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TRENDS IN THE SCIENCE AND APPLICATIONS OF PECTINS

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The literature on the chemistry and application of pectins is reviewed. The principal themes are the search for nontraditional sources of pectins with novel structural elements and the deeper understanding of the structure and physicochemical properties of well studied pectins from traditional sources (apple, beet, citrus). Custom preparation of pectins with a desired set of properties that have practical application in medicine, biology and the food industry has been demonstrated. Chemical modification of pectins is a promising trend.

Key words: pectins, structure, properties, preparation, application.

Pectins are biopolymers found in the primary cell walls of many living plant cells. They have been widely studied [1-3]. The composition and structure of pectins are still not completely understood although pectins were discovered over 200 years ago. The structure of pectins is very difficult to unravel because the pectin can change during isolation from plants, storage, and processing of plant material. In addition, impurities can accompany the main components.

At present pectin is thought to consist mainly of galacturonic acid (GalA) units. Rhamnose (Rha) is a minor component of the pectin backbone whereas other neutral sugars such as arabinose (Ara), galactose (Gal), and xylose (Xyl) occur in the side chains. A chain of several hundred α-(1→4)-bonded GalA units with a varying degree of esterification (DE) is a typical fragment. Pectin is known to be capable of forming aqueous gels. This property is widely used in the food industry [4, 5].

The magnitude of DE has the greatest influence of many pectin characteristics on its gelation properties. Pectins are divided into two groups according to their gelation properties. These are pectins with a high DE, which can form gels, and those with a low DE, which exhibit a reduced tendency to form gels. Therefore, the estimated DE of various pectins has significant commercial value because it can be correlated with the gelation properties of a particular pectin and its gels and with its suitability for use in the food industry.

In continuation of previous research [6], we have searched, collected, and preliminarily analyzed the scientific, industrial, and patent literature on the structure, chemistry, physical chemistry, and processing of pectin.

The principal topics in this area are:

-structural studies of pectins from traditional sources (citrus, apple, beet): determination of the monosaccharide composition, the sequence of monosaccharide linkage, the structures of the main backbone and the side chain, and the sites of attachment of the side chains and individual fragments;

-structural studies of pectins from nontraditional sources;

-studies of the conformational and molecular-weight characteristics of pectic substances;

-studies of the DE of pectins and the gelation properties and gel-forming processes correlated with it;

-the industrial production of pectin from traditional raw materials (for yield optimization and property modification);

-the search for new plant sources of pectin;

-the chemical modification of pectins;

-applications of pectins (food industry, medicine).

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STRUCTURAL STUDIES OF PECTINS

The composition and structure of pectic polysaccharides is a recurring theme of detailed studies using a broad range of modern research methods. Chemical hydrolysis and enzymes easily decompose pectic polysaccharides into their individual fragments. The latter method is becoming an analytical tool for studying pectin structures owing to the highly specific action of enzymes.

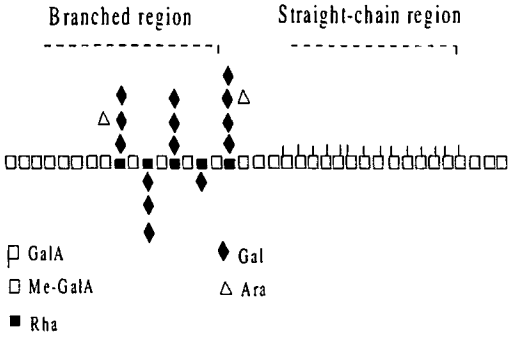
A general model has been proposed for apple, citrus, and beet pectins, which typically consist of alternating linear (smooth) (1→4)-GalA chains and branched (hairy) regions, which contain most of the neutral sugars. The principal pectins from these sources differ in molecular weight (citrus > apple > beet) and Rha content (beet > apple > citrus). Homogalacturonans with DE 72-120, 91-108, and 114-138 have been isolated from apple, beet, and citrus pectins, respectively.

