Involvement of the Complex Polysaccharide Structure of Pectin in Regulation of Biological Functions

Saki Gotoh¹, Kohji Kitaguchi² and Tomio Yabe¹, ², ³*

¹ The United Graduate School of Agricultural Science, Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan
² Department of Applied Life Science, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan
³ Center for Highly Advanced Integration of Nano and Life Sciences (G-CHAIN), Gifu University, 1-1 Yanagido, Gifu, 501-1193, Japan

ABSTRACT

The water-soluble dietary fiber, pectin, is a polysaccharide present in the cell wall of terrestrial plants. It is a polymer of D-galacturonic acid polymerized by alpha-1,4 linkages and has side chains composed of 13 different sugars. Due to its complex chemical structure, pectin has a number of physical characteristics, including gelling properties and viscosity, and has physiological functions in vivo. The degree of esterification of the pectin main chain affects gelling. In addition, the side chain structure has been shown to affect antitumor activity and regulation of intestinal immunity, which are well-known physiological functions of pectin, and the rhamnogalacturonan-I region is particularly important. This review discusses the correlations between the molecular structure of pectin and its functions. Not only that, we will discuss the mechanisms and physiological significance of small intestinal villus morphological changes, which is still a mysterious function of pectin.

Keywords
biological function, pectin, pectin structure, rhamnogalacturonan-I

1. Introduction

Pectin is a complex polysaccharide ubiquitously present in the primary cell wall and middle lamellae of all terrestrial plants, including fruits and vegetables. It is the most complex polysaccharide in nature, providing adhesion and stability between plant tissues and cells [1]. Pectin is an industrially valuable polysaccharide and a typical water-soluble dietary fiber. As it has various properties, including gelling ability, water retention, viscosity, and cation binding properties, it is used as a gelling agent for jams and jellies, a stabilizer in acidic beverages, and as a thickening agent. It is used not only in the food and beverage industry, but also in cosmetics and pharmaceuticals. Pectin is not degraded by human digestive enzymes and is not absorbed as a nutrient in the small intestine. However, in recent years, it has been shown to have physiological activities, such as lowering lipid and cholesterol levels, suggesting that it may reduce the risk of progression of various diseases, such as cancers and gastrointestinal diseases [2,3,4]. Pectin has a complex molecular structure, consisting of a polymer of galacturonic acid (GalA) with linear covalent bonds. It is composed of three polysaccharide regions: homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II). In addition, the side chains have oligosaccharides consisting of 13 types of monosaccharides [5,6]. Furthermore, the structure of pectin differs depending on the source and extraction conditions. This review presents the molecular structures of pectins that have been elucidated to date and discusses
the effects of structural differences on the physicochemical and physiological functions of pectin. In particular, we described the cellular responses through the direct interaction between the small intestinal epithelium and pectin, and focused on the truth that pectin-induced morphological changes of small intestinal villi is a beneficial phenomenon for the host.

2. Structural properties of pectin

2.1 Fundamental structure of pectin

Pectin is a polysaccharide with a complex structure, the exact chemical structure of which is still under investigation. Although the binding form and positional relationships are unknown, pectin has different structural regions linked to each other by covalent bonds. The reported regions are HG, xylogalacturonan (XGA), apiogalacturonan, RG-I, and RG-II (Fig. 1) [7]. HG, RG-I, and RG-II are well known as the main structural regions, and their ratios vary depending on the plant species from which they are derived. In general, HG constitutes about 65% of pectin, RG-I constitutes 20%–35%, and RG-II constitutes less than 10% [8,9].

Figure 1: Schematic diagram of pectin structure

HG is the most abundant polysaccharide in the primary cell wall, and its fundamental skeleton is a linear structure in which α-GalA is bound in an α-(1→4) configuration. It is a critical factor in regulating the strength of plant cell walls and is deeply involved in softening during fruit maturation. HG is biosynthesized in plant cells. First, it is synthesized in a highly methylesterified state in the intracellular Golgi apparatus and secreted into the cell wall. Subsequently, HG is completed by partial de-esterification by enzymes belonging to the pectin methylesterase family [10]. The carboxy groups of the GalA residue are partially methylesterified with C-6, and the hydroxy groups of O-2 and O-3 are partially acetylated. The substitution rates of methoxy and acetyl groups for GalA residues in pectin are expressed in terms of the degree of methoxylation (DM) and degree of acetylation (DAc). These values vary widely depending on the plant origin and affect the properties of pectin.

RG-I has a repeating disaccharide unit in the main chain, in which GalA and 1-rhamnose (Rha) are alternately bound [→4]-α-D-GalA-(1→2)-α-L-Rhap→. In addition, it has a structure in which side chains consisting of arabinose (Ara) and galactose (Gal) are bound to the C-4 position (rarely C-3 position) of the Rha residue. As side
chains, arabinan composed mainly of α-1,5 bonds of Ara, galactan composed of β-1,4 bonds of Gal, and arabinogalactan of their complex are bound [11]. Galactoarabinan has also been reported as another side chain [12].

