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The production of galacturonic acid enriched fractions and their functionality

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ABSTRACT

Vegetable tissues discarded at harvesting or after industrial processing constitute a valuable and renewable source of biopolymers and bioactive compounds. Upgrading of vegetable waste can not only reduce pollution but also add value to the commodity production. Plant cell walls are an important source of galacturonic acid compounds The present paper reviews some selected bibliography published in the last years concerning the production of pectin and oligosaccharides based on galacturonic acid, and their functionality with special reference to their effect on health.

In relation to pectin it can be concluded that, in addition to their ability to thicken and form gels, they are soluble dietary fibers. They can also act as prebiotics, but more systematic studies must be performed to elucidate the effect of their chemical structure on health. With respect to pectic oligosaccharides rich in galacturonic acid, they have been proposed as prebiotics and immunity enhancers, but the absence of standardized techniques for their production and purification, the ample variety of substrates involved and the lack of systematic studies about their structure does not allow to conclude nowadays about the structure-function relationship and it is a must to deepen these studies to help to the rational usage of oligosaccharides in functional foods.

1. Introduction

Vegetable tissues discarded at harvesting or after industrial processing constitute a valuable and renewable source of biopolymers and bioactive compounds (Goñi & Hervert-Hernández, 2011).

Polymerized anhydrogalacturonic acid in which some of the carboxylic acid groups are methyl esterified is the main constituent of pectin (Nussinovitch, 1997) which can be isolated from plant tissues by means of disruption of cell wall and middle lamella. It is well known that pectin acts modifying food rheology and texture due to its thickening and gelling capacity (Kjøniksen, Hiorth, & Nystrom, 2005). Pectin is a soluble dietary fiber that has also direct and indirect nutritional and physiological actions (Dongowski, Lorenz, & Anger, 2000) in relation to human health.

Partial hydrolysis of pectin by chemical and/or enzymatic methods leads to the production of pectin-derived oligosaccharides (POS), which have been proposed as having important physiological properties, including their prebiotic activity (Mandalari et al., 2007; Mussatto & Mancilha, 2007).

The present paper reviews some selected bibliography published in the last years concerning the production and properties of pectin and oligosaccharides based on D-galacturonic acid, with the purpose of contributing to: a) systematizing the information, and b) the understanding of the effect of the different substrates used and the procedures followed for their production, on the functionality of these compounds.

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2. Pectin

D-galacturonic is an acid sugar. It is a monosaccharide containing six carbon atoms and its structure corresponds to the oxidized form of galactose. In its open form, it has an aldehyde group at C1 and a carboxylic acid group at C6.

Polymerized anhydrogalacturonic acid in which some of the carboxylic acid groups are methyl esterified is the main constituent of pectin (Nussinovitch, 1997). The pectins are abundant in the primary cell walls and the middle lamellae of plants.

The structural classes of the pectic polysaccharides include homogalacturonan (HG), xylogalacturonan (XGA), apiogalacturonan (AGA), rhamnogalacturonan II (RG-II) and rhamnogalacturonan I (RG-I) (Caffall & Mohnen, 2009).

The HG, RG-I and RG-II domains can be covalently linked to form a pectic network throughout the primary cell wall matrix and middle lamellae. HG is a linear homopolymer of α-1,4-linked anhydrogalacturonic acid and is thought to contain some 100–200 galacturonic acic (GalA) residues and is present in the cell wall in a form that has 70–80% of GalA residues methylesterified at the C-6 carboxyl (Willats, McCartney, Mackie, & Knox, 2001) but esterification degree depends on plant species, plant tissues and ripeness state (Hyodo et al., 2013) and can be modified by isolation technique. The removal of methyl ester groups within the cell wall matrix results in HG capable of being cross-linked by calcium and form gels. GalA residues in HG can be O-acetylated, predominantly at C-3. The substitution of the C-3 of GalA with residues of xylose produces a domain known as xylogalacturonan. Substitution of GalA with apiose at C-2 or C-3 results in apiogalacturonan. RG-I is an acidic pectic domain consisting of as many as 100 repeats of the disaccharide

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α-1,2-L-rhamnose- α-1,4-D-galacturonic acid which has been isolated from a wide range of plants and is ramified principally with arabinan, galactan and arabinogalactan (Mohnen, 2008). RG-II is a branched pectic domain containing a backbone of around 9 GalA residues that are α-1,4-linked and is substituted by 4 heteropolymeric side chains that contain eleven different sugars including apiose, aceric acid and 2-keto-3-deoxy-D-manno-octulosonic acid (KDO) (Albersheim, Darvill, O'Neill, Scols, & Voragen, 1996; Willats et al., 2001; Gullon et al., 2013).

Pectin is present in an ample variety of plant tissues and can exert different actions. For example, Njoroge et al. (2014, 2015) reported the influence of its structure and cross-linking on the development of hard to cook behavior of common beans.

