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Characterization of pectic polysaccharides extracted from apple pomace by hot-compressed water

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ABSTRACT

Response surface methodology (RSM) was used to optimize the extraction of pectic polysaccharides from apple pomace by hot-compressed water, by which the optimum levels of the parameters were obtained as follows: extraction temperature 140 °C, extraction time 5 min, S:W ratio 1:14. Compared with commercial pectin, the *Mw*, galacturonic acid content, DM and protein of the extracted pectic polysaccharides were lower while ash content and neutral sugars were higher. The endothermic transition temperature and fusion heat of the extracted pectic polysaccharides was lower than commercial one according to DSC analysis. For its rheological properties, it was found that the viscosity of the extracted pectic polysaccharides solution was slightly lower than commercial pectin at lower shear rate region while it decreased sharply when the shear rate increased. Besides, both G' and G'' moduli of the extracted pectic polysaccharides were lower than the commercial pectin's possibly because of weaker polymer chain interaction, which was also reflected in gel textural properties. However, the extracted pectic polysaccharides showed higher in vitro antioxidant capability and inhibitory effect on HT-29 colon adenocarcinoma cells than commercial pectin.

1. Introduction

Apple pomace, which is mainly composed of apple peel, core, seed and pulp remains, is the main by-product in cider and apple juice processing industries and accounts for about 25–30% of the original fruit mass (Shalini & Gupta, 2010; Vendruscolo, Albuquerque, Streit, Esposito, & Ninow, 2008). Apple pomace contains about 80% of moisture and is a rich source of carbohydrate, acids, vitamin C and minerals, which make it quite perishable and become a severe environmental problem because of it's vast amounts (Shalini & Gupta, 2010). China now is the largest apple exporter and leading apple juice concentrate (AJC) producer worldwide (Gale, Huang, & Gu, 2010), thus approximately one million tons of apple pomace are annually produced (Wang, Sun, et al., 2007). However, only small amounts of apple pomace are used for deep-processing, the majority of apple pomace are not efficiently utilized yet (Wang, Sun, et al., 2007). Actually, many attempts have been made to utilize apple pomace for several value-added products, such as polyphenol, enzymes, single cell protein, aroma compounds, ethanol, organic acids, polysaccharides, and mushroom culture (Cetkovic et al., 2008; Thakur, Singh, & Handa, 1997). However, pectin is still considered as one of the most reasonable way for apple pomace utilization both from an economical and from an ecological point of view (Schieber, Stintzing, & Carle, 2001).

Pectin is a family of complex polysaccharides that contain 1,4-linked α-D-galacturonic acid (Gal*p*A) residues, in which homogalacturonan, rhamnogalacturonan-I, and rhamnogalacturonan-II were isolated and characterized as major pectin (Willats, Knox, & Mikkelsen, 2006). Pectin has been widely used in food, cosmetic and pharmaceutical industries as stabilizer, gelling agent and thickener (Voragen, Coenen, Verhoef, & Schols, 2009; Willats, Knox, & Mikkelsen, 2006). Recently, pectin is used for cardiovascular disease theraphy, induction of prostate cancer cells apoptosis, colon-specific drug delivery, anti-inflammation, probiotic growth promotion and even a new raw material for porous materials production (Das & Ng, 2010; Jackson et al., 2007; Licht et al., 2010; Popov et al., 2011; Theuwissen & Mensink, 2008; White, Budarin, & Clark, 2010).

Commercially, pectin is usually extracted from citrus peel and apple pomace by hot acid method (Liu, Cao, Huang, Cai, & Yao, 2010). Besides, other extraction methods, such as enzymatic, microwave-assisted, ultrasound-assisted, extrusion and alkaline extraction, were reported (Kost'alova, Hromadkova, & Ebringerova, 2010; Ptichkina, Markina, & Runlyantseva, 2008; Shin, Kim, Cho, & Hwang, 2005; Wang, Chen, et al., 2007; Zykwinska, Rondeau-Mouro, Garnier, Thibault, & Ralet, 2006). Hot-compressed water, also called subcritical water, was proved to be effective for extraction of pectin from citrus peel (Hoshino, Tanaka, Terada, Sasaki, & Goto, 2009; Tanaka, Takamizu, Hoshino, Sasaki, & Goto, 2012;

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Ueno, Tanaka, Hosino, Sasaki, & Goto, 2008), however, the detailed chemical composition as well as the functional characteristics of the extracted citrus peel pectin were not completely investigated. Moreover, as one of the two main feedstocks for pectin production, apple pomace was not investigated for pectin extraction by hot-compressed water and the optimal conditions were unknown. Further, the physicochemical and functional properties of the extracted pectic polysaccharides from apple pomace were focused on and simultaneous comparison was carried out between the extracted pectic polysaccharides and commercial apple pomace pectin in this work.

2. Materials and methods

2.1. Feedstock

Apple pomace provided by Shaanxi Haisheng Fresh Fruit Juice Co. Ltd. was used as raw material in all experiments. Apple pomace was dried at 105 °C for 24 h thereafter it was comminuted and sieved (100 mesh pass) before experiments. Commercial pectin was bought in the market produced by Yantai Andre Pectin Co. Ltd. China (apple pomace as feedstock).