RG-II is the most complex structural region in the pectin molecule that exists as part of HG. Oligosaccharide side chains with four different structures are bound to the HG main chain consisting of α-1,4-linked D-GalA residues [13]. The side chains have very specific sugar residues, such as apiose, aceric acid, 3-deoxy-lyxo-2-heptulosaric acid (DHA), and 3-deoxy-manno-2-octulosonic acid (KDO). Although pectin molecules have different structures depending on the plant species, RG-II is very well conserved among plants throughout evolution. RG-II can be crosslinked by forming a boric acid diester with the HG of another pectin molecule [14,15].

Furthermore, among the GalA main chains of pectin, HG without side chains is classified as the smooth region, and RG-I and RG-II in which side chains consisting of various neutral sugars are densely distributed are classified as the hairy region.

2.2 Pectin gelling properties

Various polysaccharides obtained from fruits, vegetables, and plants are used in fields such as the healthcare and the food industry. The extracted pectin is widely used in the food industry as a gelling agent, thickener, stabilizer, and emulsifier in the production of confectionery, jam, and jelly.

Pectin gelation is closely related to the methoxylation of galacturonic acid in the pectin backbone [16] and results from hydrogen bonds, hydrophobic interactions, and ionic bonds (Fig. 2). High methoxy (HM) pectin with a DM of 50% or higher forms a gel in the presence of high concentrations of sucrose under acidic conditions [17,18]. On the other hand, low methoxy (LM) pectin with a DM of less than 50% forms gels through calcium bridges between carboxy groups in the presence of calcium and other divalent metal ions [18,19]. DM, which affects pectin's gelling ability and gelling properties, varies greatly depending on the plant species from which pectin is derived and the extraction methods used.

![Figure 2: Pectin gelation mechanism](image)

HM pectin gels are formed by hydrogen bonding of methylesterified GalA at high sugar content and low pH, while LM pectin gels are formed by bonding of GalA with divalent cations, such as Mg²⁺ and Ca²⁺.

HM, high methoxy; LM, low methoxy; GalA, galacturonic acid.

Most of the pectins currently used industrially are derived from citrus peels (85.5%) due to their high contents. In addition, some apple pomace and beet pulp-derived products have also been developed on an industrial scale [20].
The extraction process of pectin usually uses strong acids such as hydrochloric acid or nitric acid. It is extracted with citric acid as well. In addition to these acidic conditions, it is well extracted at 70 °C to 100 °C and 0.5 to 5 hours. Its yield is improved by high temperature, long extraction time, and low pH [21,22].

Most of the natural pectin widely used in industry is HM pectin, and LM pectin is produced through a demethylation process. On the other hand, passion fruit peels [23], banana peels [24,25], pomegranate peels [26], and pea hulls [27] have also been reported to contain low DM as natural pectins. The reason why low DM pectin is produced in the natural state is unknown, but it could be due to hydrolysis of esters during the extraction process with strong acids, such as nitric acid, citric acid, and sulfuric acid.

3. Bioactive sites within the pectin molecular structure

There have been many reports on the physiological functions of pectin from various plant species, including antioxidant activity [28,29,30], antihypertension effects [31], plasma cholesterol normalizing activity [32,33], antitumor activity [34], and suppression of food allergies [35,36]. These physiological functions are closely related to the complex chemical structure of the pectin molecule. It has been suggested that specific structural regions and compositions within pectin are required to exert physiological activity when pectin is ingested. Therefore, to better understand the physiological functions of pectin, it is crucial to elucidate the structures responsible for the observed beneficial effects. Here, we divided pectin into the main chain structure made of GalA polymer and side chain structures made of neutral sugars, and explore the effects of each pectin region on physiological functions in the intestinal tract.

3.1 Relationship with main chain structure

Pectin forms a gel in the gastrointestinal tract. It has been suggested that this property causes a feeling of satiety in humans by delaying digestion in the intestinal tract and is a mechanism of beneficial health effects, such as improvement of cholesterol and lipid metabolism and prevention of diabetes. Pectin is roughly classified into HM pectin and LM pectin by the DM of GalA that forms the main chain. As DM is closely related to the gelling ability and viscosity of pectin, it affects the physiological activity of pectin through the mechanism of action caused by physicochemical interactions. For example, pectin has a cholesterol-reducing effect as a bioregulatory effect, but it has been reported that lipid digestion and absorption decrease with increases in DM [16]. High molecular weight pectin increases the viscosity of the intestinal contents by forming a complex gel that traps water and lipids inside. Increased methoxylation results in an increase in the overall hydrophobicity of the pectin molecule and a decrease in the number of basic functional groups. Increased non-polar groups increase bile salt binding by hydrophobic attraction, resulting in a reduced ability to interact with the lipid droplet surface. Thus, by inhibiting lipase adsorption and limiting the diffusion of lipids and lipase, fat absorption is suppressed, and the effect is more pronounced in high DM pectins [16].

The gelling properties of pectin have been shown to be effective against dyspepsia in several clinical studies. Oral supplementation of pectin to children and infants was shown to reduce acute intestinal enteritis and significantly delay or reduce diarrhea [37,38]. These effects have been shown to involve not only the physical properties of pectin, but also its prebiotic effects.