The main uses for pectins are as gelling agents, thickening agents and stabilizers in foods. Pectins have a great capacity for water retention and can gel under adequate conditions. The consumption of soluble polysaccharides which increase the viscosity can delay and reduce the concentration of glucose in blood because of the restricted access of amylases to starch. Soluble polysaccharides can also reduce the levels of total cholesterol and low density lipoproteins (LDL) cholesterol in blood (Brouns et al., 2012).

Emulsifying properties have been also reported and are attributed to the proteins that are associated to pectins (Saha & Bhattacharya, 2010; Ngouémazong, Christiaens, Shpigeman, Van Loey, & Hendrickx, 2015).

According to their degree of methylation, pectins can be classified as high methoxyl pectins, HMPs (50% esterified or higher) or low methoxyl pectins, LMPs (less than 50% esterified). HMPs form gels in acidic and high soluble solid conditions whereas LMPs gel in the presence of divalent ions such as calcium, being this capacity of great interest in low caloric value foods (Seshadri, Weiss, Hulbert, & Mount, 2003). For the high methoxyl pectins, chain association occurs by means of junction zones that are stabilized by hydrogen bonds between non-dissociated carboxyl and secondary alcohol groups and by hydrophobic interactions between methoxyl groups. This phenomena changes food viscosity and can produce gelation. It is well known that HMPs form gels at acid pH in presence of a large amount of sugars which reduce the water activity. For LMPs, hydrogen-bonded intermolecular complexes play a prominent role in the chain association process. For this type of pectins, the gelation occurs in the presence of divalent cations such as calcium, which act as a bridge between pairs of carboxyl groups of different pectin chains (Fraeye, Duvetter, Doungla, Van Loey, & Hendrix, 2010; Kjøniksen et al., 2005).

Carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by the endogenous enzymes in the small intestine of humans, are considered as dietary fiber compounds (Philips & Cui, 2011). This definition can be extended to carbohydrate polymers with three to nine monomers (Lupton, Betteridge, Loek, & Pijls, 2009). Non-processed carbohydrates transit the large intestine where they become food for the commensal bacterial community. The species within the gut microbiota use a variety of strategies to process and scavenge both dietary and host-produced carbohydrates such as mucins. They produce secondary metabolites and fermentation derived compounds that can influence cell proliferation and apoptosis, modulate the immune response and can alter host metabolism impacting on obesity, diabetes, inflammatory bowel disease, colon cancer, gastrointestinal infections, and potentially many health problems (Cockburn & Koropatkin, 2016).

Pectins are a type of soluble fiber commonly present in vegetal tissues, and this review will focus on innovative methods proposed for their isolation, on the use of non-traditional substrates for their production and on the functional properties they have in relation to health.

2.1. Isolation of pectins

The biopolymer network present in plant tissues must be partially disrupted to enable extraction of pectin, for example, through the use of calcium-chelating agents, alkali or acid. Extraction conditions can alter pectin composition, structure and physiological properties. Pectin extraction from raw material is usually performed on citric peels or apple bagasse remaining from industrialization of these fruits, under acidic conditions (pH 1.5–3.0) and at high temperatures (70–90 °C) using hydrochloric acid, nitric acid or sulfuric acid. The raw acid extract is separated, in general, from the residue by filtration or centrifugation and pectin is precipitated with alcohol. Purification, drying and milling yield powdered pectin (Vriesmann, Teófilo, & de Oliveira Petkowicz, 2011).

The acidic conditions generally used by the industry generate a high amount of effluents and their treatment represents an additional cost that promotes the development of more environmentally friendly procedures.

The use of non pectolytic enzymes for pectin extraction is an alternative to industrial acidic extraction. These enzymatic techniques have been described by different authors. For example, Ptichkina, Markina, and Rumyantseva (2008) obtained pectin from an alternative source such as pumpkin (*Volzhskaya Grey* variety) using cellulase and pectinesterase activities. Fissore, Matkovic, Wider, Rojas, and Gerschenson (2009), Fissore, Ponce et al. (2009), and Fissore et al. (2011) studied the isolation of butternut (*Cucurbita moschata*) and red beet (*Beta vulgaris)* pectin by means of the use of cellulase and hemicellulase activities. Campbell (2006) studied the optimized production of pectin form watermelon (*Citrullus lanatus*) using cellulase. A more detailed explanation of these researches is given in Table 1.

Other authors studied the pectin extraction with the help of different techniques. For example, Chen, Hu, Yao, and Liang (2016) proposed the extraction from processed pomelo peels previously submitted to an essential oil extraction, with the help of microwaves and using hydrochloric acid (Table 1) observing a diminishing of processing time. Freitas de Oliveira et al. (2016) evaluated by response surface methodology, the effect of ultrasound power intensity and of the temperature used, on the pectin extraction from passion fruit peel using nitric acid and concluded that ultrasound increased pectin yield (Table 1). Pereira et al. (2016) studied the extraction of pectins from pomegranate peels with citric acid, a more environmentally friendly acid (Table 1).

