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Extraction and Purification of Pectin from Agro-Industrial Wastes

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Abstract

With the advent of science and technology, agro-industrial wastes are converted into various value-added products to meet the demands of increasing population. In recent years, natural polymers have evoked tremendous interest due to easy conversion into value-added products. Apart from various natural polymers, pectin occupied a prominent place due to diverse pharmaceutical and therapeutic applications. Excess utilisation of pectin, the gap between production and demand is widening. To fulfil this gap various techniques are adopted for obtaining high yield pectin from various agro-industrial wastes. This chapter will be focusing on extraction and purification of pectin from various agro-industrial wastes, considered as main environmental pollutants.

Keywords: agro-industrial, waste, pectin, pharmaceutical, therapeutic

1. Introduction

Pectins are complex branched polysaccharides present in the primary cell wall of plants [1]. It is a highly valued food ingredient commonly used as a gelling agent and stabilizer [2]. It is usually extracted by chemical or enzymatic methods from fruits [3]. Pectin is considered as the most complex macromolecule in nature, since it can be composed of up to 17 different monosaccharides containing more than 20 different linkages [4].

Pectins are enriched with repeated units of methyl ester galacturonic acid [4]. They form chemically stable and physically strong skeletal tissues of plants when combined with proteins and other polysaccharides [5]. They are usually produced in the initial stages of primary cell wall growth and make one third of the cell wall in both monocots and dicots [6]. Pectin is significantly reduced or absent in non-extendable secondary cell walls and is the only major class of plant polysaccharide largely limited to primary cell walls [7]. Pectin imparts strength and flexibility to the cell wall, apart from number of fundamental biological functions such as signalling, cell proliferation, differentiation, cell adhesion and maintaining turgor pressure of cell [8]. Pectins are involved in regulating mobility of water and plant fluids through the rapidly growing parts [6]. It also influences the texture of fruit and vegetables [9]. Apple pomace

and orange peel are the two major sources of commercial pectin due to the poor gelling behaviour of pectin from other sources [6].

Pectin is one of the most important polysaccharides due to its increasing demand in the global market, reaching a total production capacity of around 45–50 Million tonnes per annum. While the demand in 2011 was approximately 140–160 Million tonnes per annum, earning the interest of industry in this complex polysaccharide processing [10]. Pectins have received considerable attention as a high fibre diet that benefits health by reducing cholesterol and, serum glucose levels and acting as anticancer agents [11]. Pectins have shown promising results as drug carriers for oral drug delivery and are widely used for various bio-medical applications [5]. In addition, pectin has been described as an emerging prebiotic with the ability to modulate colon microbiota [12]. Considering above properties and applications, pectin has gained immense priority in the global biopolymer market with great potential and opportunities for future developments.

2. Structure and properties of pectin

One of the most abundant macromolecules present in the primary cell wall of the plants is pectin; their presence is detected in the matrix as well as in the middle lamellae [7]. Pectin is highly rich with galacturonic acid (GalA), that forms the backbone of three more domains found along with pectin that are homogalacturonan (HGA), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) [13]. About 70% of pectin is mainly composed of galacturonic acid (GA) [14]. Pectin is made of three polysaccharides that are covalently linked together, thus forming pectin networks in the cell wall matrix and the middle lamellae [15, 16].

Homogalacturonan (HG) takes up about 60–65% of the total pectin [3, 17], with a backbone of alpha-1,4-linked GalA residues, these GalA residues are methyl esterified which has an important role in the physical properties of pectin [4]. The presence of HG is seen to be present in approximately 100 GalA residues, but there are cases when its detected interspersed within other pectin polysaccharide [14]. On the other hand rhamnogalacturonan-I (RG-I) backbone which contributes 20–35% of pectin is composed of repeated and alternating groups of L-rhamnosyl and D-galacturonosyl residues [18]. There can be as many repeats as 300 of this disaccharide in case of sycamore cells, which are cultured in suspension [3, 16, 19]. The rhamnosyl residues have side chains of sugars which are mainly consisting of either galactosyl or arabinosyl residues [5]. The GalA residue of RGI unlike HGA are mostly not methyl esterified [20].

