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Pectic-oligosaccharides prepared by dynamic high-pressure microfluidization and their *in vitro* fermentation properties

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ABSTRACT

Pectic-oligosaccharides (POSs) were prepared from apple pectin by dynamic high-pressure microfluidization (DHPM). Operating under selected conditions (pectin concentration 1.84%, solution temperature 63 °C, DHPM pressure 155 MPa and number of cycles 6 passes), 32.92% of the pectin was converted into POS. The resulting POS contains 29.56% galacturonic acid and 58.53% neutral sugars. The prebiotic properties of POS were then 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. Moreover, the effects of POS on the growth of these bacteria and production of short-chain fatty acids are comparable to those of the most studied prebiotic, fructooligosaccharide. These results indicated that the POS prepared by DHPM has a potential to be an effective prebiotic.

1. Introduction

In the last decade, a number of novel dietary carbohydrates have been introduced as ingredients for food applications, responding to the growing awareness among consumers of the link between health, nutrition and diet. One import group is formed by the non-digestible oligosaccharides which may function as prebiotics. Several oligosaccharides with prebiotic properties, such as fructooligosaccharides, galactooligosaccharides, and fructans, are commercially available (Rastall & Hotchkiss, 2003), but there has been a considerable increase in the demand for production of "second generation" of novel prebiotic ingredients in recent years (Hernandez-Hernandez, Luz Sanz, Kolida, Rastall, & Javier Moreno, 2011).

It was reported that pectic-oligosaccharides (POSs) could be an excellent candidate for second-generation prebiotics (Hotchkiss, Olano-Martin, Grace, Gibson, & Rastall, 2003). Its parent material, pectin, is a family of complex and heterogeneous polysaccharides that widely present within the primary cell wall and intercellular regions of higher plants. Some studies have been carried out for preparing POS from kinds of source species, such as sugar beet pectin (Meyer et al., 2011), orange peel wastes (Martínez, Yáñez, Alonsó, & Parajó, 2010), and olive by-products (Lama-Muñoz, Rodríguez-Gutiérrez, Rubio-Senent, & Fernández-Bolaños, 2012) in the recent years.

Generally, POS was obtained according to three strategies: (1) extraction from plants (Ducasse, Williams, Meudec, Cheynier, & Doco, 2010); (2) synthesis (Nemati, Karapetyan, Nolting, Endress, & Vogel, 2008); (3) depolymerization of the polysaccharides. The depolymerization was regarded as the most competitive method because a wide variety of oligomers can be obtained from one polymer (Courtois, 2009). Basically, acid hydrolysis (Hu, Liu, Wang, & Ding, 2009), enzymatic hydrolysis (Zheng & Mort, 2008) and physical degradations are three frequently used depolymerization methods, where physical degradation was considered to be an efficient and environmentally friendly method. Physical methods such as γ-ray irradiation (Byun, Kang, Jo, Kwon, Son, & An, 2006) have been applied to prepare POS.

Dynamic high-pressure microfluidization (DHPM) is an emerging dynamic high-pressure homogenization technology, which generated powerful shear, turbulence, impaction, and cavitation forces simultaneously (Chen, Huang, Tsai, Tseng, & Hsu, 2011; Liu, Liu, Xie, Liu, Liu, & Wan, 2009). This technology has been proven to be a promising physical method to manipulate the molecular weight of polymers (Tsai, Tseng, & Chen, 2009). In our previous research, it was found that pectin being DHPM-treated induced serious degradation of high methoxyl pectin (Chen et al., 2012), indicating this method may be an alternative method to produce the POS, and need to be further developed.

The present study was conducted to optimize the production of POS by novel DHPM technology through a response surface methodology experiment. The prebiotic of the resulting POS was

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Table 1
Four-factor, three-level Box–Behnken design used for RSM and experimental data of the investigated response.