Huang, Jeffrey, Zhang, and Huang (2012) studied the effect of the use of different ionic liquids in combination with microwaves on pectin yield by means of response surface methodology. The ionic liquids are solvents composed of organic cations and inorganic or organic anions, and have excellent characteristics for extracting and high microwave-absorbing ability. The highest yield was obtained with 1-butyl-3-methylimidazole chloride (25% w/w, dry basis) and with the following conditions of extraction: an extraction temperature of 88 °C, an extraction time of 9.6 min, and a liquid-solid ratio of 22.7 mL/g. The use of reflux in a water bath at 88 °C instead of microwaves, gave origin to a yield of 13% w/w dry basis after a treatment time of 2 h.

The direct interaction of microwaves with the ionic liquid solutions and the free water molecules present in the cells probably resulted in the rupture of the cells and release of intracellular products

Table 1
Production of pectin from different substrates.

Sustrate	Procedure	Fraction yield and composition	Properties/Advantages	References
Pumpkin (*Volzhskaya Grey* variety)	Treatment with enzymatic complex (cellulose, xylanase, beta-glucosidase, endopolygalacturonase, pectinesterase) 2 mg/mL. Time: 3 h,. pH: 5.0. Temperature: 45 °C.	Polysaccharide yield: 14%w/w, dry basis. Degree of methoxylation (DM): 53%. Molecular mass: 45 kDa.	Gel formation with 60% w/w sucrose at pH 3. Gel strength: 10 kPa.	Ptichkina et al. (2008)
Butternut (*Cucurbita moschata*)	Enzymatic treatment with cellulase and hemicellulase activities. Temperature: 30 °C, Time: 20 h pH: 5.2.	Yield: 4–7% w/w, dry basis.	Butternut pectin showed thickening capacity. Pseudoplastic behavior and delaying of glucose absorption *in vitro*.	Fissore, Matkovic et al. (2009) and Fissore, Ponce et al. (2009)
Red beet (*Beta vulgaris)*	Basic treatment and enzymatic treatment with cellulase activities. Temperature: 30 °C, Time: 20 h pH: 5.2.	Yield: 10–20% w/w, dry basis. Pectin with low DM. Galacturonic acid content: 54% w/w, dry basis.	Beetroot pectin gelled in the presence of calcium	Fissore et al. (2011)
Watermelon (*Citrullus lanatus*)	Enzymatic (cellulase) techniques. Solid(liquid ratio: 0.18 g/mL. Enzyme loading: 9.7 FPU/g. Time: 2 h pH: 4.0 (citric acid). Temperature: 50 °C.	Yield: 34% w/w, dry basis, of low methoxyl pectins. Galacturonic acid content: 47% w/w, dry basis.	–	Campbell (2006)
Pomelo peels previously submitted to an essential oil extraction	Hot solvent microwave extraction (HSME) using hydrochloric acid. Microwave power: 520 W. Time: 5.6 min. Water pH value:2.0. Time. 5.6 min.	Yield: 3% w/w, dry basis.	HSME method allowed a diminishing of processing time and an increase in yield in comparison to traditional acidic solution method (HCl aqueous solution at pH 2.0, temperature 90 °C, 90 min).	Chen, Hu et al. (2016)
Passion fruit peel	Nitric acid extraction assisted by ultrasound. Dried peel/extractant ratio: 1:30. Time of sonication: 10 min. Power intensity: 644 W/cm^2. Temperature: 85 °C.	Yield: 13% w/w, dry basis. Galacturonic acid content 67% w/w, dry basis. DM: 60%.	Extraction yield increased 1.6 fold when the extraction was assisted by ultrasound with respect to conventional extraction (dried peel/extractant ratio 1:30, temperature 85 °C, 10 min).	Freitas de Oliveira et al. (2016)
Pomegranate peels	Citric acid extraction. Time: 120 min. Temperature: 88 °C. pH 2.5.	Yield: higher than 8% w/w, dry basis. DM: equal or higher to 54%.	The use of citric acid prevents the generation of environmentally unfriendly (corrosive) effluents, requiring special treatments.	Pereira et al. (2016)

into the solvent achieving a higher extraction efficiency in shorter extraction times.

Pectin was extracted by Chen, Fu, and Luo (2015) from sugar beet pulp using subcritical water combined with ultrasonic-assisted treatment. Maximum yield of 25% w/w, dry basis, was obtained with a liquid/solid ratio of 44.03, an extraction temperature of 121 °C, an extraction time of 30 min and an extraction pressure of 11 MPa. The pectin contents of galacturonic acid and arabinose were 59 and 21.66% w/w dry basis, respectively.