The gut microbiota interacts with the intestinal immune barrier and influences the health of the host [39]. A balanced composition of the gut microbiota is essential for preventing the development of diseases and maintaining the health of the organism. Dietary fiber has a beneficial effect on the intestinal immune barrier through the gut microbiota [40]. One of the mechanisms is the production of short-chain fatty acids (SCFAs), which are metabolites
resulting from the assimilation of dietary fiber, including pectin, by intestinal bacteria. In the intestine, large amounts of acetic, propionic, and butyric acids are mainly produced; SCFAs inhibit the growth of pathogenic bacteria and promote the growth of useful intestinal flora by maintaining a low pH in the intestine [41]. Pectin acts to alleviate immune system disorders by stimulating the flora and the production of SCFAs (Fig. 3). These effects in the intestinal tract have been shown to be DM-dependent.

![Figure 3: Effects of structural features of pectin on biological functions](image)

Each region of pectin is responsible for the regulation of various biological functions according to its structural characteristics. The DM of GalA that composes the HG region is involved in the gelation of pectin and thus affects the digestion rate of ingested food. It also influences the activation of intestinal flora and the production of SCFAs. The RG-I and RG-II regions, which have many branching structures, are involved in the regulation of functionality, especially through direct interaction with small intestinal epithelial cells. They cause receptor-mediated immune responses, strengthening of the mucosal layer, and elongation of intestinal villi, which in turn affect small intestinal homeostasis and nutrient absorption efficiency.

HG, homogalacturonan; RG-I, rhamnogalacturonan-I; RG-II, rhamnogalacturonan-II; SCFA, short-chain fatty acids; TLRs, Toll-like receptors; GalA, galacturonic acid; Rha, rhamnose; Api, apirose; Ara, arabinose; Gal, galactose; Xyl, xylose; Fuc, fucose.

The DM of pectin has been reported to be an important regulator of the composition of bacterial flora [42]. It has been suggested that pectin with low DM is digested faster than pectin with high DM, thus acting faster on the bacterial flora and inducing microbial flora growth [43,44,45]. It has also been reported that the amount of propionic acid produced in the feces is higher with HM pectin of DM 53 compared to LM pectin of DM 29 [46]. On the other hand, it has been reported that the total SCFA concentration in the cecum of rats treated with LM pectin is higher than that of rats treated with HM pectin [44].

The functions of pectin in the intestinal tract are exerted not only by physicochemical and prebiotic interactions, but also by direct interactions with receptors that recognize pectin. In particular, pectin inhibits the Toll-like receptors (TLRs), TLR2 and TLR4. TLR2-1 inhibition was more effective with LM pectin than with HM pectin [47]. On the other hand, TLR4 inhibition showed the opposite trend, where higher DM pectin effectively inhibited TLR4 activation of macrophages by LPS [48]. It has also been reported that high DM pectin inhibits iNOS and COX2
expression in LPS-activated macrophages more efficiently than low DM pectin [48]. The DM of pectin also affects direct interaction, exhibiting antioxidant and immunomodulatory effects.

3.2 Relationship with side chain structures

The biological activities of pectin include anti-inflammatory [48,49], antioxidant [50,51], and antitumor activities [52]. These effects of pectin are known to vary depending on the DM of the pectin backbone. Not only that, it has been shown that they depend on the structural features of the pectin side chains, such as the proportion of neutral sugars that make up the side chain and the different binding modes (linear or branched). Thus, the importance of side chains has been suggested. The branched structure is particularly important and has been attributed to type I arabinogalactan [53].

Type I arabinogalactan has a β-1,4-galactan skeleton and is covalently bound to the RG-I region of pectin. A D-Gal with a β-1,4 bond is the main chain, and L-Ara with an α-1,5 bond is the side chain and is bound to the O-3 and O-4 positions of Rha in the RG-I region [54].

RG-I pectin has a protective effect on the mucosal layer of mice with DSS-induced colitis [55]. RG-I protects the mucosal layer and prevents activation of the inflammatory response by maintaining the number of mucus-secreting goblet cells and the expression of MUC-1. The RG-I region is one of the most useful bioactive sites in the pectin molecule, and galactan side chains are more active against colitis than arabinan [56]. On the other hand, there are reports that it promotes the pathology of colitis, suggesting that not all RG-I regions have anti-inflammatory effects [57].

More recent studies have shown that the branching structure of the RG-I region is important for immunological activities [58,59,60]. RG-I and RG-II pectins activate macrophages and dendritic cells. In particular, stronger activity has been reported for RG-I pectin, suggesting that the galactan and arabinan structures contribute to the activation of immune responses [61,62]. Many studies have shown that the branching structure of the RG-I region influences the function of immune cells. It has been reported that TNF-α and IL-10 secretion are decreased by removing the side chain structure of sweet pepper pectin [63]. Furthermore, the addition of the degradation product of the main chain of pectin suppressed IL-6 production in macrophage cell lines stimulated via TLRs [64]. However, the addition of only the GalA polymer, the main chain of pectin, showed no inhibitory effect [64]. These results emphasize the importance of the side chains of pectin in contributing to the immune response and in exerting anti-inflammatory effects. Galectin-3 is a receptor that recognizes pectin [65]. Galectin-3 is a protein that is expressed extracellularly or intracellularly by various cell types. It is particularly overexpressed in many cancer cells and is involved in the health and disease of the organism [66]. Pectin and galectin-3 are bound by the interaction of galactose or arabinan in the RG-I and RG-II regions of pectin with the lectin domain of galectin-3 [65]. It has been suggested that pectin exhibits anti-metastatic and anti-tumor effects in cancers through this interaction. Among them, the β-1,4-galactan side chain antagonizes galectin-3 and is considered to be more useful [65].