Common pectin from apple pomace, citrus rind, and sugar beets contains a straight-chain homogalacturonic region and a branched region. The latter is rich in neutral sugars, mainly Ara, Gal, and Rha. The sensitivities (lability) to acid hydrolysis of the glycoside bonds vary in pectins: GalA—GalA > GalA—Rha > Rha—GalA > neutral sugar—neutral sugar. This enabled the homogalacturonic region to be isolated and its minimal length to be estimated [7].

Homogalacturonan is constructed from more than 100 successive α-(1→4)-bonded GalA units [7] in which the carboxyl group is esterified to varying extents (up to 70%). In addition, the secondary hydroxyl of GalA is acetylated in pectin from certain plant species (apple) [8].

However, recent studies found that Rha units are present in the homogalacturonan linear region. Blocks of approximately 25 GalA units are separated by a single Rha unit. It was proposed that namely these units lead to "loops" in the molecule [9]. Other researchers demonstrated that the linear homogalacturonic region contains one Rha unit per 200 GalA molecules [7].

The general structure of pectin is shown below:



One of the most significant recent investigations of pectin structure is a report on the homogalacturonan portion of citrus pectin. At least 40 GalA units per single Rha molecule were found. The homogalacturonan contains ca. 15% of the total Rha. The remaining 85% of the Rha occurs in the branched portion [10].

The structure of the branched portion has been very thoroughly studied. The Rha occurs in the main chain whereas Ara and Gal are found in the side chains and are mainly C-4-bonded through Rha to the pectin backbone. Rhamnogalacturonan from traditional sources has a nonuniform molecular weight and side-chain composition of neutral sugars. The backbone of this portion contains 4-bonded α-D-GalA and α-L-Rha bonded to it through the 2-position in variable sequences. However, the size of the oligomers forming these sequences is less than the postulated length of the branched portion. The study of these deviations in the branching sequence is important for commercial pectins because the branching structure and the DE control the functional properties, especially gel formation, of these macromolecules.

Acid hydrolysis of rhamnogalacturonan produces more than two fractions. The neutral side chain is rapidly hydrolyzed to form low-molecular-weight oligomers whereas the straight-chain portion gives a fraction of intermediate molecular weight that is rich in GalA and Rha.

Acid-hydrolysis products are studied and identified by dialysis, ion-exchange chromatography (IEC), and NMR spectroscopy of the sugars in order to compare the structures and characteristics of the rhamnogalacturonan fraction.

The rhamnogalacturonan fraction consists of a series of linear homologs that are oligomers with the well defined sequence [4)-α-D-GalA-(1→2)-α-4Rha-(1→] and Rha at the reducing terminus. On the other hand, oligomers prepared using rhamnogalacturonase [11] have GalA at the reducing terminus and Rha partially replaced by Gal. The degree of polymerization

of the oligomer is 20 (10 repeating GalA—Rha moieties). In apple pectin, 78% of the Rha occurs in such fragments. Later this fragment was found to be common to all pectins. However, the length of this fragment is still under question. The length might be overestimated owing to systematic uncertainties related to the use of globular proteins and dextrans as standards. The population distribution of branched fragments in various plant (apple) pectins from cell walls, their stability toward enzyme-induced decomposition, and the presence and distribution of subunits internal and external to the branched portion have been studied in detail [12].

Primary cell walls of plants have yielded not only the polysaccharides described above, i.e., monogalacturonan and rhamnogalacturonan I (RG-I), but also rhamnogalacturonan II (RG-II) [13, 14]. Plant cell walls contain 1-8 mass % of rhamnogalacturonan II. It is the principal soluble polysaccharide of plant cells and is stable toward certain enzymes, in contrast with homogalacturonan and rhamnogalacturonan I.

