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Pectin extracted from apple pomace and citrus peel by subcritical water

Xin Wang, Quanru Chen, Xin Lü*

College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi Province 712100, China

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ABSTRACT

Subcritical water was used to extract pectin from citrus peel and apple pomace, in which the effect of extraction temperature on properties of the pectins was investigated. The maximum yield of citrus peel pectin (CPP) and apple pomace pectin (APP) were 21.95% and 16.68% respectively. No significant differences were found in FTIR spectra of CPP and APP. According to DSC analysis, the endothermic property of pectin was affected by extraction temperature while the exothermic property of pectin was only affected by its constituents and raw material. The pectin solutions exhibited shear-thinning properties and tended to be more elastic ($G' > G''$) with frequency increase according to rheological analysis, which was also reflected in hydrogel analysis. Moreover, both CPP and APP scavenged more than 60% DPPH radical and 80% ABTS radical in vitro and the highest proliferation inhibition rates of colon cancer cell HT-29 by CPP and APP were 76.45% and 45.23% respectively.

1. Introduction

Pectin is a natural high molecular compound widely-existing in cell wall and middle lamella structure of all higher plants (Qiu, Tian, Qiao, & Deng, 2009). Pectin is usually considered as a complex polysaccharide which consists of α-1,4-linked D-galacuronic acid, which is partly methyl esterified, and the side chain contains various neutral sugars, such as L-rhamnose, L-arabinose, and D-galactose (Mohnen, 2008; Xie, Li, & Guo, 2008). Pectin properties include gelatification, thickening and stabilization, giving it widespread use in food, medical, chemical, textile and other industrial fields (Sato et al., 2011). It is also reported that pectin had several biological and physiological functions, such as reduction of serum cholesterol (Brown, Rosner, Willett, & Sacks, 1999), delay of gastric emptying (Schwartz et al., 1988), immune-modulation (Inngjerdingen et al., 2007) and inducing apoptosis of colon cancer cells (Olano-Martin, Rimbach, Gibson, & Rastall, 2003).

Generally the main feedstocks for commercial pectin production are apple pomace and citrus peel (Willats, Knox, & Mikkelsen, 2006). China, now is the largest apple and condensed apple juice producer in the world (Qiu et al., 2009) as well as increasing yields of orange and orange juice (Xie et al., 2008). Thus large amounts of apple pomace and citrus peel, as the primary waste product of the juice manufacturing, are produced annually, which lead to wasting of resources and create environmental problems. Numerous attempts have been made to utilize them as a source of dietary fiber (Sudha, Baskaran, & Leelavathi, 2007), animal feed (Joshi & Sandhu, 1996), polyphenol (Li, Smith, & Hossain, 2006), and biofuel (Edwards & Doran-Peterson, 2012). Among them, pectin extraction is thought to be the most reasonable way for the apple pomace and citrus peel to be utilized (Shalini & Gupta, 2010). Pectin is industrially produced at acidic conditions with elevated temperature (Koubala et al., 2008). Acidic wastewater and environmental concerns make alternative extraction methods including ultrasound (Zhang et al., 2013), enzymatic (Ptichkina, Markina, & Rumyantseva, 2008), microwave (Fishman & Cooke, 2009) and subcritical water (Ueno, Tanaka, Hosino, Sasaki, & Goto, 2008) attractive.

Subcritical water is the water under subcritical temperatures and pressures with dielectric constant and the ion product greatly changed (Marshall & Franck, 1981; Teo, Tan, Yong, Hew, & Ong, 2010), which has proved to be effective for hydrolysis of lignocellulosic material (Heitz et al., 1986) and pectin extraction from citrus peel (Carr, Mammucari, & Foster, 2011; Ueno et al., 2008). However, detailed reporting on the characteristics of pectin extracted by subcritical water has not been found, which was focused in this study.

In this study, the pectin of apple pomace and citrus peel was extracted by subcritical water without acid or alkaline addition, after which the physicochemical properties, rheological properties, gel properties and bioactive activities of the pectins were investigated.

* Corresponding author. Tel./fax: +86 29 8709 2195.
E-mail address: xinlu@nwsuaf.edu.cn (X. Lü).

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2. Materials and methods

2.1. Materials and reagents

Apple pomace was provided by Shaanxi Haisheng fresh fruit juice Co. Ltd., China and oven-dried at 105 °C for 24 h. Citrus reticulata was purchased from a local supermarket and the peel was stripped manually and oven-dried at 105 °C for 24 h. Whereafter apple pomace and citrus peel were ground to pass through a 100-mesh sieve and stored in desiccators at room temperature for latter extraction.

Galacturonic acid, arabinose, rhamnase, xylose, galactose, glucose, mannose, DPPH and ABTS•+ were purchased from Sigma chemical Co. (St. Louis, MO, USA). All other chemicals used in this study were analytical grade.

2.2. Extraction of pectin by subcritical water

An autoclave with 500 mL working volume was used for pectin extraction by subcritical water, in which a thermocouple and a pressure gage were used to assay the temperature and pressure inside the reactor. The raw material and distilled water were added into the reactor with the solid to liquid ratio of 1:30. The extraction temperature was set at 130 °C, 150 °C, 170 °C for apple pomace pectin extraction and 100 °C, 120 °C, 140 °C for citrus peel pectin extraction according to preliminary experiments. The extraction time was set for 5 min. All the experiments were performed in triplicate, with the average value reported.