It has been reported that the effects of pectin on oxidative stress, such as cytoprotective effects against heavy metal ion-induced oxidative stress and cytotoxicity, also depend on the side chain structure and composition of the RG-I region. In particular, it has been suggested that Gal residues in the side chain play an important role [67,68,69]. It has also been suggested that Ara residues in the RG-I region contribute to prebiotic activity [70].

These reports indicate that galactan is the major bioactive site in the RG-I region. They also suggest that the regulation of neutral glycans in the RG-I region, such as arabinan and arabinogalactan, is important for the bioactivity of pectin.
4. Morphological changes in the small intestinal villi by pectin

Many actions and functions of pectin in the intestinal tract have been reported. Here, we focus on the morphological changes of small intestinal villi caused by ingested pectin. It has been reported that feeding a diet containing pectin causes villi to become irregular in rats and chickens [71,72], and that crypts become deeper in the jejunum and ileum of rats [73]. Thus, many animal experiments have shown the effects of pectin. However, the molecular mechanisms and significance of these effects are still unclear and subject to debate. Further elucidation of these issues is expected to lead to the discovery of new physiological functions of pectin in the intestinal tract. In this review, we summarize the mechanism of pectin-induced morphological changes of small intestinal villi and the effects of villus elongation on the host (Fig. 4).

Figure 4: Effects of pectin on intestinal villi
In the intestinal tract, pectin, which interacts with fibronectin, is recognized by the cell-surface receptor α5β1-integrin. The stimulation of pectin alters the structure of HS on the cell surface, leading to the secretion of Wnt protein, a cell growth factor. The Wnt protein secreted inside the villus induces overgrowth of proliferating cells in the crypts, leads to changes in the morphology of the villi of the small intestine. The elongation of small intestinal villi affects the efficiency of nutrient absorption in the small intestine.

4.1 Molecular mechanisms that cause morphological changes of small intestinal villi

In order for pectin, which is not digested and absorbed in the small intestine, to change intestinal morphology, the molecular structure of pectin moving through the intestine may be recognized by the epithelial cells of the small intestine. It has been shown that heparan sulfate (HS), which is ubiquitously present on the cell surface, is involved in the mechanism of recognizing pectin and causes changes in the morphology of the small intestine [74]. HS is a type of glycosaminoglycan, a linear polysaccharide composed of repeating polymerized disaccharide units of N-acetylglucosamine and glucuronic acid or iduronic acid. HS plays a role in transmitting changes in the extracellular environment to the inside of the cell, and depending on the pattern of sulfation formed, the interacting proteins are switched. Therefore, HS is known to be involved in the regulation of cellular function and various physiological functions [75].

When intestinal epithelial cells recognize pectin, the expression levels of the desulfatases Sulf-1 and Sulf-2 are changed, indicating that the sulfated structure of HS is altered [74]. Furthermore, as it has been reported that enzyme-treated pectin interacts with fibronectin [76], receptors that recognize pectin have also been explored. It has been
reported that pectin bound to fibronectin enhances the expression of Sulf-2 by introducing ERK1/2 signal through the cell-surface receptor α5β1-integrin [74] (Fig. 4).

The relationships between the pectin-induced changes in HS on the cell surface and the changes in small intestinal villi due to cellular responses have also been reported. HS is known to act as a receptor for bioactive substances, such as Wnt and TGF-β, depending on its sulfated structure, and functional modification of these proteins may be involved in villus changes. Wnt3a has been identified as a cell growth factor that is secreted upon pectin stimulation in co-culture model systems in vitro, such as the small intestinal environment [77]. Wnt3a binds strongly to HS on the cell surface, but the binding is weakened by the reaction with pectin. At the same time, stimulation of small intestinal epithelial-like cells by pectin showed proliferation of crypt-like cells. These have been suggested that pectin changes the structure of HS on the small intestinal epithelium cell surface and weakens its interaction with Wnt3a, thereby increasing the amount of secreted Wnt3a inside the villus and overgrowth crypt cell proliferation (Fig. 4).

With regard to the pectin-induced morphological changes in the small intestine, we have reported that the active site is located in the complex pectin molecular structure. We added variously treated pectins to differentiated Caco-2 cells, which are small intestinal epithelial-like cells, and compared the effects of cell secretions on crypt-like cells. The results showed that different amounts of negative charge in the pectin main chain did not affect the activity, but it was altered by the RG-1 side chain of pectin [78]. That is, the structure of the pectin side chain is involved in the morphological changes in the small intestine, indicating the importance of arabinogalactan.

Thus, the mechanism of morphological change of small intestinal villi by pectin feeding is gradually being elucidated. Further development is expected by demonstrating the effects of changes in the pectin structure on elongation of the villi, the secretion of cell growth factors, and the strength of binding to pectin recognition receptors.

4.2 Effects of pectin-induced morphological changes in small intestinal villi on living organisms

The small intestinal epithelium is the most important place for the body to absorb nutrients and functional ingredients in foods. The intestinal mucosa is covered with numerous villi, which transport nutrients into blood vessels through intracellular and intercellular pathways and circulate them throughout the body. Therefore, the extension of small intestinal villi by pectin is expected to expand the sites of nutrient absorption and increase the efficiency of nutrient absorption in the small intestine (Fig. 4).