Rhamnogalacturonan-II (RG-II) is one of the highly conserved and complex structure which consist of distinct regions within HG, which makes up about 10% of the pectin [3], they have side chains of four different types with a particular sugar residue like aceric acid, apiose-3-deoxy-lyxo-2-heptulosaric acid, and 3-deoxy-manno-2-octulosonic acid. The HG residues along with nine of the GalA residues are attached to these side chains [3, 5]. There are other substituted HG residues that make up pectin such as xylogalacturonan and apiogalacturonan whose expression is restriction. Even a minor mutation in R-II structure can lead to defects in the plant growth like dwarfism, thus suggesting its importance for normal growth of plant [3]. RG-I being highly branched in nature thus, called as the hairy region of pectin on the other hand HGA domain are known as the smooth region [7]. It is generally believed and noticed that there is covalent linkage within the pectin polysaccharides and pectin degrading enzymes are needed to separate and isolate HG, RG-I and RG-II from each other [21, 22]. Due to their similarity in

HG and RG-II backbone structure composing of 1-4-linked alpha-D-GalA residues, they are likely to be linked covalently but there are no reports of RG-I to be covalently linked with HG [23].

3. Properties of pectin

3.1 Colloidal

Pectin precipitates as a solid gel on treating with a dehydrating agent like alcohol. They are extremely sensitive to dehydration and get effected by any other hydrophilic colloids as well, thus they are known to be insoluble in most of the bio-colloids. The negative charge of pectin depends on the number of free carboxyl group that is mainly responsible for its precipitation [24].

3.2 Solubility

Based on solubility pectins are of two types i.e., water soluble and water insoluble. Factors affecting the solubility of pectin are pH, temperature, nature of the solute and concentration of the solute [6, 13]. Pectin attains stability at a pH of 4 [17]. The solubility of pectin also depends on its composition like monovalent cation of pectin are soluble in water whereas di or trivalent are insoluble in water.

3.3 Gelation

One of the most interesting properties of pectin is its ability to form gel in the presence of either acid or calcium or sugar, this enables them to be used in many food industries [15]. Hydrogen bonding and hydrophobic interactions between polymer chains stabilizes the pectin polymer [9].

4. Extraction and purification of pectin from agrowaste

Pectin is a high molecular weight polysaccharide that is present in almost all plants and help in maintaining the integrity of cell structure. Pectin is used in food industries to increase the viscosity of food products such as beverages, jams and jellies. It also has implications in pharmaceutical industry, especially in drug formulations, as an excipient due to its characteristics in release kinetics. Due to increased demand for pectin in food, pharmaceutical and therapeutic applications, thus, require efficient extraction processes. In order to increase the yield of pectin, various extraction methods have been adapted to obtain insoluble pectin present in the middle lamellae of plant cells, one of them being heating in acidic medium that makes insoluble pectin as soluble. Ripening of fruits also converts insoluble pectin into soluble pectin. Pectin can be extracted from various kinds of fruits, but the most commercial form of pectin is extracted from the peels of citrus fruits by alcohol precipitation [9, 25]. Citrus fruits contain 0.5–3.5% pectin which is largely present in the peel of fruits [26].

Pectin has been isolated from various plants such as apple [27], citrus peel, carrots [28], sugar beet pulp [29, 30], sunflower heads [31], papaya [32] and oranges [33]. The most commonly used method for extracting pectin from plant tissue is by heating the plant sample in acidified water. The addition of extra chelating agents such as EDTA to the extraction mixture helps in easy release of pectin from cell wall. Care should be taken not to perform a long period of direct heating as it may lead to the thermal

