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Prebiotic potential of pectins and pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative evaluation

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A B S T R A C T

Sugar beet pulp (SBP) and lemon peel wastes (LPW) were used to obtain two mixtures of pectic oligosaccharides (denoted as SBPOS and LPOS, respectively). Oligogalacturonides in LPOS showed a larger molecular weight, higher degree of methylation and lower degree of acetylation than the ones in SBPOS. The suitability of pectic oligosaccharides, pectins from SBP and LPW and commercial FOS for causing prebiotic effects were compared by *in vitro* fermentation and fluorescence *in situ* hybridization using human faecal inocula and eight different probes. The joint populations of bifidobacteria and lactobacilli increased from 19% up to 29%, 34% and 32% in cultures with LPOS, SBPOS and FOS, respectively. *Faecalibacterium* and *Roseburia* also increased their counts with all the substrates (especially with LPOS). The highest concentrations of organic acids were observed in media containing oligosaccharides. This work confirms that pectic oligosaccharides present better prebiotic properties than pectins, and similar or better than FOS.

1. Introduction

Along the last decades, the consumer's interest in health and overall quality of life has increased, boosting the demand for natural products and food ingredients with functional and/or health properties. Despite the current commercial availability of a range of ingredients for functional foods, additional research and development efforts are needed to enhance their efficiency (Muthaiyan, Hernandez-Hernandez, Moreno, Sanz,

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& Ricke, 2012) and to develop new products with improved and/or additional properties. Among them, prebiotics have attracted more interest due to their benefits on the gastrointestinal microbiota.

In this field, pectin and pectin-derived oligosaccharides (POS) have been identified as emerging prebiotics with improved properties, such as their superior ability for modulating microbiota (including the increases in bacterial species like *Faecalibacterium prausnitzii* or *Roseburia intestinalis*) and for reaching and causing effects on the distal part of the colon (where there are increased risks of colon cancer and ulcerative colitis), compared to other commercial non-digestible oligosaccharides such as fructooligosaccharides (FOS) or galactooligosaccharides (GalOS) (Gullón et al., 2013).

Citrus peels and apple pulp are the major sources of pectin, which is also abundant in agroindustrial subproducts such as sugar beet pulp, peach peels, or pulps of grapes and pumpkin. Less frequently, pectin can also be extracted from by-products of the manufacture of starch from potatoes, sunflower heads in oil production and onions (Ovodov, 2009).

Pectin is a complex hetero-polysaccharide, and its proportion in a given feedstock is affected by several factors, including the botanic and anatomic plant origin, the age and the grade of ripening (Voragen, Coenen, Verhoef, & Schols, 2009). Pectin is mainly made up of the following structural polymers: (1) homogalacturonan, a linear polymer consisting of 1,4-linked D-galacturonic acid (GalA); (2) xylogalacturonan, a substituted homogalacturonan in which the α-1,4-linked D-galacturonate backbone is substituted at position O-3 with a β-D-xylopyranose; (3) rhamnogalacturonan I, formed by repeating sequences of the disaccharide α(1-4)-D-GalA-α(1-2)-L-rhamnose with a variety of different glycan chains (mainly arabinan and galactan) attached to the rhamnose residues; and (4) rhamnogalacturonan II, a backbone of homogalacturonan with complex side chains attached to the GalA residues (Willats, Knox, & Mikkelsen, 2006).

The suitability of pectin for specific applications is governed by the structural features, including molar mass, neutral sugar content, proportions of “smooth” and “hairy” regions, or the degree of methylation and acetylation (DM and DA), which can vary greatly from one raw material to another (Sila et al., 2009; Willats et al., 2006). Pectin is a food ingredient widely used as a gelling agent and stabilizer (Funami et al., 2011). On the other hand, it is also possible to use it in the formulation of drugs employed for treating gastrointestinal disorders, diabetes, cholesterol or high blood pressure (Matsumoto et al., 2008).

In recent years, several studies have been focused on POS production from several feedstock using a number of technologies, including enzymatic hydrolysis (Concha & Zúñiga, 2012; Martínez, Yáñez, Alonso, & Parajó, 2012b), acid hydrolysis (Hu, Liu, Wang, & Ding, 2009), hydrothermal processing (Gómez, Gullón, Yañez, Parajó, & Alonso, 2013) or physical degradation (Byun et al., 2006). Purification of the raw POS mixtures (for example, to achieve food-grade products) can be carried out by membrane filtration (Gómez et al., 2013, 2014).

It has been reported that POS intake (*in vitro* and *in vivo*) by a number of bacteria results in the generation of short chain fatty acids (SCFA). SCFA exert a number of healthy effects (Gullón et al., 2013), including the reduction of the proliferation of harmful bacteria as *Clostridium* (Jun, Lee, Song, & Kim, 2006). POS also show antioxidant properties, being effective for inhibiting the proliferation of several cancers, and can induce apoptosis in cancer cells (Jackson et al., 2007), prevent ulcers, cause anti-inflammatory effects and increase the urinary excretion of toxic metals (Burana-Osot, Soonthornchareonnon, Hosoyama, Linhardt, & Toida, 2010; Holck et al., 2011).

As the colonic microbiota plays a key role on host health, a deep understanding of the fermentation dynamics of the bacterial populations is of key importance. The investigations on the effects of prebiotics on gut health should not be just limited to assess selected bacterial groups, but the greatest possible part of the microbial ecosystem. According to Scott, Martin, Duncan, and Flint (2014), only by monitoring total population shifts will we improve our understanding of the mode of action of prebiotics and our ability to determine their true role in promoting health.

Beneficial microbes like *Bifidobacterium*, *Lactobacillus* and *Eubacterium* usually ferment carbohydrates, do not produce toxins and may cause a range of benefits for the host, including enhancement of the immune system and competitive inhibition of pathogens (Binns, 2013). Oppositely, the microbial metabolism of proteins in the large intestine can result in the formation of harmful metabolites, including nitrosamines (Scott, Gratz, Sheridan, Flint, & Duncan, 2013).

On the other hand, even if some commercial prebiotics are selectively metabolized by bifidobacteria and lactobacilli in the proximal part of the colon, the development of improved products causing healthy effects on the distal part is still an open question in the field of prebiotic research (Gullón et al., 2014).

The effects of POS on the microbiota depends on their chemical and physicochemical features, and can be conveniently assessed by key factors such as the consumption kinetics, the distribution of metabolic products and the effects caused on bacterial populations (Gullón et al., 2013).

This work provides an experimental assessment on the prebiotic properties of POS and pectins obtained from lemon peel wastes (LPW) and sugar beet pulp (SBP) by *in vitro* fermentation assays with human faecal cultures. FOS were also included in this study as positive control. The chemical characterization of the refined POS was studied by a combination of techniques, including HPAEC-PAD/UV, GC-FID and HPSEC, whereas the prebiotic potential was assessed by measuring the substrate consumption, the generation of SCFA and the changes in the microbial population.

2. Materials and methods

2.1. Raw material

LPW and SBP samples were kindly supplied by Indulleida (Lleida, Spain) and Azucarera Ebro (La Bañeza, Spain), respectively. These samples were mixed in a single lot (one for each kind of raw material) to avoid variations in the composition. When necessary, pieces of this lot were defrosted, milled and used for analytical determinations or processing according to the methods listed below.