After subcritical water extraction, the water soluble portion was retrieved by filtration and the filtrate was collected for alcohol precipitation. The precipitate was washed by 5% (v/v) HCl in 60% isopropyl alcohol and anhydrous ethanol in turn for three times, whereafter it was dried at 105 °C for 24 h. The pectin yield was calculated according to equation (1).

$$\text{Pectin Yield(wt\%)} = \frac{\text{Pectin(g)}}{\text{Raw Materials(g)}} \times 100\% \quad (1)$$

2.3. Chemical composition and molecular weight analysis

The galacturonic acid was determined by modified carbazole method (Bitter & Muir, 1962). The degree of methyl esterification was determined by the titration of free carboxyl groups before and after basic hydrolysis (Liu, Cao, Huang, Cai, & Yao, 2010). The protein content was determined via the Bradford method (Carlsson, Borde, Wölfel, Åkerman, & Larsson, 2011). Ash content was determined by incinerating dried samples at 575 °C for 8 h in a muffle furnace.

Neutral monosaccharides were released from pectin 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. The sodium borohydride was added into the solution at room temperature for 1.5 h, then acetic anhydride and pyridine were added to catalyze esterification reaction in boiling water 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 et al., 2010). Gas chromatography (Shimadzu 2014C) with a high performance capillary column, DB-17 (30 m × 0.25 mm ID, 0.25 μm film thickness, Agilent) was used to determine the neutral monosaccharides derivatization products.

The molecular weight (*Mw*) of pectin samples were determined by gel-permeation chromatography (GPC) as described by the references (Jia, Zhang, Lan, Yang, & Sun, 2013; Ying, Han, & Li, 2011). Waters HPLC apparatus (Waters Co. Ltd., USA) equipped with three Ultrahydrogel linear columns (7.8 × 300 mm) in a series and a model 2414 refractive index detector was used, by which the *Mw* was investigated and calculated according to the calibration curve ($\text{LgMw} = -0.1316x + 10.94$, x means retention time, $R^2 = 0.9938$) obtained by using various standard dextrans.

2.4. Fourier transform infrared spectroscopy

An FT-IR spectrometer (Bruker Vetex70 FTIR instrument, Germany) was employed to investigate the characteristic spectra of the extracted pectins. Dried sample (1 mg) and potassium bromide (100 mg) was mixed, ground and pressed into tablets, thereafter it was scanned within the range of 4000–400 cm^{-1} (Park, Khan, & Jung, 2006).

2.5. Thermal analysis

Differential scanning calorimetry (DSC Q2000 TA system, USA) was used to investigate the thermal properties of the pectins according to described method (Sharma & Ahuja, 2011). 5 mg dried and finely ground pectin sample was added into a standard aluminum crucible and immediately sealed. The crucible was heated from 40 °C to 300 °C at a heating rate of 10 °C/min in dynamic inert nitrogen atmosphere (50 mL/min). Simultaneously, an empty standard aluminum crucible was used as reference.

2.6. Rheological properties

The rheological properties of pectin were determined by rheometer (AR1000, TA instruments, USA) with a 20 mm parallel plate. The solution for rheological tests was prepared by mixing pectin with distilled water (2%, w/w). The sample solutions were subjected to steady-shearing at 25 °C with the shear rates ranged from 0.02-100 s^{-1}. Oscillatory measurements were used to determine the storage modulus (G') and loss modulus (G'') of pectin solutions. Strain sweep (0.01–100% at 1 Hz) was applied to test the linear viscoelastic region of the samples. And the frequency dependence of G' and G'' was determined by a frequency sweep (0.1–10 Hz at 1% strain) (Zhang et al., 2013).

2.7. Gel properties

Gel preparation and gel properties assay were according to described method (Angioloni & Collar, 2009; Piermaria, de la Canal, & Abraham, 2008). Briefly, 0.5 g pectin sample was dissolved in 17 mL distilled water, whereafter 35 g sucrose was added into the solution and the pH was adjusted to 3 by 12.5% citric acid solution. The mixture solution was placed at 4 °C for 24 h. Before textural analysis, the prepared pectin gels were placed at room temperature for 0.5 h. A Texture Analyser TA.XT Plus (Stable Micro Systems, UK) was used to determine the textural properties of the pectin gel. The puncture test was performed using a cylindrical probe (5-mm radius, PC-0.5R). A standard one-cycle program was used to compress the gels at 0.1 mm/s test speed, 1.0 mm/s pre-test speed, 1.0 mm/s post-test speed, and 1 g originating force. The test was stopped when a 10-mm depth of penetration had been reached. The whole procedure was repeated three times at 20 °C.

2.8. DPPH radical scavenging activity

The antioxidant activity of the pectins were determined based on scavenging activity of the stable DPPH free radical (Rha et al., 2011). 2 mL pectin solution (0.5, 1.5, 2.5, 3.5, 4.5 mg/mL) was added into 100 μM DPPH in ethanol (4 mL). Then the mixture was placed in the dark at room temperature for 30 min, whereafter the