Freitas de Oliveira et al. (2015) studied the extraction of pectin from passion fruit peel using moderate electric field and compared the results with the ones obtained with conventional heating extraction methods. In both cases, the galacturonic acid content and the degree of esterification obtained was high. They observed that pH exerts an important effect on yield and that using a peel/extractant (nitric acid solution) ratio of 1:30 (w/v), voltage of 50 V, temperature of 30 °C and time of 15 min, the yield obtained was 7% w/w dry basis at a pH of 5.0. Galacturonic acid content of the product was 62% w/w dry basis, and degree of methoxylation (DM) was 81%. In the case of conventional extraction using a peel/extractant ratio of 1:30 (w/v), temperature of 85 °C and pH 2.0, for a time of 60 min, the yield was 11% w/w dry basis and the GalA content of the product was 70% w/w dry basis and the DM was 87%. They concluded that moderate electric field is an efficient, time saving, and eco-friendly method for the extraction of pectin from passion fruit peel, especially for pectin with a high esterification degree and a GalA content higher than 65% w/w dry basis.

Ognyanova et al. (2016) isolated a pectic polysaccharide from rosehip fruits (*Rosa canina* L.) using a citric aqueous solution (1% w/v). They found that the pectic fraction isolated mainly consisted of GalA (45% w/w dry basis) but also contained galactose (5.5% w/w dry basis) and arabinose (4.7% w/w dry basis). The DM was 62% and the acetylation degree was 10% and it presented molecular weight populations of 10–100 kDa. These researchers performed an enzymatic fingerprinting using a combination of pectin lyase and endo-polygalacturonase combined with the use of liquid chromatography (LC)-hydrophilic interaction liquid chromatography (HILIC)-mass spectrometry (MS)/evaporative light scattering detector (ELSD) and high performance anion exchange chromatography (HPAEC), to obtain detailed information about the structure and level of GalA oligomers released. All this with the object of concluding about the relation between the isolation method applied and the characteristics of the pectin obtained.

Guo, Meng, Zhu, Zhang, and Yu (2015) proposed the metal precipitation as an effective method for the purification of pectins. Sugar beet pectins were precipitated by means of copper obtaining two fractions, copper-precipitated pectins (CPP) and copper-unprecipitated pectins (CUP). The isolated fractions were structurally different because CUP contained markedly higher neutral sugars (NS) and protein contents, but lower GalA and acetyl ester group contents than CPP. However, no appreciable difference was observed in terms of DM. Elution profiles on high performance size exclusion chromatograph showed that CPP exhibited a monomodal-like molecular weight distribution (MWD), while CUP demonstrated a multimodal-like MWD pattern composed of three relatively broad peaks, and a narrow peak that was associated with a high intensity of UV signal at 278 nm. They concluded that copper ions selectively bind the anionic regions among pectin chains, thereby separating pectic saccharides from non-uronide compounds like low molecular weight carbohydrates and free proteins. The poor affinity of copper ions toward CUP might be ascribed to the absence of typical homogalacturonan (HG) regions.

Adetunji, Adelunke, Orsat, and Raghavan (2017) wrote an excellent review in which there were evaluated some novel techniques

for pectin production such as enzyme-assisted extraction, subcritical water extraction, microwave-assisted extraction and ultrasound-assisted extraction. They concluded that for the integration of these techniques in industrial production it is a must to have additional information concerning their scale up, the capability of their inclusion in a continuous process and that a drawback of these technologies is the risk of their potential impact on the increased cost of the final product.

2.2. Pectin and its functional properties

Cobs-Rosas, Concha-Olmos, Weisntein-Oppenheimer, and Zúniga-Hansen (2015) isolated from defatted rapeseed cake and by different extraction methods, pectic substances and studied their antiproliferative activity and biological activity on cancer cell lines. The isolation process consisted of sequential treatment with alkalized water (pH ~ 8), EDTA (0.01 M) and alkaline protease (Alkalase 2.4L) was used for desproteinization. Pectic substances extracted from rapeseed cake exhibited antiproliferative activity on MCF-7 breast cancer cell line and Caco-2 colorectal carcinoma cell line. EDTA and alkalized water treatment, yielded acid pectins with different antiproliferative profiles. While EDTA produced pectic extract exhibiting high antiproliferative activity at the highest concentration, alkalized water produced pectic extract that displayed an effect which was dependent on the tested cell line. For Caco-2 cells their effect was comparable to the one of EDTA, while on the MCF-7 cells this extract showed the second best response. These results show that different methods of extraction yield products that might differ on their activity when tested on certain cell lines.

Rubio-Senent, Rodriguez-Gutierrez, Lama-Muñoz, and Araanzazu-Gracía (2015) studied the production of water soluble polysaccharides from olive mill wastewater, which is a subproduct of olive oil industry. They used ethyl alcohol precipitation with 40 and 80% (v/v) EtOH. The isolated pectic materials presented a high molecular size and a low percentage of methyl esterification and acetylation. In comparison with commercial citrus pectin, the extracts had better oil holding capacity and similar emulsifying activity. They also showed a higher capacity for binding bile acids and glucose than commercial product, properties that might help in the reduction of cholesterol levels and in the delaying of the adsorption of intestinal glucose. The high polyphenol content and high antioxidant activities of the raw and purified polysaccharides suggested that they have the potential to function as antioxidant dietary fiber, combining the beneficial physiological effects of both dietary fiber and antioxidant in a single material.