Material	Extraction process	Pectin (%)	References
Cacao pod husk (<i>Theobroma cacao</i>)	Acid extraction	3.7–8.6	[34]
Mangosteen rind (<i>Garcinia mangostana</i>)	Chemical treatment	23.5	[35]
Durian rind (<i>Durio zibethinus</i>)	Acid extraction	2.1–10.25	[36]
Orange peels (<i>Citrus sinensis</i>)	Acid extraction	0.2–6	[37]
Lemon peels (<i>Citrus limon</i>)	Acid extraction	0.8–8	[37]
Dragon fruit peels (<i>Hylocereus undatus</i>)	Ultrasound assisted	1.89–7.65	[38]
Banana-stem, leaf, peel (<i>Musa acuminata</i>)	Alcohol precipitation	4–13.8	[39]
Orange peel	Alcohol precipitation	7.9	[39]
<i>Cucumis melo</i>	Aqueous acid extraction alcohol precipitation	4.53	[40]
Cocoa peel	Microwave assisted	42.3	[41]
Apple Pomace	Acid extraction	12.9–20.9	[27]
Lime-peel and pulp	Microwave assisted extraction under pressure	8–17.9	[42]
Watermelon rind	Acid and enzymatic extraction		[43]
Orange peels	Acid extraction	5.4–26.3	[44]
Sweet potato peels	Acid extraction	2.59–5.08	[45]
Orange peel	Ultrasound assisted	20.92	[46]
Orange peels (<i>Citrus sinensis</i>)	Acid extraction	29.41	[47]
Kaffir lime peel (<i>Citrus hystrix</i>)	Chemical and acid extraction	61.8	[48]
<i>Punica granatum</i> peels	Acid extraction	27	[49]
Orange peel (<i>Citrus sinensis</i>)	Acid extraction	45.5	[50]
Lemon (<i>Citrus limon</i>)	Acid extraction	2.7–16.7	[51]
Orange (<i>Citrus sinensis</i>)	Acid extraction	1.6–15.9	[51]
Grape (<i>Citrus paradisi</i>)	Acid extraction	2.3–15.7	[51]
Orange peel	Water-based extraction	2.2	[52]
Sweet potato peel (<i>Ipomoea batatas</i>)	Alkaline extraction	16.78	[45]
Tomato waste	Ultrasound assisted	15.1–35.7	[53]
Pumpkin peels	Soxhlet extraction	6.8–7.7	[54]
Lemon pomace	Acid extraction	10.3–13.1	[55]
Jackfruit waste (<i>Artocarpus heterophyllus Lam</i>)	Acid and chemical extraction	12–15	[27]
Lemon peel wastes	Aqueous extraction		[56]
Citric waste	Acid extraction	78	[57]
Apple peel waste (<i>Malus pumila. Cv Amri</i>)	Acid and chemical extraction	1.21	[58]
Horse eye bean peel (<i>Mucuna urens</i>)	Acid extraction	4.4	[59]
Banana peel	Acid extraction	11.31	[60]
Mango peel	Acid extraction	18.5	[60]
Grapefruit peel	Alcohol extraction	25	[33]

Material	Extraction process	Pectin (%)	References
Saba banana peel (<i>Musa acuminata</i> × <i>Musa balbisiana</i>)	Acid extraction	17.05	[61]
Passion fruit peels	Acid extraction	2.25–14.6	[62]
Citrus peel	Acid extraction	25.5	[63]
Pumpkin waste (<i>Cucurbita maxima</i>)	Acid hydrolysis	2.90	[39]
Mango peel	Acid extraction	20.8	[64]
Jackfruit wastes (<i>Artocarpus integer</i>)	Optimised acid extraction	38.42	[65]
<i>Citrus depressa</i> endocarp	Acid extraction	4.1	[66]
Orange peel	Acid extraction	7.3–52.9	[67]
Jackfruit waste (<i>Artocarpus heterophyllus</i>)	Chemical and acid extraction	8.9–15.1	[27]

Table 1.
 Methods of extraction of pectin from various agrowaste compounds.

degradation of the polymer. Extraction process of pectin is carried out under reflux using acidified water at 97°C for 30 min. The hot acid extract was then filtered using a cheese cloth to remove the pulp. The filtrate was then cooled to 4°C and precipitated using double the volume of ethanol. The solvent precipitate mixture is then mixed till the pectin floats and removed by using cheese cloth followed by drying [27].