2.2. Extraction of pectic polysaccharides by hot-compressed water

An autoclave with 500 mL working volume was used for pectic polysaccharides extraction by hot-compressed water, which is water under subcritical temperature and pressure. A thermocouple and a pressure gauge were used to assay the temperature and pressure inside the reactor. Apple pomace (10 g) and distilled water were added into the reactor, whereafter the nitrogen gas was inputted to exclude oxygen for prevention of oxidation reaction. Then the reactor was tightly enclosed into an electric heater and the extraction time was counted after the temperature inside the reactor reached designed temperature. After the extraction, the reactor system was immediately quenched to room temperature by an inside cooling coil. All the experiments were performed in triplicate, with the average value reported.

After hot-compressed water extraction, water-soluble portion was retrieved by filtration and the filtrate was collected for precipitation by anhydrous ethanol (final ethanol ratio was approximately 75%). The precipitated pectic polysaccharides were washed several times by anhydrous ethanol by which small molecular weight compounds, such as phenolic compounds and degradation compounds (furaldehyde as example) were removed. The precipitated and washed pectic polysaccharides were dried at 105 °C for 24 h. The pectic polysaccharides yield and polygalacturonic acid yield (calculated according to galacturonic acid content in precipitated pectic polysaccharides) were calculated according to Eq. (1) and Eq. (2) as follow:

$$\text{Pectic polysaccharides yield}(\%) = \frac{\text{Pectic polysaccharides}(g)}{\text{Apple pomace}(g)} \times 100 \quad (1)$$

$$\text{Polygalacturonic acid yield}(\%) = \frac{\text{Polygalacturonic acid}(g)}{\text{Apple pomace}(g)} \times 100 \quad (2)$$

2.3. Experimental design

Response surface methodology (RSM) was widely used for various process optimization (Im & Zoh, 2013; Salari, Chayjan, Khazaei, & Parian, 2013; Wang, Li, et al., 2013). This method can deal with two or more factors at several levels, by which a wide range of experimental conditions can be comprehensively investigated in limited trials. The RSM was used in present study to optimize process parameters for pectic polysaccharides extraction by hot-compressed water, through which maximum pectic polysaccharides yield and maximum polygalacturonic acid yield could be obtained. The Box–Behnken experimental design as a rotatable second-order designs based on three-level incomplete factorial designs was adopted for the optimization process, in which three central points were used for estimation of the experimental error as well as to investigate the suitability of the proposed model. Coding of the independent variables was done according to Eq. (3),

$$x_i = \frac{(X_i - X_0)}{\Delta X_i} \quad (3)$$

where x_i and X_i were the dimensionless coded and the actual values of the independent variable i, X_0 was the actual value of the independent variable i at the central point and ΔX_i was the step change of X_i.

The temperature (X_1, °C), extraction time (X_2, min) and S:W ratio (X_3) were selected as the independent variables according to preliminary experiments. The range and the levels of both coded and actual values of the independent variables were listed in Table 1. The performance of the process could be described by the following quadratic polynomial Eq. (4).

$$Y = \beta_0 + \sum_{i=1}\beta_i x_i + \sum_{i=1}^{n}\beta_{ii}x_i^2 + \sum_{i=1}^{n}\sum_{j>i}^{n}\beta_{ij}x_i x_j \quad (4)$$

where Y represented response variable, β_0, β_i,β_{ii}, β_{ij}, were intercept coefficient, linear term, quadratic term and interaction term, respectively. x_i, x_j were coded levels of independent variables. Y represented pectic polysaccharides yield and polygalacturonic acid yield as dependent variables. The Design Expert 8.00 (trial) software was used for the regression analysis and numerical optimization.

2.4. Analytical methods

2.4.1. Molecular weight determination and chemical composition analysis

The molecular weight (abbreviated as *Mw*) of samples were determined by gel-permeation chromatography (GPC) as described in the references (Jia, Yang, & Sun, 2013; Ying, Han, & Li, 2011). Waters HPLC apparatus (Waters Co. Ltd., USA) equipped with three Ultrahydrogel linear columns (7.8 mm × 300 mm) in series and Waters 2414 refractive index detector were used to determine the *Mw*, in which various dextrans were used as standards.

After the protein was removed by sevag method (Staub, 1965), galacturonic acid was determined by modified carbazole method (Bitter & Muir, 1962). The degree of methylation was determined by the titration of free carboxyl groups before and after basic hydrolysis (Schultz, 1965). The protein content was determined via the Bradford method (Bradford, 1976). Ash content was determined by incinerating pectic polysaccharides samples at 575 °C for 8 h in a muffle furnace.

Neutral monosaccharides were released from pectic polysaccharides by acid hydrolysis with trifluoroacetic acid (2 M) at 120 °C for 1.5 h, whereafter trifluoroacetic acid was removed by rotary evaporation at 60 °C. Then the sodium borohydride was added into the solution at room temperature for 1.5 h, after which gas chromatography was used to determine alditol-acetate derivatization products of the monosaccharides (Blakeney, Harris, Henry, & Stone, 1983; Masmoudi, Besbes, Ben Thabet, Blecker, & Attia, 2010). Gas chromatography (Shimadzu 2014 C) with a high performance capillary column, DB-17 (30 m L × 0.25 mm ID, 0.25 μm film thickness, Agilent) was used to determine the neutral monosaccharides' derivatives.