In fact, in rats fed pectin, the height of villi and the depth of crypts in the jejunum and ileum are increased, along with the amounts of maltose absorbed [79]. In addition, an increase in the production rate of crypt cells and activities of mucosal enzymes, such as sucrase and alkaline phosphatase, have also been observed [79]. Similarly, rapid drug absorption in the small intestine has also been shown [80]. Even in the cecum, pectin also affects the absorption of minerals. It has been shown that potassium, magnesium, and calcium fluxes are increased in rats fed pectin [81].

On the other hand, it has been shown that viscous pectin slows down the rate of intestinal absorption of glucose and may inhibit the absorption of bile acids and minerals by forming a gel [82]. It has also been reported that the ingestion of pectin does not affect the absorption of most minerals except magnesium [83,84]. It has also been reported that the specific activity of alkaline phosphatase is reduced in the upper jejunum of rats fed pectin [85].

The effects of pectin on nutrient absorption in the intestinal tract have been shown to vary depending on the measurement point (amount or rate of absorption) and site. Most of these studies were animal experiments using rats, and the effects of pectin on the human intestinal tract will need to be carefully studied in the future.
5. Conclusion

Fig. 3 shows the bioregulatory functions of the intestinal tract by each structural region constituting pectin. Based on their complex molecular structure and physical properties, pectins have been shown to exhibit prebiotic effects and to directly interact with biomolecules to modulate physiological functions. The direct interaction between pectin and cells in vivo is thought to be mediated by receptors that recognize pectin molecules, and proteins that bind to pectin have been reported. It has been suggested that the active site in the pectin molecular structure that binds to the receptor is the side chain, but the details are not yet clear. A variety of pectin-degrading enzymes have been discovered. It is expected that further research will elucidate the details of pectin structures that exhibit physiological activity. In particular, clarification of the correlation between the biological effects of morphological changes in small intestinal villi and the specific structure of pectin will clarify the importance of ingesting pectin and further expand the possibility of industrial use of pectin.

REFERENCES

[1] Lunn J and Buttriss JL (2007) Carbohydrates and dietary fibre. Nutr. Bull., 32(1): 21–64.
[2] Zhang W, Xu P and Zhang H (2015) Pectin in cancer therapy: A review. Trends Food Sci. Technol., 44(2): 258–271.
[3] Noreen A, Nazli Z i. H, Akram J, Rasul I, Mansha A, Yaqoob N, Iqbal R, Tabasum S, Zuber M and Zia KM (2017) Pectins functionalized biomaterials; a new viable approach for biomedical applications: A review. Int. J. Biol. Macromol., 101: 254–272.
[4] Wikiera A, Irla M and Mika M (2014) Health-promoting properties of pectin. Postepy. Hig. Med. Dosw., 68: 590–596.
[5] Vincken JP, Schols HA, Oomen RJFJ, McCann MC, Ulvskov P, Voragen AGJ and Visser RGF (2003) If homogalacturonan were a side chain of rhamnogalacturonan I. Implications for cell wall architecture. Plant Physiol., 132(4): 1781–1789.
[6] Elias I and Raz A (2019) Pleiotropic effects of modified citrus pectin. Nutrients. 11(11): 1–18.
[7] Scheller HV, Jensen JK, Sørensen SO, Harholt J and Geshi N (2007) Biosynthesis of pectin. Physiol. Plant., 129: 283–295.
[8] Mohnen D (2008) Pectin structure and biosynthesis. Curr. Opin. Plant Biol., 11(3): 266–277.
[9] Zandleven J, Sørensen SO, Harholt J, Baldman G, Schols HA, Scheller HV and Voragen AJ (2007) Xylogalacturonan exists in cell walls from various tissues of Arabidopsis thaliana. Phytochemistry, 68(8): 1219–1226.
[10] Wolf S, Mouille G and Pelloux J (2009) Homogalacturonan methyl-esterification and plant development. Mol. Plant., 2(5): 851–860.
[11] Voragen AGJ, Coenen G-J, Verhoeof RP and Schols HA (2009) Pectin, a versatile polysaccharide present in plant cell walls. Struct. Chem., 20(2): 263–275.
[12] Yu L, Yu C, Zhu M, Cao Y, Yang H, Zhang X, Ma Y and Zhou G (2015) Structural analysis of galactoarabinan from duckweed. Carbohydr. Polym., 117: 807–812.
[13] Ridley BL, O’Neill MA and Mohnen D (2001) Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry, 57(6): 929–967.
[14] Ishii T and Matsunaga T (2001) Pectic polysaccharide rhamnogalacturonan II is covalently linked to homogalacturonan. Phytochemistry, 57(6): 969–974.
[15] Ishii T, Matsunaga T, Pellerin P, O’Neill MA, Darvill A and Albersheim P (1999) The plant cell wall polysaccharide rhamnogalacturonan II self-assembles into a covalently cross-linked dimer. J. Biol. Chem., 274(19): 13098–13104.
[16] Naqash F, Masoodi FA, Rather SA, Wani SM and Guni A (2017) Emerging concepts in the nutraceutical and functional properties of pectin — A review. Carbohydr. Polym., 168: 227–239.
[17] Thakur BR, Singh RK and Handa AK (1997) Chemistry and uses of pectin — A review. Crit. Rev. Food Sci. Nutr., 37(1): 47–73.
[18] Thibault J-F and Ralet M-C (2003) Physico-chemical properties of pectins in the cell walls and after extraction. In: Advances in Pectin and Pectinase Research (Voragen F, Schols H and Visser R, eds.). pp.91–105. Springer, Dordrecht.
[19] Han W, Meng Y, Hu C, Dong G, Qu Y, Deng H and Guo Y (2017) Mathematical model of Ca2+ concentration, pH, pectin concentration and soluble solids (sucrose) on the gelation of low methoxyl pectin. Food Hydrocollo., 66: 37–48.
[20] Ciriminna R, Chavarria-Hernández N, Inés Rodríguez Hernández A and Pagliaro M (2015) Pectin: a new perspective from the biorefinery standpoint. Biofuel. Bioprod. Biorefin., 9(4): 368–377.
[21] Mellinas C, Ramos M, Jiménez A and Garrigós MC (2020) Recent trends in the use of pectin from agro-waste residues as a natural-based biopolymer for food packaging applications. Materials, 13(3): 673.