Pectin is also extracted from dried sugar beet pulp after treating with acidified medium followed by purification through alcohol precipitation. Xin Huang et al., slightly modified the traditional method, where the sample was diluted with deionized water and was made acidic (pH –1.2) by using HCl. The sample was then heated to 90°C for 3 h and cooled to 40°C (pH –4.5) with 25 g/100 g ammonia. The mixture was then filtered using a Buchner funnel and pectin was precipitated using ethanol [29]. The ethanol is removed by squeezing with nylon cloth and washed several times followed by drying.

The carrot pomace is also used for pectin extraction by treating with hot aqueous citric acid solution adjusted to the desired pH. The pectin yield was maximum at the following optimum conditions: pH –1.3; temperature 90°C; heating time 79.8 min. Under these conditions, the extraction yield of carrot pomace pectin was found to be 16.0%. The extract mixture was then allowed to cool, filtered and precipitated by using ethanol in the ratio 2:1 [28]. Dried papaya peel can be used in pectin extraction where the majority of the lipids, and soluble pigments are removed by treating with ethanol and acetone. It is repeatedly homogenised with 95% ethanol and filtered until the filtrate becomes clear. The final filtration was done with the residue homogenised in acetone and dried overnight to obtain the alcohol insoluble residue (AIR). The majority of the pectin in the papaya AIR is present as chelator soluble pectin (CSP) followed by sodium carbonate soluble pectin (SSP) and water-soluble pectin (WSP). The WSP fraction is first obtained from the AIR by boiling it in water and filtering it. The remaining residue is treated with 0.05 M cyclohexane trans-1,2-diamine tetra-acetic acid (CDTA) in 0.1 M potassium acetate (pH 6.5) for 6 h at 28°C and filtered to give the CSP fraction. The residue is then treated with 0.05 M sodium carbonate solution having 0.02 M NaBH₄ for 16 h at 4°C, and subsequently for 6 h at 28°C. The solution when filtered gives the SSP fraction of the AIR [32].

Agro-industrial wastes can be used as the raw material for the production of industrial low and high methoxy pectin. The alcohol insoluble material (AIM) produced from dried agrowaste by boiling it with 3 volumes of ethanol for 25 min

and continuous washing with 70% ethanol to remove impurities such as pigments, free sugars, etc. Sunflower heads also act as potential sources for pectin extraction. The heads are washed by hot distilled water through a mesh or cheese cloth and the pectin was precipitated by addition of 1 M nitric acid at 1:5 acid:filtrate ratio [34]. The mixture was maintained for 1 h at 5°C and was washed six times in ethanol solvent at 1:2 gel:solvent ratio to remove the impurities and to increase pH by removing the acid [31]. The washed pectin gel can be dried in a vacuum oven at 55°C for 16 h. The dried pectin flakes are ground into a powder for further use (**Table 1**).

5. Characterization

Large amounts of fruit wastes that are being generated can be disposed effectively by manufacturing beneficial by-products like pectin. Pectin is used to increase foaming power of gases, as agglutinator, as filler in pharmaceutical preparations and also in food industry. The use of pectin for different purposes depends on its characters like acetyl value, degree of esterification, uronic acid content and methoxyl content, etc. [68]. The amount of anhydrouronic acid gives the purity of pectin which is not less than 65% for pectin that is used commercially [69].

5.1 Qualitative tests

Colour reader can be used to measure the colour of extracted pectin by placing lens of reader on sample powder. The colour of the extracted samples can be compared with that of commercial pectin. Solubility of pectin in different solvents is measured i.e., solubility in cold and, hot water and alkali like NaOH.

5.2 Quantitative tests

Acetyl content and equivalent weight of pectin can be estimated using NaOH whereas methoxyl content can be estimated by saponification and titration. Degree of esterification can be calculated from methoxyl content and anhydrouronic acid content. After acid hydrolysis, sugar separation can be achieved by thin layer chromatography. Intrinsic viscosity of pectin is measured by dissolving pectin in water and by preparation of solutions of various concentrations [27, 32, 60].