RG-II was first isolated from sycamore (*Acer pseudoplatanus*) cell walls using fungal endogalacturonase [15]. The presence of RG-II in cell walls of Douglas-fir (*Pseudotsuga menziessi*) [2], rice (*Oryza sative*) [3], garlic (*Allium*) [16], kiwi fruit (*Actinidia deliciosa*) [17], radish [18], roots of *Bupleurum falcatum* [19], leaves of *Arabidopsis thaliana* [20] and *Panax ginseng* [21], pulp of sugar beets [22], red wine [23], apple (*Malus domestica*), tomato (*Solanum licopersicum*), and carrot (*Daucus carota*) [24] juices, and other sources has been reported.

Several groups of researchers have studied RG-II. The RG-II isolated from sycamore cell walls contains 11 different glycosyl units, including such rarely encountered ones as apiose, 3-C-carboxy-5-deoxy-L-xylose (aceric acid), 3-deoxy-D-manno-octulosonic acid (KDO), 3-deoxy-D-lixo-heptulosaric acid (DHA), and the methylated sugars 2-O-methyl-L-fucose and 2-O-methyl-D-xylose. Most of the glycosidic bonds and rings in RG-II are also unusual with the exceptions of β-D-GalA, β-L-arabinofuranose, α-L-arabinopyranose, and the fully substituted Rha. Some of the glycosidic portions of RG-II are O-acetylated.

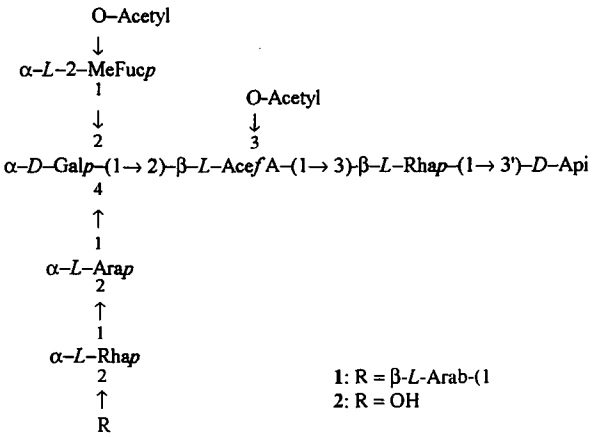
The RG-II backbone is a chain of at least seven 1,4-bonded α-D-galacturonosyl moieties with four oligosaccharides in the side chains [25]. It was recently shown that RG-II exists in plant cell walls as a dimer, the fragments of which are cross-linked by a borate diol-diester located on one of the apioses [26, 27].

The structure of RG-II was investigated further using selective acid hydrolysis in combination with decomposition by endo- and exopolygalacturonases. The mono- and oligosaccharides were identified using HPLC, anion-exchange chromatography, and mass spectrometry.

Chemical fragmentation enabled four oligoglycoside side chains, in particular, the acid-hydrolysis product heptasaccharide **2**, to be isolated and structurally characterized [28]. Later, additional refined structural information was obtained for the fragments of RG-II. Selective acid hydrolysis of RG-II (trifluoroacetic acid) produced at least seven other oligosaccharides that are structurally related to **2**. The full structure of one of these, octasaccharide **1**, and partial structures of the six remaining oligosaccharides were determined. Furthermore, the locations of the O-acetyl groups on the chains of **2** and **1** were proved. It was found that the main backbone of RG-II consists of more than 11 1,4-bonded α-D-GalA units [13, 29].

Further research on fruit-juice pectins that were treated with enzymes during preparation confirmed the validity of the proposed structural model relative to the presence of the same monosaccharide units, their quantitive ratio, and the bonding sequence. The sugar composition was studied by size exclusion chromatography (SEC) on Sephacryl S-200 [24].

The structures of **1** and **2** are illustrated below:



The structure of the modified branched region from apple cell walls was studied after saponification, deacetylation with acetylesterase, and use of SEC. A fraction enriched in Xyl and GalA where the Xyl is β-(1→3)-bonded to GalA was observed, isolated, and characterized. The degree of methylation in this fraction was calculated to be of the order of 39. It was demonstrated that the methyl esters are distributed evenly among the substituted and unsubstituted galacturonic units [30].

