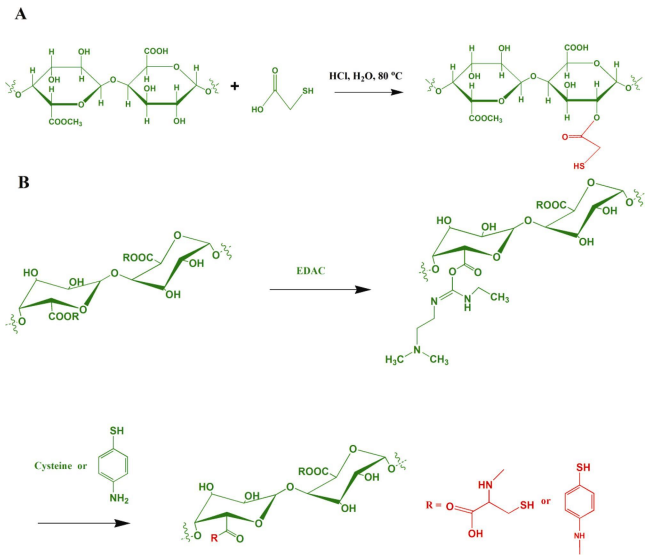


R = H, OH or Carbon radicals

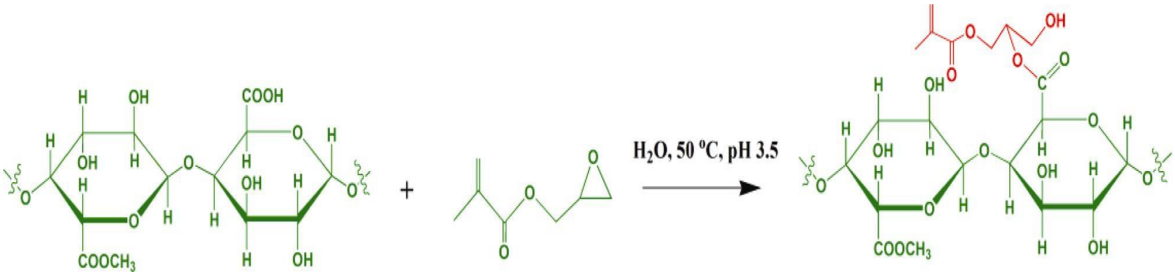
Scheme 4 Reaction scheme for quaternization of pectin with 3-chloro-2-hydroxypropyltrimethylammonium chloride (Fan et al., 2012a).



Scheme 5 Thiolated pectin prepared by formation of ester or amide bonds. (A) thiolated pectin was synthesized by esterification of pectin with thioglycolic acid in the presence of hydrochloric acid (Sharma & Ahuja, 2011). (B) thiolated pectin was synthesized by a covalent attachment with cysteine (Majzoob et al., 2006) or 4-aminothiophenole (Perera et al., 2010).



Scheme 6 Schematic representation of the reaction between pectin and glycidyl methacrylate (Maior et al., 2008).



Scheme 7 β-elimination reaction of pectin molecules.