6. Applications

Pectin being a great inert, biodegradable and biocompatible complex, is widely used in various fields such as in textiles, food industries as gelling agents, pharmaceuticals and other products as well [70]. Pectin are used as biomaterials in gene delivery [71], application in oral drug delivery [72], as edible coating for food packaging [25], biomass yield and bio refinery [21, 22]. It also has applications in tissue engineering as scaffolds [73], in paper and textile industries for the preparation of ultracentrifugation membrane [74].

6.1 As food product

From the very early period of time, pectin had become one of the major natural constituents of human food, and they have been widely used as a gelling agent for jams and jellies. In jam processing, fruits are cooked properly in order to release juice and pectin which converts the proto-pectin into soluble pectin [19]. Pectins

are also used as a substitute of sugar in jams that are made without sugar, using LM (low methoxy) pectin due to its stability in acidic condition. Pectin is widely used for making instant jellies for bakery production these are made with the use of HM (high methoxy) pectin that are thermally stable, the only difference between HM and LM pectin is the amount of pectin in the formula, LM requires a higher amount than that of HM [75]. Other food products like artificial cherries [15], are used to make different kinds of gel puddings that is made of pectin present in the fruit syrup and cold milk [25]. Edible coating of food material is also made of pectin [25]. Pectin is used in beverages as a beverage clouding agent like in diabetic soft drinks [76]. Pectins are also used in the fruit preparation of yogurt to make it more soft and to obtain the partial gel texture [77].

6.2 Biomedical application

6.2.1 Pectin as films

The blending of the natural and synthetic polymers is one of the promising areas of development, this gives new polymeric material with better durability and resistance. Materials like sponge, hydrogels, encapsulating drugs etc. are produced by polymer films [32]. Due to development and discovery of natural polymers scientist have started to form bio-based material rather than synthetic one due to its physiochemical properties like biodegradability, this shift is mainly caused due to the environmental issues and concern regarding the heavy use of plastic [78]. Films of pectin are used to encapsulate and thicken food, and in pharmaceuticals [29]. Hoagland et al. made pectin films with glycerol and lactic acid to prevent fungal contamination on the laminated films [47]. The similar kind of products were made by Fisherman et al., where an edible pectin blend film were plasticized with glycerol, they also suggested that the glass transition at about 50°C was large in case of pectin films, which indicates that the films were fairly flexible at room temperature [79]. Liu et al., made different varieties of biofilms one each with pectin, fish skin gelatin and soybean flour protein which in turn resulted in a composite film that showed an increase in stiffness as well as the strength, whereas decrease in water solubility and water vapour transmission rate when compared to the film that was made with pectin alone. They thus suggested that the tensile strength can be improved by crosslinking the films with methanol or glutaraldehyde [80]. A bio-reactive substance for tissue regeneration was developed by Liu et al., which was composed of pectin or PGLA matrix, which demonstrated that pectin was able to carry signals to molecules, further they suggested that the pectin also helped in the adhesion of the cell and promotes cell proliferation [35]. Some researches have reported the use of pectin membranes as a wound dressing material [81].

6.2.2 Drug delivery

In recent years, biomedical application especially in case of drug delivery system, the use of natural polymers is preferred over the other types due to their inert nature and its biocompatibility. Pectin as the natural polymer is a new developed interest for drug delivery application due to its properties of gel formation in acidic condition, its mucoadhesiveness and its ability to dissolve in basic environment [36]. These properties of pectin are applied in different ways such as the mucoadhesiveness helps in targeting and controlling the drug delivery especially in the nasal and gastric environment, where as its ability to dissolve in basic condition helps in the release of colon related drugs and the formation of gel helps in increasing the contact time of drug in gastric condition [35, 36]. Recent studies have shown the