Chen, Jin et al. (2016) studied by response surface methodology, the obtention of pectic polysaccharides from tangerine peels using microwave-assisted extraction(MAE) with powers ranging from 600 W to 800 W. The optimal extraction conditions, using water as extractant, were a microwave power of 704 W, an extraction temperature of 52.2 °C, and an extraction time of 41.8 min and they obtained a yield of 19.9% w/w dry basis. The use of anion exchange and gel filtration chromatographies allowed to purify the pectin. The content of total carbohydrates, uronic acid and protein were 89.2%, 44.8%, and 6.5% w/w dry basis, respectively. Sugar analysis showed that the pectin was constituted by (w/w dry basis) 42.5% of galacturonic acid, 23% w/w dry basis of arabinose, 20% of galactose, 6.9% of rhamnose, 4.2% of glucose and 3.5% of mannose. The antioxidant capacity was evaluated observing that pectin isolated had a high ferric reducing antioxidant power (FRAP) value, a strong radical scavenging activity against $^{\bullet}OH$ and $^{\bullet}$2,2-diphenyl-1-picrylhydrazyl (DPPH), but a weak scavenging activity against $^{\bullet}O_2$ and that the activity was affected by its monosaccharide composition, molecular weight and extraction methods.

It is well known that pectin is fermented in the large intestine to SCFA and gases. Decomposition of the polysaccharide pectin occurs during the following main steps: (i) macromolecular pectin, (ii) (unsaturated) oligoGalA, (iii) monogalacturonic acid or its rearrangement products, and (iv) SCFA and gases. Dongowski et al. (2000) studied the capacity of microorganisms present in complete human fecal flora as well as of cultures of defined species to ferment citrus pectins of high molecular weight and with different degrees of methoxylation. The pectin-degrading microorganisms obtained from human feces *Bacteroides thetaiotaomicron* alone and in co-culture with *Escherichia coli* were tested as pectin-degrading microorganisms. The levels of oligogalacturonic acids formed during the assay were estimated by using high performance thin-layer chromatography and high-performance anion-exchange chromatography. In the case of the complete fecal flora, the oligocompouds changed permanently but after 24 h, no oligogalacturonic acids were detected. The pectin-degrading activities of pure cultures of *B. thetaiotaomicron* were lower than the pectin-degrading activity of a complete fecal flora. Cocultures of *B. thetaiotaomicron* and *E. coli* exhibited intermediate levels of degradation activity and no pectin-degrading activity was found in pure cultures of *E. coli*. Additionally, the rate of pectin degradation was affected by the degree of esterification of the substrate. The disappearance of oligogalacturonic acids in the later stages of fermentation with both the complete fecal flora and *B. thetaiotaomicron* was accompanied by an increased formation of short-chain fatty acids. Low methoxyl pectins were fermented *in vitro* more efficiently than high methoxyl pectins. The authors concluded that it seems to be possible to extend the region of intense formation of SCFA from the proximal into the distal parts of the colon by using dietary fibers like pectin with special structural parameters.

The classification of a food ingredient as a prebiotic requires a scientific demonstration that the ingredient resists gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption, is fermented by the intestinal microflora and stimulates selectively the growth and/or activity of intestinal bacteria associated with health and wellbeing (Roberfroid, 2007). Olano-Martin, Gibson, and Rastall (2002) compared the *in vitro* fermentation properties of pectins and oligosaccharides derived from them in pure and mixed faecal cultures. They concluded that the degree of methylation plays an important role in the fermentation properties of pectins. Citrus pectin with a degree of methylation of 66% and apple pectin with a degree of methylation of 8% were studied and it was evaluated the growth of Bifidobacteria, Lactobacilli, Clostridia and Bacteroides. Both of the pectins (1% w/w concentration in the media) significantly increased of approximately 0.7 ($p < 0.01$) the number of bifidobacteria. Also LMP significantly increased ($p < 0.01$) the number of Bacteroides. There was a clear influence of the DM on the fermentations, with highly methylated carbon sources giving lower growth rates than the lower methylated ones.

Ferreira, Passos, Madureir, Vilanova, and Coimbra (2015) stated that pectin is an immunostimulatory polysaccharide because it is capable of interacting with the immune system and enhance specific mechanisms of the host response and that its activity depends on its structure. For example, a higher degree of acetylation, due to acetyl groups localized in the galacturonan backbone, had been associated to a lower activity, which increased after deacetylation procedure.