The studied nontraditional sources of pectin include seeds of *Nicandra physalodes* [31], roots of *Angelica acutiloba* [31] and *Bupleurum falcatum* [31, 32], leaves of *Panax ginseng* [34], and others, many of which are widely used in traditional Eastern folk medicine.

The pectins of *Angelica acutiloba* and *Bupleurum falcatum* [31-33] contain such rarely encountered sugars as 2-methylfucose, 2-methylxylose, apiose, and aceric acid. Pectin from these sources has a high content of α-D-(1→4)-galacturonan and a low content of fragments similar to RG-II. The structure of the part of RG-I that is stable toward endogalacturonase has been determined. The following structure was proposed:

→ 2)-Rha-(1 → 4)-GalA-(1 → ...4)-[→ 2)-Rha-(1]→ 4-GalA-(1 → ... and .. 4)→ GalA-(1 → 2)-[→ 4)-Rha-(1]→....

The structural features of pectin polysaccharides isolated from ginseng, which have been studied in detail, include a longer chain of neutral sugars and a different type of substitution of individual sugar fragments than have been found in previously studied polysaccharides [34].

The pectin-like arabinogalactans of red wine have a uronide component that contains an elevated content of glucuronic acid (3-7%) [35]. Studies of pectin from tomato cell walls by atomic microscopy confirm that pectin structure is difficult to study [36]. The pectin molecule contains an unusual and previously unknown branching structure that differs from that found previously using enzymatic cleavage of the neutral side chains and analysis of the sugar residues. The branches had lengths from 30 to 170 nm and were relatively linear. An attempt was made to interpret the nature and sequence of formation of these long branched side chains.

MOLECULAR WEIGHT AND CONFORMATION OF PECTINS

The study of pectins as natural high-molecular-weight polymers composes a significant portion of structural studies in which traditional chemical methods of studying high-molecular-weight compounds have been used.

The molecular weight, dispersity, and stability of pectin solutions are known to depend significantly on the nature and type of extractant used to isolate it and the extent of thermal treatment used to prepare it [37, 38]. The viscosity of dilute pectin solutions is one of the most important characteristics that enables the molecular weight and physicochemical and conformational properties of pectin polysaccharides to be estimated. The viscosity and density of dilute pectin solutions have been studied [39-41].

The viscosity characteristics [h] of cotton, beet, apple, and citrus pectins have been determined by viscosimetry to be 0.6-1.29 [42]. The molecular weights of several pectins have been determined using these values: beet, 14,000; cotton, 15,500; apple, 37,000; grape, 21,000; citrus, 25,800.

The effect of temperature on the viscosity of aqueous solutions of pectic acid and several of its derivatives has been studied. The activation parameters of viscous flow have been obtained taking into account polymer—solvent interaction [43].

The supramolecular structure of solutions of various pectin samples was established by studying the turbidity spectrum, which provides information about conformational differences of biopolymers and the solubility and presence of a gel fraction. The content of the latter is especially significant in pectin extracts from cotton bolls [42]. The molecular homogeneity of pectins from various plant sources was determined and its population distribution was estimated by ultracentrifugation. Samples of cotton and apple pectins exhibit a narrow range of molecular weights and lack of low-molecular-weight fractions [42].

The molecular weights and conformations of citrus pectin with DE 64.5% and [η] 1.849 dl/g [44] were completely analyzed using results from sedimentation studies. Molecular weights of various degrees of averaging and the distribution of molecular weights were calculated: $M_z = 2.25\times10^5$, $M_w = 1.47\times10^5$, and $M_z/M_w = 1.53$. A quantitative estimate of the flexibility of citrus pectin macromolecules yielded the Kuhn segment length ($A = 120 \pm 4$ Å) and the hydrodynamic chain parameter ($d = 11.0 \pm 2$ Å) [44]. The optical density (OD) of a series of citrus pectins was studied. The ODs depend linearly on the –COOH concentration in the biopolymer [45].