[22] Dranca F and Oroian M (2018) Extraction, purification and characterization of pectin from alternative sources with potential technological applications. Food Res. Int., 113: 327–350.

[23] Kulkarni SG and Vijayanand P (2010) Effect of extraction conditions on the quality characteristics of pectin from passion fruit peel (Passiflora edulis f. flavicarpa L.). LWT - Food Sci. Technol., 43(7): 1026–1031.

[24] Hippi Emaga T, Ronkart SN, Robert C, Wathelet B and Paquot M (2008) Characterisation of pectins extracted from banana peels (Musa AAA) under different conditions using an experimental design. Food Chem., 108(2): 463–471.

[25] Oliveira TJS, Rosa MF, Cavalcante FL, Pereira PHF, Moates GK, Wellner N, Mazzetto SE, Waldron KW and Azeredo HMC (2016) Optimization of pectin extraction from banana peels with citric acid by using response surface methodology. Food Chem., 198: 113–118.

[26] Abid M, Cheikhrouhou S, Renard CMGC, Bureau S, Cuvelier G, Attia H and Ayadi MA (2017) Characterization of pectins extracted from pomegranate peel and their gelling properties. Food Chem., 215: 318–325.

[27] Gutöhrlein F, Drusch S and Schalow S (2020) Extraction of low methoxylated pectin from pea hulls via RSM. Food Hydrocoll., 102: 105609.

[28] Nara K, Yamaguchi A, Maeda N and Koga H (2009) Antioxidative activity of water soluble polysaccharide in pumpkin fruits (Cucurbita maxima Duchesne). Biosci. Biotechnol. Biochem., 73(6): 1416–1418.

[29] Torralbo DF, Batista KA, Di-Medeiros MCB and Fernandes KF (2012) Extraction and partial characterization of Solanum lycopersicum pectin. Food Hydrocoll., 27(2): 378–383.

[30] Košťálová Z, Hromádková Z and Ebringerová A (2010) Isolation and characterization of pectic polysaccharides from the seeded fruit of oil pumpkin (Cucurbita pepo L. var. Styriaca). Ind. Crops Prod., 31(2): 370–377.

[31] Baluja Z and Kaur S (2013) Antihypertensive properties of an apple peel — Can apple a day keep a doctor away? Bull. Pharm. Med. Sci., 1(1): 9–16.

[32] Mokady S (1973) Effect of dietary pectin and algin on blood cholesterol level in growing rats fed a cholesterol-free diet. Ann. Nutr. Metab., 15(4–5): 290–294.

[33] Trumbo P, Schlicker S, Yates AA and Poos M (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J. Am. Diet. Assoc., 102(11): 1621–1630.

[34] Wikiera A, Grabacka M, Byczynski L, Stodolak B and Mika M (2021) Enzymatically extracted apple pectin possesses antioxidant and antitumor activity. Molecules, 26(5): 1434.

[35] Blanco-Pérez F, Steigerwald H, Schülke S, Vieths S, Toda M and Scheurer S (2021) The dietary fiber pectin: health benefits and potential for the treatment of allergies by modulation of gut microbiota. Curr. Allergy Asthma Rep., 21(10): 1–19.

[36] Kerperien J, Jeurink P V., Wehkamp T, van der Veer A, van de Kant HJG, Hofman GA, van Esch ECM, Garssen J, Willemsen LEM and Knippels LMJ (2014) Non-digestible oligosaccharides modulate intestinal immune activation and suppress cow’s milk allergic symptoms. Pediatr. Allergy Immunol., 25(8): 747–754.

[37] Rabbani GH, Teka T, Zaman B, Majid N, Khatun M and Fuchs GJ (2001) Clinical studies in persistent diarrhea: Dietary management with green banana or pectin in Bangladeshi children. Gastroenterology, 121(3): 554–560.

[38] Triplehorn C (2002) A rice based diet with green banana or pectin reduced diarrhoea in infants better than a rice alone diet. BMJ Evid. Based Med., 7(2): 55.

[39] Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W and Pettersson S (2012) Host-gut microbiota metabolic interactions. Science, 336(6086): 1262–1267.

[40] Cui J, Lian Y, Zhao C, Du H, Han Y, Gao W, Xiao H and Zheng J (2019) Dietary fibers from fruits and vegetables and their health benefits via modulation of gut microbiota. Compr. Rev. Food Sci. Food Saf., 18(5): 1514–1532.