3. Pectic oligosaccharides

While natural monosaccharides are comprised of a single saccharide unit generally with 5- or 6-carbon atom skeleton, the oligosaccharides are less clearly defined. According to Linhardt and Bazin (2001) they may consist of 2–10 glycosidically linked monosaccharide units. Other authors like Ridley, O'Neill, and Mohnen (2001) and Willems and Low (2016) proposed that oligosaccharides are molecules constituted by two to about twenty monomers.

Partial hydrolysis of pectin by chemical and/or enzymatic methods leads to the production of pectin-derived oligosaccharides (POS), substituted or non-substituted, which have been proposed as a new class of prebiotics. POS includes oligogalacturonides (OGalA), galactooligosaccharides (GalOS), arabinooligosaccharides (AraOS), rhamnogalacturonoligosaccharides (RhaGalAOS), xylooligogalacturonides (XylOGalA) and arabinogalactooligosaccharides (AraGalOS) (Gullon et al., 2013).

In this review, it is discussed some recent literature concerning POS rich in galacturonic acid.

Oligosaccharides resist digestion until the colon and are claimed to behave as dietary fibers and prebiotics (Mussatto & Mancilha, 2007). These effects improve gut microecology including bacterial populations, biochemical profiles and physiological effects, influencing host immunity and metabolism (Bindels & Thissen, 2016). According to Babbar. Dejonghe, Gatti, Sforza, & Elst. (2016b), these oligosaccharides have health effects and are active ingredients adequate for functional food formulation because they have physiological benefits and reduce the risk of chronic disease beyond basic nutritional functions.

3.1. Production of pectic oligosaccharides and their functionality

Gullón, Gullón, Sanz, Alonso, and Parajó (2011) subjected apple pomace samples to simultaneous saccharification and fermentation to yield a medium containing mainly lactic acid and oligosaccharides. After purification and concentration, oligogalacturonides were quantitatively recovered. As a result of these treatments, the mass fraction of oligomers in the product increased from 0.360 up to 0.677% w/w dry basis. The refined product showed prebiotic potential by means of *in vitro* fermentability assays performed with individual microbial strains or human fecal inocula (Table 2).

Combo, Aguedo, Goffin, Wathelet, and Paquot (2012) studied the individual efficiency of six commercial pectinase preparations (Endopolygalacturonase M2, Pectinase, Viscozyme L, Pectinex Ultra SP-L, Pectinase 62L and Macer8 FJ) in catalyzing the liberation of POS from polygalacturonic acid. All enzymes generated oligogalacturonic acid with different degrees of polymerization (DP). The authors evaluated by high performance anion exchange chromatography with pulsed amperometric detection, the POS with DP ranging from 1 to 3 due to the unavailability of other commercial standards. They informed that hydrolysis of polygalacturonic acid with these enzymes allowed the release of the monomer and of several oligomers, depending on the hydrolysis conditions and concluded that reaction times should be controlled if large amounts of monomers are not wanted. Data for endopolygalacturonase M2, which was the more efficient enzyme preparation for production of POS, are reported in Table 2.

Combo et al. (2013) studied the enzymatic hydrolysis of sugar beet pectin using endopolygalacturonase and pectinmethylesterase to produce pectic oligosaccharides. Three POS were obtained for different treatment times (2, 5, 15 min). After only 2 min of digestion, commercial pectinases produced a mixture of oligogalacturonic acid

Table 2
Production of pectic oligosaccharides (POS) from different substrates.

Sustrate	Procedure	Purification/Fractionation	Fraction composition	Properties	References
Apple pomace	Saccharification and fermentation using *Lactobacillus rhamnosus* (ATCC-9595) strain, cellulases from *Trichoderma reesei* and *β*-glucosidase from *Aspergillus niger*	Purification and concentration of oligosaccharides by ion exchange (lactic acid removal) and discontinuous diafiltration (Lactate, arabinose, NaCl elimination)	Oligogalacturonides Oligomer concentration in final stream: 81 kg/m^3	Prebiotic	Gullón et al. (2011)
Polygalacturonic acid	Enzymatic Endopolygalacturonase. Temperature: 40 °C. pH: 5.5. Time: 2 h		Oligogalacturonic acid with different degree of polymerization (DP). Yield: 18% w/w digalaturonic acid and 58% w/w of trigalacturonic acid (dry basis)	–	Combo et al. (2012)
Sugar beet	Enzymatic. Endopolygalacturonase and pectinmethylesterase Time: 2 min		Galacturonic acid (GalA) amount: 59% w/w dry basis. Molecular weight: 1.8–422 kDa. DP: 1–9.	Low intrinsic viscosity. Anionic structure with capacity for electronic interactions	Combo et al. (2013)
Apple pectin	Dynamic high pressure microfluidization (pectin concentration 1.84%, solution temperature 63 °C, pressure 155 MPa, number of cycles 6 passes)	Ultrafiltration with a 5000 Da molecular weight cut-off	Oligogalacturonides containing 29% w/w GalA and 58% w/w neutral sugars (dry basis) in a quantity of 606 mg POS/ 100 mL flow.	Prebiotic	Chen et al. (2013)
Orange peel	Enzymatic. Multi-enzyme preparation from *Aspergillus japonicus* PJ01	Membrane fractionation	Fractions rich in glucose, arabinose, and GalA (13–24% w/w total monosaccharides). Molecular weight of pectin oligosaccharides (POS): fraction POS1 <1 kDa; 1 kDa < fraction POS2 < 3 kDa; fraction POS3 > 3 kDa.	Prebiotic and antimicrobial. POS2 showed the highest prebiotic properties POS1 and 2 showed the highest antimicrobial activities	Pei-Jun et al. (2016)