use of LM pectin for nasal drug delivery due to its mucoadhesive property they have a tendency to bind to the mucin with the help of hydrogen bond [82]. Its use in the production of fentanyl (painkiller) has also been seen that help in treating cancer pain which needs rapid drug release [83, 84]. An alternative for smoking cessation are the nasal pectin containing nicotine [85]. As pectin have resistance towards proteases and amylases it has been highly preferred as an encapsulating nanoparticle for drug delivery as most of the proteins are easily degraded by our digestive enzymes and thus to protect these drugs the use of pectin as an outer cover that cannot be degraded in the gastrointestinal tract are preferred for colon and oral drugs [86]. Studies have shown that pectin is able to inhibit cancer metastasis and primary tumour growth in many animal related cancer [87, 88]. Gal-3 is one of the important factors controlling cancer progression and metastasis, and pectin has the ability to recognise these Gal3 components [89]. In a study, citrus pectin was used to target Gal3 that could inhibit the metastatic successfully [87, 90].

6.2.3 As gene delivery and nanoparticles

The treatment of any genetic disorder is called gene therapy as it deals with the defected genes that are responsible for the disorder; these are treated by replacing the defective gene, silencing the unwanted gene expression or by substituting missing genes and these are carried out with the help of viral or non-viral vectors [36]. The use of non-viral vectors is preferred over viral due many reasons like biocompatibility, minimal toxicity and immunogenic reactions of our body [71]. These non-viral vectors are made of polymers of polycationic, chitosan or even pectin. It has been observed that the use of carbohydrate mediated products have better binding capacity, to facilitate the uptake by target cell [91–93]. Pectins were found to be suitable as a coating substance for b-PEI [94, 95]. Opanasopit et al. has also observed the formation of pectin nanoparticle which in turn helps to entrap the DNA for transfection [96]. Katav et al., modified pectin with the help of three different amine groups and these complexes were able to bind with plasmid DNA and there efficiency to transfect or their potential as a non-viral gene delivery carrier was compared and suggested that modified pectin has a promising role in gene delivery [71]. Similar type of study was conducted by Opanaopit et al., where pectin ability as a nanoparticle for gene delivery were studied and the study suggested the potential use of pectin as delivery vector to be safe [96]. Pectin has also been used as wound dressing material in the form of pectin-chitosan based nanoparticles. It has the ability to create an acidic environment in which the bacteria cannot grow. Burapapadh et al. developed a pectin based nanoparticle to improve and enhance the drug dissolution of ITZ (Itraconazole) [97].

6.2.4 Pectin-based scaffolds

Scaffolds are 3-D biomaterials that are porous in nature and are designed to be applied in various fields, few of its basic functions are to promote cell adhesion, to allow enough nutrients and gases transportation and mainly for tissue engineering [32]. Tissue engineering mainly involves the use of biocompatible scaffolds materials to act as a support matrix or to be used as a substrate for delivery of some compounds. There has been a great research going on to promote tissue reconstructions. Coimbra et al., prepared pectin based scaffolds to be used for bone tissue engineering [98]. Similar study was performed by Munarin et al., who examined the use of pectin as injectable biomaterial for bone tissue engineering [99]. Ninan et al. were also able to fabricate biopolymer scaffold of pectin and other compounds using the technique of lyophilisation, thus suggested the use of pectin as ideal polymeric matrix for tissue engineering [73, 100, 101].

7. Conclusion

Pectin is one of the most extensively studied natural biodegradable polymer. In spite of its availability in a large number of plant species, commercial sources of pectin are very limited. There is, therefore, a need to explore other sources of pectin or modify the existing sources to obtain pectin of desired quality attributes. Current knowledge of the molecular basis of pectin has helped us to understand some aspects of this complex polysaccharide. Extensive studies must be carried out to find out more about the biological pathways to devise various efficient means of pectin extraction that are scalable and can be commercialized. The large variety of applications as well as the increasing number of studies on pectin suggests that the potential of pectin as novel and versatile biomaterial will be even more significant in the future. As the research and development continues in pectin-based products, we expect to see many innovative and exciting applications.

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Conflict of interest

The authors would like to declare that there was no conflict of interest in this work.

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