[41] Macfarlane GT and Macfarlane S (2012) Bacteria, colonic fermentation, and gastrointestinal health. J. AOAC Int., 95(1): 50–60.

[42] Larsen N, De Souza CB, Krych L, Cahú TB, Wiese M, Kot W, Hansen KM, Blennow A, Venema K and Jespersen L (2019) Potential of pectins to beneficially modulate the gut microbiota depends on their structural properties. Front. Microbiol., 10: 223.

[43] Tian L, Bruggeman G, van den Berg M, Borewicz K, Scheurink AJW, Bruijninx E, de Vos P, Smidt H, Schols HA and Gruppen H (2017) Effects of pectin on fermentation characteristics, carbohydrate utilization, and microbial community composition in the gastrointestinal tract of weaning pigs. Mol. Nutr. Food Res., 61(1): 1600186.
[44] Dongowski G, Lorenz A and Proll J (2002) The degree of methylation influences the degradation of pectin in the intestinal tract of rats and in vitro. J. Nutr., 132(7): 1935–1944.

[45] Mao G, Li S, Orfilla C, Shen X, Zhou S, Linhardt RJ, Ye X and Chen S (2019) Depolymerized RG-I-enriched pectin from citrus segment membranes modulates gut microbiota, increases SCFA production, and promotes the growth of Bifidobacterium spp., Lactobacillus spp. and Faecalibacterium spp. Food Funct., 10(12): 7828–7843.

[46] Gulpil M, Arrigoni E and Amadô R (2005) Influence of structure on in vitro fermentability of commercial pectins and partially hydrolysed pectin preparations. Carbohydr. Polym., 59(2): 247–255.

[47] Sahasrabudhe NM, Beukema M, Tian L, Troost B, Scholte J, Bruininx E, Bruggeman G, van den Berg M, Scheurink A, Schols HA, Faas MM and de Vos P (2018) Dietary fiber pectin directly blocks toll-like receptor 2-1 and prevents doxorubicin-induced ileitis. Front. Immunol., 9: 355.

[48] Chen CH, Sheu MT, Chen TF, Wang YC, Hou WC, Liu DZ, Chung TC and Liang YC (2006) Suppression of endotoxin-induced proinflammatory responses by citrus pectin through blocking LPS signaling pathways. Biochem. Pharmacol., 72(8): 1001–1009.

[49] Goldman H, Bergman M, Djaldetti M, Orlin J and Bessler H (2008) Citrus pectin affects cytokine production by human peripheral blood mononuclear cells. Biomed. Pharmacother., 62(9): 579–582.

[50] Mateos-Aparicio I, Mateos-Peinado C, Jiménez-Escrig A and Rupérez P (2010) Multifunctional antioxidant activity of polysaccharide fractions from the soybean byproduct okara. Carbohydr. Polym., 82(2): 245–250.

[51] Yang B, Zhao M, Prasad KN, Jiang G and Jiang Y (2010) Effect of methylation on the structure and radical scavenging activity of polysaccharides from longan (Dimocarpus longan Lour.) fruit pericarp. Food Chem., 118(2): 364–368.

[52] Cipriani TR, Mellinger CG, Bertolini MLC, Baggio CH, Freitas CS, Marques MCA, Gorin PAJ, Sasaki GL and Iacomini M (2009) Gastroprotective effect of a type I arabinogalactan from soybean meal. Food Chem., 115(2): 687–690.

[53] Wu D, Zheng J, Mao G, Hu W, Ye X, Linhardt RJ and Chen S (2020) Rethinking the impact of RG-I mainly from fruits and vegetables on dietary health. Crit. Rev. Food Sci. Nutr., 60(17): 2938–2960.

[54] Maria-Ferreira D, Nascimento AM, Cipriani TR, Santana-Filho AP, Watanabe P da S, Sant’Ana D de MG, Luciano FB, Bocate KCP, van den Wijngaard RM, Werner MF de P and Baggio CH (2018) Rhamnogalacturonan, a chemically-defined polysaccharide, improves intestinal barrier function in DSS-induced colitis in mice and human Caco-2 cells. Sci. Rep., 8: 12261.

[55] Markov PA, Popov S V., Nikitina IR, Ovodova RG and Ovodov YS (2011) Anti-inflammatory activity of pectins and their galacturan backbone. Russ. J. Bioorg. Chem., 37(7): 817–821.

[56] Meièrjerk M, Rösch C, Taverne N, Venema K, Gruppen H, Schols HA and Wells JM (2018) Structure-dependent immunomodulation by sugar beet arabinoxylans via a SYK tyrosine kinase-dependent signaling pathway. Front. Immunol., 9: 1972.

[57] Nascimento GE, Winnischofer SMB, Ramirez MI, Iacomini M and Cordeiro LMC (2017) The influence of sweet pepper pectin structural characteristics on cytokine secretion by THP-1 macrophages. Food Res. Int., 102: 588–594.

[58] Ishisono K, Yabe T and Kitaguchi K (2017) Citrus pectin attenuates endotoxin shock via suppression of Toll-like receptor signaling in Peyer’s patch myeloid cells. J. Nutr. Biochem., 50: 38–45.
[65] Gao X, Zhi Y, Sun L, Peng X, Zhang T, Xue H, Tai G and Zhou Y (2013) The inhibitory effects of a rhamnogalacturonan I (RG-I) domain from ginseng Pectin on galectin-3 and its structure-activity relationship. J. Biol. Chem., 288(47): 33953–33965.