showing DPs between 1 and 9 (Table 2). The POS obtained were more sensitive to thermal degradation and showed lower crystallinity than the parent pectin.

Chen et al. (2013) prepared pectic oligosaccharides (POS) from apple pectin by using an innovative technique, dynamic high-pressure microfluidization. Operating under selected conditions (Table 2), 33% of the pectin was converted into POS. The prebiotic properties of POS were evaluated using a fecal batch culture fermentation. The POS increased the number of Bifidobacteria and Lactobacilli, and produced a higher concentration of acetic, lactic, and propionic acid than their parent pectin. Furthermore, POS decreased the number of Bacteroides and Clostridia while their parent pectin increased them. The effects of POS on the growth of these bacteria and production of short-chain fatty acids were comparable to those of the fructooligosaccharides. Olano Martin et al. (2002), stated that POS are a better prebiotic candidate than the pectins, because molecular size affects the prebiotic potential and that pectins can have their functional properties improved by partial hydrolysis to POS.

Pei-jun, Jin-lan, Zhen-yuan, & Yang. (2016) studied the production of POS from orange peel through hydrolysis using enzymatic techniques (Table 2). The hydrolyzate was fractionated via membrane separation into three different molecular weight fractions which presented prebiotic and antimicrobial properties. The size of the oligomers had an impact upon the growth of probiotic bacteria, being the oligomer POS2 (DP: 6–8) which presented the intermediate molecular weight, the one that showed the highest prebiotic properties. When the three fractions were compared, it was observed that POS2 was the one that presented the highest GalA content. The main component of this fraction was glucose and GalA ranked the second component followed by arabinose and galactose.

According to Ridley et al. (2001), the minimum size of OGalAs required for most of the biological activities reported and the minimum size required for the formation of a Ca^{2+} structure, coincide at a degree of polymerization of, approximately, 10.

Willems and Low (2016) studied the effect of enzyme treatment (commercial enzyme preparation) and processing on the oligosaccharide (degree of polymerization, DP, of 1–20) profile of industrial samples representing the major stages of pear juice processing. It was found that the majority of polysaccharide hydrolysis and oligosaccharide formation occurred during enzymatic treatment at the pear mashing stage and that the remaining processing steps had minimal impact on the carbohydrate profile of pear juice. Chromatographic analysis confirmed the presence of the following carbohydrates from pectin, D-galacturonic acid and D-galacturonic methyl ester, linear (DP 2 and 3) polymers of D-galacturonic acid and D-galacturonic acid methyl ester. The authors remarked that enzymatic treatment of juices could be tailored so as to increase its soluble fiber content, which could result in a final product with improved health properties.

Gómez, Gullon, Yáñez, Schols, and Alonso (2016) used lemon peel wastes to obtain POS containing liquors by hydrothermal treatment at 160 °C for 326 min followed by a purification performed through a two step membrane process (discontinuous diafiltration and concentration). Membrane purification was performed using a spiral wound membrane of regenerated cellulose of 1 kDa molecular weight cut off. They observed a high oligogalacturonides content (0.624 kg/kg non volatile compounds), together with significant amounts of arabino-oligosaccharides (0.139 kg/kg non volatile compounds) and galacto-oligosaccharides (0.094 kg/kg non volatile compounds). *In vitro* fermentability tests showed that the joint populations of bifidobacteria and lactobacilli increased from 19% up to 29% in cultures with lemon POS. It was also observed an enhancement of the growth of *Roseburia intestinalis* group. The short chain fatty acid profile showed the following order: acetate > butyrate > propionate. The authors concluded that lemon oligosaccharides might have potential as prebiotic ingredients for a variety of functional foods.