Run order	Variables				Response
	X_1	X_2	X_3	X_4	Y
1	1.75 (0)	140.00 (0)	70.00 (+1)	6.00 (+1)	521.7
2	1.75 (0)	140.00 (0)	65.00 (0)	5.00 (0)	573.5
3	1.75 (0)	120.00 (−1)	65.00 (0)	6.00 (+1)	518.3
4	1.75 (0)	140.00 (0)	60.00 (−1)	4.00 (−1)	518.7
5	1.75 (0)	120.00 (−1)	60.00 (−1)	5.00 (0)	443.9
6	1.75 (0)	160.00 (+1)	65.00 (0)	6.00 (+1)	593.7
7	1.75 (0)	140.00 (0)	65.00 (0)	5.00 (0)	571.7
8	1.75 (0)	160.00 (+1)	70.00 (+1)	5.00 (0)	529.1
9	1.50 (−1)	140.00 (0)	65.00 (0)	6.00 (+1)	435.9
10	1.50 (−1)	120.00 (−1)	65.00 (0)	5.00 (0)	316.3
11	1.75 (0)	140.00 (0)	65.00 (0)	5.00 (0)	565.1
12	2.00 (+1)	140.00 (0)	70.00 (+1)	5.00 (0)	477.5
13	2.00 (+1)	140.00 (0)	60.00 (−1)	5.00 (0)	535.3
14	2.00 (+1)	120.00 (−1)	65.00 (0)	5.00 (0)	410.7
15	1.50 (−1)	160.00 (+1)	65.00 (0)	5.00 (0)	425.5
16	2.00 (+1)	160.00 (+1)	65.00 (0)	5.00 (0)	543.7
17	2.00 (+1)	140.00 (0)	65.00 (0)	6.00 (+1)	539.1
18	1.75 (0)	140.00 (0)	70.00 (+1)	4.00 (−1)	506.9
19	1.50 (−1)	140.00 (0)	60.00 (−1)	5.00 (0)	353.3
20	1.75 (0)	140.00 (0)	65.00 (0)	5.00 (0)	597.5
21	1.50 (−1)	140.00 (0)	65.00 (0)	4.00 (−1)	429.7
22	1.75 (0)	120.00 (−1)	65.00 (0)	4.00 (−1)	501.7
23	1.50 (−1)	140.00 (0)	70.00 (+1)	5.00 (0)	389.1
24	2.00 (+1)	140.00 (0)	65.00 (0)	4.00 (−1)	542.9
25	1.75 (0)	160.00 (+1)	65.00 (0)	4.00 (−1)	581.7
26	1.75 (0)	160.00 (+1)	60.00 (−1)	5.00 (0)	578.1
27	1.75 (0)	120.00 (−1)	70.00 (+1)	5.00 (0)	469.3
28	1.75 (0)	140.00 (0)	65.00 (0)	5.00 (0)	567.5
29	1.75 (0)	140.00 (0)	60.00 (−1)	6.00 (+1)	551.7

evaluated through enumerating of selected human gut microflora including *Bifidobacteria*, *Lactobacilli*, *Bacteroides*, *Clostridia* and *Eubacteria*, and analysing the concentration of short-chain fatty acid in a fecal batch culture fermentation test. Fructooligosaccharides (FOS), the most extensively studied prebiotics and the current market leader, were chosen as a positive control.

2. Materials and methods

2.1. Materials

Apple pectin (8471, Sigma–Aldrich, Shanghai, China) were dispersed in phosphate buffer and treated enzymatically for starch degradation, using α-amylase (A6211 from *Aspergillus oryzae*, Sigma–Aldrich, Shanghai, China) and amyloglucosidase (10115 from *Aspergillus niger*, Sigma–Aldrich, Shanghai, China) according to methods used by Urias-Orona, Rascón-Chu, Lizardi-Mendoza, Carvajal-Millán, Gardea, and Ramírez-Wong (2010). Then the digestion products were removed by a 48 h dialysis (molecular weight cut-off 6000–8000) with continuous change of distilled water. After dialysis, the retentates were freeze-dried. The galacturonic acid content of purified pectin was 71.68% determined by the m-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), and the degree of methoxylation (DM) was 70.76% determined by a titrimetric method (FCC, 1981). All of the other chemicals were of analytical reagent grade.

2.2. Preparation of POS by DHPM

2.2.1. DHPM treatment

Pectic-oligosaccharides were prepared by dynamic high-pressure microfluidization according to our previous method (Chen et al., 2012). Purified pectin was dispersed in deionized water and stirred gently at room temperature to achieve complete solubilization. Then the solution was adjusted to pH 1.0 using 0.5 M H_2SO_4 and placed in a water bath to achieve the required temperature. The solution was then treated in an M-100EH-30 microfluidizer (Microfluidics Co., Newton, USA). After DHPM, the reaction mixture was neutralized with calcium carbonate to precipitate the higher molecular weight species that are not soluble in neutral solution, and the resulting precipitate was removed by centrifugation (Du, Song, Hu, Liao, Ni, & Li, 2011). The supernatant was circulated through a concurrent ultrafiltration with a cut-off size of 5000 Da (Pall Gelman, Ann Arbor, MI) to remove the species that their molecular weight were larger than 5000 Da, and permeates were collected, freeze-dried and weighted.

2.2.2. Experimental design

The response surface methodology (RSM) was adopted to study the effect of pectin concentration (X_1), DHPM pressure (X_2), solution temperature (X_3) and number of cycles (X_4) on the mass of POS (Y). A three level four factor Box–Behnken design (BBD) was used in this study. A total of 29 experiments, consisting of 5 replicates with combinations of different levels of each independent variable, were generated using the software Design Expert version 8.0.5 (Stat-Ease, Inc., Minneapolis, USA) (Table 1). The range for pectin concentration, DHPM pressure, solution temperature and number of cycles was set at 1.5–2.0%, 120–160 MPa, 60–70 °C, and 4–6 passes, respectively, based on our preliminary experiments. Individual experiments were carried out in randomized order to minimize the effect of unexplained variability in the experimental responses due to extraneous factors.

Experimental data were fitted to a quadratic polynomial model. The general form of the quadratic polynomial model was as follows:

$$\Upsilon = \beta_0 + \sum_{i=1}^{4}\beta_i X_i + \sum_{i=1}^{4}\beta_{ii}X_i^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{4}\beta_{ij}X_iX_j \quad (1)$$