The tendency to associate in dilute solution of highly methoxylated commercial citrus pectin in phosphate buffer was studied using light scattering. Model calculations confirmed that a bimodal system consisting of the molecularly disperse main component and small quantities of particles of higher molecular weight provides an adequate description of pectin samples [46-48].

Pectin derivatives, in particular, metal derivatives, continue to be studied in order to resolve several structural issues. Although the primary structure of pectin is well known, the secondary, tertiary and quaternary structures in gels or solutions are still poorly defined.

Pectins cross-linked by Ca ions are important for the organization of polysaccharides in plant cell walls. The structure of the aggregated chains is described by the "egg—carton" model [49]. In this model the joined segments are situated in identical chains and the fusion zones are formed by chains of galacturonan in the 2_1-spiral conformation (two monosaccharides per spiral turn). The Ca^{2+} ions are situated in the space between neighboring spiral polysaccharide chains, similar to an egg in a carton, and form complexes with O atoms [50]. Thus, the conformation of cross-linked parts of the chain in gels differs from that of the free pectin chains in solution [51].

The hydrated gel has the 2_1-spiral structure according to ^{13}C NMR. This is in contrast to the 3_1-spiral structure of dry Ca-polygalacturonan [50, 51]. Transitions from the 2_1-spiral dimer to the 3_1-spiral oligomer through intermediate spiral aggregate forms can be seen in concentrated samples of Ca^{2+}-pectin [52]. Other divalent cations also form gels. However, the conformation of the chains in these gels has not been conclusively defined.

Methyl esters are known to have a great influence on ion binding and the manifestation of gel-forming properties. The esters carry a negative charge and interfere sterically with the aggregation of chains. The degree to which this effect is manifested and the geometry of the bound fragments depend mainly on the nature (size) of the cation and the type of pectin [53].

The chain conformation in the cross-linking zone has been studied using various methods such as circular dichroism, small-angle scattering, mass spectrometry, and Fourier-IR spectroscopy [52]. Results from the last method confirmed that the metal coordination of divalent metals to the pectin chain using K pectates and pectinates as an example agrees with the egg—carton model. The Fourier-IR data are consistent with a weak interaction of Mg^{2+} with K pectate even in the instance where a gel is not formed. The ions Ca^{2+} and Sr^{2+} form strong complexes with pectate and low-molecular-weight pectinate; the ions Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Pb^{2+} with pectinate with DE 59.1%; and Pb^{2+} and Cu^{2+} even with highly esterified pectins [54].

Studies of aqueous solutions of sodium pectate by NMR confirmed that the proposed model of the secondary pectin structure agrees with data obtained from hydrodynamic theory and molecular modeling. The optimal model seems to be an anisotropic reorientation of a doubly twisted spiral segment containing 29 monosaccharides. The average axial length of the spiral segment is 13 nm. This is in excellent agreement with values obtained by small-angle scattering and molecular mechanics. The cross-sectional hydrodynamic radius of the spiral segment is 0.8 nm whereas the covalent radius is 0.4-0.45 nm. The increased hydrodynamic radius relative to the covalent radius is consistent with counter-ion condensation according to the polyelectrolyte theory [55].

DEGREE OF ESTERIFICATION

As mentioned above, a thorough understanding of the DE is one of the most important aspects of structural research of pectins owing to the excellent correlation of the DE, the molecular conformation, and the gel-forming properties of pectins, which define the suitability for use in the food industry.

Classical titration is currently most commonly used to determine the DE of pectins [56-58]. An alternate method is the loss of methanol through cleavage of the ester by enzymes or treatment with base or acids with subsequent determination of the methanol produced by chromatography [59].

A SEC method with refraction and conductivity detectors for determining the DE of pectins was recently described [60, 61]. The quantity of carboxylates was determined as mmol of pectin carboxylates per gram of pectin and was standardized using a known compound. However, the method is limited by the need for preliminary determination of the anhydrogalacturonic acid content in the sample.