Babbar, Baldassarre et al. (2016) used onion skins for the enzymatic production of pectic oligosaccharides (POS) with a targeted degree of polymerization (DP). As the pectin present in onion skins is calcium bounded, the proposed extraction method was based on sodium hexametaphosphate because it can loosen the homogalacturonan-calcium complex. The process was based on a two stage process consisting of a chelator based crude pectin extraction followed by a controlled enzymatic hydrolysis. Various enzymes were used, (Viscozyme L(V-2010), a multienzyme complex), Pectinase and Endo-polygalacturonase M2) and it was observed that the EPG-M2 was the most appropriate for tailored POS production. The highest amount of DP2 and DP3 was obtained at a time scale of 75–90 min with an EPG-M2 concentration of 26 IU/mL. At these conditions, the yield was ≃3.0% dry basis for DP2 and ≃5.5% dry basis. for DP3, respectively. The maximum DP4 production of ≃5.5% dry basis. was obtained with 5.2 IU/mL at times of 15–30 min. As the pectin present in onion skins is mainly constituted by homogalacturonan, oligosaccharides obtained were mainly OGalA. A total yield of 21% of galacturonic acid was obtained without the formation of monosaccharides. It was observed the presence of different forms of oligomers in the pectic digests of onion skins and, in general, the higher the DP, the higher the possibility of having a methylated form, due to the fact that the presence of at least one methyl group becomes statistically more likely with more galacturonic residues present. Acetylated forms were not observed in the digests because acetylation is practically absent in the onion peels.

Bustamante-Vargas et al. (2015) used the commercial pectinase Rohapect®DA6L from *Aspergillus niger* immobilized in rigid polyurethane foam (RFPU) for the obtention of POS. The yield of immobilization was of 178.64% when it was calculated taking into consideration the total experimental activity of the immobilized enzyme and the total activity of lyophilized enzyme offered to the immobilization. The optima reaction conditions for free and immobilized pectinase were pH 3.5 and 37 °C and pH 4.5 and 55 °C, respectively. To determine the stability to the storage of both free and immobilized pectinases, the enzymes were stored dry at 4 °C and the activities were determined periodically during 229 days. Considering the initial activity as 100%, it was observed that during the period of evaluation, the immobilized biocatalyst presented an hyperactivation, showing a residual activity of 198% after 229 days. These results suggest that the support offers a protection to the enzyme allowing an increase of pectinolytic activity, as a result of improvement of the microenvironment created by the polymeric lattice which protected the enzyme from adverse effects. The immobilization process did not change the affinity of the enzyme for the substrate. The immobilized pectinase in RFPU was reused consecutively by 6 catalytic cycles in the hydrolysis of pectic oligosaccharides, keeping 35% of its initial activity. The immobilization procedure is a potential alternative for enzymatic processes occurring in industrial reactors and can help in the development of enzymatic processes at industrial scale.

Leijdekkers, Huang, Bakx, Gruppen, and Schols (2015) studied the separation and characterization of complex mixtures of pectic oligosaccharides which often requires the use of multiple analytical techniques. They demonstrated that the coupling of hydrophilic interaction chromatography (HILIC) to traveling-wave ion mobility mass spectrometry (TWIMMS) enabled the simultaneous separation and characterization of complex mixtures of various isomeric pectic oligosaccharides. Labeling of oligosaccharides with 3-aminoquinoline (3-AQ) improved MS-ionization efficiency of the oligosac-

charides and reduced the complexity of the product ion mass spectra, without losing resolution of the HILIC separation. Isomeric structures were distinguished using TWIMMS. This integrated approach is very interesting because it allows to know the structure of galacturonic acid oligosaccharides produced with different techniques, information with fundamental implications for the understanding of the effect of different isolation techniques on products structural and functional characteristics.

4. Conclusions and future trends

D-galacturonic is a monosaccharide containing six carbon atoms that constitutes the polygalacturonic acid and is the main constituent of pectin.

Pectin is present in an ample variety of plant tissues and can exert different actions, being used as a gelling agent, thickening agent and stabilizer in foods. Its viscosity enhancing effect can reduce the concentration of glucose and cholesterol in blood. The acidic conditions generally used by the industry for its production generate a high amount of effluents and more environmentally friendly procedures like the use of citric acid or of non pectolytic enzymes have been recently developed. Also hot solvent microwave extraction, ultrasounds and subcritical water extraction had been suggested to diminish procedure time and energy consumption and/or to increase yield. But changes in process modify the properties of the product and more systematic studies must be performed to completely evaluate alternative techniques in aspects like their effect on pectin functional properties as well as their potential hidden impacts on the environment.

In the last years, it has been observed an increased interest on the production, purification, and chemical characterization of pectic oligosaccharides (POS). Partial hydrolysis of pectin by chemical and/or enzymatic methods leads to the production of POS, in particular, oligosaccharides rich in galacturonic acid, and pectin-containing agricultural by-products can be used for that purpose. These oligosaccharides had been proposed as prebiotics and immunity enhancers, contributing in this way to human and animal health. Anyhow, an important barrier for their industrial production is the absence of standardized techniques for their production and purification and the ample variety of substrates involved. Also the lack of systematic studies about their structure does not allow to understand the structure-function relationship. Future research must be performed on these subjects, on confirmation of their physiological properties as well as on the production of these compounds in a larger scale. This research will contribute to POS use as an ingredient in the food industry and will help in the development of functional foods.

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