[66] Sciaccitano S, Lavra L, Morgante A, Ulivieri A, Magi F, De Francesco GP, Bellotti C, Salehi LB and Ricci A (2018) Galectin-3: One molecule for an alphabet of diseases, from A to Z. Int. J. Mol. Sci., 19(2): 379.

[67] Kratchanova M, Nikolova M, Pavlova E, Yanakieva I and Kussovski V (2010) Composition and properties of biologically active pectic polysaccharides from leek (Allium porrum). J. Sci. Food Agric., 90(12): 2046–2051.

[68] Rao RSP and Muralikrishna G (2006) Water soluble feruloyl arabinoxylans from rice and ragi: Changes upon malting and their consequence on antioxidant activity. Phytochemistry, 67(1): 91–99.

[69] Popov S V., Ovodova RG, Golovechenko V V., Khramova DS, Markov PA, Smirnov V V., Shashkov AS and Ovodov YS (2014) Pectic polysaccharides of the fresh plum prunus domestica l. Isolated with a simulated gastric fluid and their anti-inflammatory and antioxidant activities. Food Chem., 143: 106–113.

[70] Di R, Vakkalanka MS, Onumpai C, Chau HK, White A, Rastall RA, Yam K and Hotchkiss AT (2017) Pectic oligosaccharide structure-function relationships: Prebiotics, inhibitors of Escherichia coli O157:H7 adhesion and reduction of Shiga toxin cytotoxicity in HT29 cells. Food Chem., 227: 245–254.

[71] Tasman-Jones C, Owen RL and Jones AL (1982) Semipurified dietary fiber and small-bowel morphology in rats. Dig. Dis. Sci., 27(6): 519–524.

[72] Langhout DJ, Schutte JB, Van Leeuwen P, Wiebenga J and Tamminga S (1999) Effect of dietary high- and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chicks. Br. Poult. Sci., 40(3): 340–347.

[73] McCullough JS, Ratcliffe B, Mandir N, Carr KE and Goodlad RA (1998) Dietary fibre and intestinal microflora: Effects on intestinal morphometry and crypt branching. Gut, 42(6): 799–806.

[74] Nishida M, Murata K, Kanamaru Y and Yabe T (2014) Pectin of Prunus domestica L. alters sulfated structure of cell-surface heparan sulfate in differentiated Caco-2 cells through stimulation of heparan sulfate 6-O-endosulfatase-2. Biosci. Biotechnol. Biochem., 78(4): 635–643.

[75] Bishop JR, Schuksz M and Esko JD (2007) Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature, 446(7139): 1030–1037.

[76] Nagel MD, Verhoef R, Schols H, Morra M, Knox JP, Ceccone G, Della Volpe C, Vigneron P, Bussy C, Gallet M, Velzenberger E, Vayssade M, Cascardo G, Cassinelli C, Haeger A, Gilliland D, Liakos I, Rodriguez-Valverde M and Siboni S (2008) Enzymatically-tailored pectins differentially influence the morphology, adhesion, cell cycle progression and survival of fibroblasts. Biochim. Biophys. Acta Gen. Subj., 1780(7–8): 995–1003.

[77] Nishida M, Murata K, Oshima K, Itoh C, Kitaguchi K, Kanamaru Y and Yabe T (2015) Pectin from Prunus domestica L. induces proliferation of IEC-6 cells through the alteration of cell-surface heparan sulfate on differentiated Caco-2 cells in co-culture. Glycoconj. J., 32(3–4): 153–159.

[78] Gotoh S, Naka T, Kitaguchi K and Yabe T (2021) Arabinogalactan in the side chain of pectin from persimmon is involved in the interaction with small intestinal epithelial cells. Biosci. Biotechnol. Biochem., 85(7): 1729–1736.

[79] Chun W, Bamba T and Hosoda S (1989) Effect of pectin, a soluble dietary fiber, on functional and morphological parameters of the small intestine in rats. Digestion, 42(3–4): 22–29.

[80] Brown RC, Kelleher J, Walker BE and Losowsky MS (1979) The effect of wheat bran and pectin on paracetamol absorption in the rat. Br. J. Nutr., 41(3): 455–464.

[81] Seal CJ and Mathers JC (1989) Intestinal zinc transfer by everted gut sacs from rats given diets containing different amounts and types of dietary fibre. Br. J. Nutr., 62(1): 151–163.

[82] Mudgil D and Barak S (2013) Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: A review. Int. J. Biol. Macromol., 61: 1–6.

[83] Mansoor Baig M, Burgin CW and Cerda JJ (1983) Effect of dietary pectin on iron absorption and turnover in the rat. J. Nutr., 113(12): 2385–2389.

[84] van der Aar PJ, Fahey GC, Ricke SC, Allen SE and Berger LL (1983) Effects of dietary fibers on mineral status of chicks. J. Nutr., 113(3): 653–661.

[85] Brown RC, Kelleher J and Losowsky MS (1979) The effect of pectin on the structure and function of the rat small intestine. Br. J. Nutr., 42(3): 357–365.