A method was proposed for determining the intramolecular distribution of the DE that includes analysis of the pectin by gradient IEC using a buffer with increasing ionic strength and concentration. However, the pectin fraction that is eluted has

a nonuniform DE. Thus, the DE values are obtained by averaging and are only approximate [62].

The possibility of using capillary electrophoresis to determine the DE was studied [63, 64] because pectins, in contrast with other polysaccharides, are charged and have UV-active chromophores. This makes it possible to study their solutions directly. A method for determining the DE was developed for aqueous pectin solutions with concentrations 0.5-5 mg/ml using phosphate buffer (pH 7.0) and UV detection (192 nm). This rapid method of quantitative analysis takes advantage of the linear dependence of the electrophoretic mobility on the average charge of the monosaccharide, which is calculated as $z = -(100 - DE)/100$. The electrophoretic mobility was not observed to depend on the intramolecular charge distribution for a fixed charge.

A method for determining the DE of pectin using capillary electrophoresis was tested on several samples that differed in structure and neutral-sugar content [65]. The standard was citrus pectin with DE 31.1-75.8%. The intramolecular DE distribution could be determined directly from the peak shapes. Regardless of the type of studied pectin (citrus, apple, beet), the DE, or the neutral-sugar content, the results agreed well with those from titration, IEC, and SEC. An advantage of the method over chromatography is the simplicity and quickness. Determination of the DE by capillary electrophoresis takes less than 2 h including sample preparation and calibration whereas IEC and SEC require at least two days.

PECTIN PRODUCTION

Methods for producing and purifying pectins are of great interest. Despite the development of modern membrane technology and a clear tendency of the largest pectin producers to prepare highly purified concentrates using membrane techniques [6, 37, 38], several traditional methods of isolating and purifying pectins continue to be studied, improved, and patented. This is apparently explained by the limited availability of membrane technology and possibly by the lack of demand for practical applications of such highly purified pectins.

Mitsubishi Rayon Co. (Japan) continues to study the extraction of pectin from apple pomace by orthophosphoric acid solutions with subsequent heating of the mixture and separation of the pectin extract. The pectin is purified using cation- and anion-exchange resins after removal of solids from the extract [66].

Depending on the desired properties of the pectin produced, the plant material is treated preliminarily with ≥ 0.1 N acid in a quantity less than the mass of plant material. This process partially demethoxylates the pectin, which is extracted using an acid solution of the same concentration [67].

The following purification methods are used depending on the pectin composition of the extract from various sources:

-purification of pectin from an aqueous extract by precipitation with 0.05-1.0 N acid or an acidic mixture of organic solvent and water [68];

-use of solids such as perlite, cellulose, and other sorbents to facilitate clarification and filtration of the pectin extract with adsorption of small extracted particles. These substances are added to the pectin extract at 100 g per 30-200 g of extract. The liquid part is clarified as usual and filtered again [69];

-treatment of the pectin source with alkaline-earth metal compounds under conditions favoring a chemical reaction. The alkaline-earth cation in the pectate is replaced by H using ion exchange after the pectate is separated into fractions of differing pectin content. Removal of water from the fraction produces a dry product that is stable to storage [70];

-the ability to form pectates through reaction with other metal ions (Al^{3+}, Cu^{2+}, $Fe^{2+(3+)}$) is used to improve the purification of pectin. The precipitates of Al, Cu, or Fe pectates, which are prepared by treatment of the pectin extract with solutions of these metal salts, are washed with dilute mixtures of sulfuric-acid and organic solvents without dissolution of the precipitates. The purified and treated pectin precipitate is separated. An additional quantity of Ca and Ba pectates can be obtained from the acidic wash solution by treatment with Ca or Ba hydroxide. The pectin is isolated by acids and organic solvents [71].

The extraction of pectins from rapeseed has been studied after basicification, defatting, and treating with 80% ethanol in order to determine the effect on this process of the extractant nature, the pH of the extracting solution, and its ionic strength. The extractants were cyclohexane-*trans*-1,2-diamine-N,N,N′,N′-tetraacetate (CDTA) and acetate or phosphate buffers.

The extract was further treated with sodium carbonate solution. The greatest pectin yield was obtained at pH 6.5 with CDTA. However, the pectin isolated under these conditions had a large uronic acid content. The main chain was not very highly branched [72].

The extraction of pectin from apricot peel by acid hydrolysis was studied. The effects of temperature, time, pH, and

required volume of liquid for extraction were investigated. It was found that only temperature affects statistically significant process parameters [73].

Pectins were produced from citrus waste at the Institute of Polymer Chemistry and Physics of the Academy of Science of the Republic of Uzbekistan. Hydrolysis and extraction of material with dilute HCl and separation of the extract by filtration, neutralization, and precipitation of pectin with ethanol were used. The hydrolysis and extraction are performed using 0.05-0.1% HCl with heating to 81-87°C in order to obtain highly esterified pectin and to increase its yield and quality. Some of the acid (0.42-0.5 of the total amount) is added at the start. The mixture is left to stand for 50-80 min. Then, 0.2-0.3 of the total amount of HCl is added. The mixture is left to stand for 35-40 min [74]. Pectin with bactericidal activity is produced by treating citrus wastes with steam. The hydrolysis and extraction are carried out at pH 1.0-1.5 and 92-96°C for 2.5-3.0 h at a ratio 1:10-1:12 [75]. Higher quality pectin with a reduced allergenicity can be produced by first distilling the essential oils from citrus wastes at 110-120°C and performing the hydrolysis and extraction for 1.5-2.0 h [76].

The chemical and physical properties of pectin obtained from *Satsuva mandarine* by acid extraction under pressure using ammonium oxalate solution as extractant have been studied. This produced pectin with the lowest molecular weight and a low content of GalA and methoxyls [77].

This same extractant was used to extract pectin polysaccharides from *Lemna minor*. Four polysaccharides, PS-1, PS-2, PS-3, and PS-4 with molecular weights 50,200-75,400, 18,800-99,700, 24,200-50,000, and 6,170-16,300, respectively, were isolated. It was found that PS-2 contains GalA, apiose, Xyl, Ara, and Rha whereas PS-4 contains GalA, apiose, and Xyl. Small quantities of fructose, mannose, and glucose were found in PS-2; Rha, fructose, Ara, mannose, and glucose, in PS-4. Pectins like PS-4 with such a high content of GalA (at least 96%) and a low DE were not previously observed in plant sources [78].

The extraction of pectins from ground apple, citrus, and apricot pulp after preliminary treatment with microwaves (2450 MHz, 0.5 kW) was studied. Such treatment gave a high yield of pectin. The isolated pectin has a high DE and a stable gel compared with controls. The effect of radiative heating depends on the type of starting material. The yield and quality of pectin depend on the degree of the plant tissue disintegration and the hydrolysis of the protopectin in addition to the rate of inactivation of pectolytic enzymes in the starting material. These results were confirmed by performing the extraction using model compounds. The results provide a basis for improving the quality of pectin produced using these technologies. Starting material prepared for future use can be irradiated before drying [79].

High-quality pectin and pigments from white grape skins were isolated in high yield using a typical sequence of operations, i.e., extraction with heating, concentration, filtration, and precipitation with alcohol. Treatment with acetone bleached the pectin and removed the pigment [80].

Gel-formation of highly methoxylated pectin that is induced by high pressure has been studied. The gel structure and strength of the intermolecular forces were studied by DSC. It was shown that pressure and temperature act synergically on gel formation of highly methoxylated pectin. The most stable and strongest gel is formed at 3.6-3.9 atm, 1.5% pectin concentration, and 43-45°C [81].

BIOLOGICAL ACTIVITY OF PECTINS

Pectins are widely distributed in nature and are important to the physiological vitality of plants. They are important to structure formation and ion and water exchange. These compounds also exhibit various biological activities and favorably effect the metabolism of man and animals because they are required nutrients.

Experimental and clinical studies of the pharmacologic and medicinal effects of pectins have recently increased greatly. Their ability to eliminate toxic substances, in particular, to bind polyvalent metal cations, has been widely studied. Highly esterified pectins, e.g., those from citrus, can be used as prophylactics for people working in highly contaminated areas and under ecological stress. Pectins are fibrous foodstuffs and enhance the throughput of the gastrointestinal tract. They change the absorption of nutrients and additives. They normalize the exchange of substances, lower the cholesterol level in blood, increase its metabolism in the liver, and inhibit peroxide oxidation of lipids [82-85]. Animal experiments showed that citrus pectin reduces glucose in the blood and plasma [86]. Pectins unquestionably are effective for curing diarrhea in infants [87].

The anticancer and/or antimetastatic activity exhibited by, for example, apple pectin, in experimental models of colon carcinogenesis and liver metastasis are very important properties [88]. The use of methoxylated apple pectin for treatment of colon cancer has been patented in Japan [89].

Fragmentation products of citrus pectin (0.1% solution) inhibited metastasis of primary tumors in animal experiments. Alternate treatment with NaOH and HCl and final adjustment of the pH to 6.3 was used to fragment (in addition to demethylate) the pectin. The average molecular weight of the resulting fragments was 10 kDa. Metastases did not grow if this preparation was used, in contrast with the control [90].

The effect of citrus pectin and pH-adjusted citrus pectin on the properties of melanoma cells bound to galectin-3 was studied. Modified citrus pectin inhibits the adhesion of B16-F1 melanoma cells to laminin and their self association [91].

The use of pectin as a matrix or carrier for biologically active components or medicinal preparations is notable. Such pectins include the reaction products of citrus pectin with antihelmintic preparations [92, 93]. The immobilization of isoniazid on pectins has been studied. It was shown that the product is more highly tuberculostatic than pure isoniazid [94]. The use of chemically modified pectins as carriers for medicinal preparations for colon treatment is also promising [95]. Cross-linked pectin, which is less soluble and less susceptible to degradation in the organism, is effective for these conditions. It has been recommended for targeted delivery of medicinal substances into the gastrointestinal tract [96]. A Ca-pectinate gel has also been proposed for this purpose [97].

Thus, preliminary analysis of available recent information on the chemistry, technology, properties, and applications of pectins suggests that research in this area remains critical. The broad capabilities of highly informative chromatographic methods (SEC, HPLC, anion-exchange chromatography), NMR, ^{13}C NMR, mass spectrometry, atomic microscopy, capillary electrophoresis, and others enabled new and previously inaccessible structural details of pectins and their derivatives to be revealed. This suggests that pectin researchers, as before, are concentrated on expanding the understanding of pectin structure and its physicochemical properties.

The starting material plays a decisive role in increasing the throughput of processes, optimizing the yield, and increasing the purity of pectins produced by the new methods. Pectins with a given set of properties that depend on the range of molecular weights, DE, and purity are prepared using complicated techniques and nontraditional extraction, methods of purification and precipitation, and mechanicochemical treatment.

The scope of research on the physiological activity of pectins and their derivatives and novel aspects of their useful biological activity has expanded.

Apparently one of the promising areas of research, in addition to the quest for pectins in nontraditional sources with new structural elements (composition, conformations), is the chemical modification of relatively well studied and available pectins from traditional sources. This modification is effected by changing the active functional groups, introducing complexed metal ions, cross-linking, etc., and by synthesizing pectin polymer—polymer systems with other polysaccharides, proteins, etc. The advantage of such approaches is the ability to enhance and vary widely the medicinal, gel-forming, and other properties of pectins.

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