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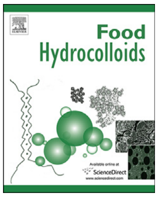

Effects of pH and high hydrostatic pressure on the structural and rheological properties of sugar beet pectin

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Water (PubChem CID: 962)
Sodium hydroxide (PubChem CID: 14798)
Hydrochloric acid (PubChem CID: 313)
Ethanol (PubChem CID: 702)
Galacturonic acid (PubChem CID: 439215)
Glycine Hydrochloride (PubChem CID: 22316)
Tris Hydrochloride (PubChem CID: 93573)
Phenol red (PubChem CID: 4766)
Sulfuric acid (PubChem CID: 1118)
Magnesium sulfate (PubChem CID: 24083)

ABSTRACT

The objective of this study was to investigate the effect of high hydrostatic pressure (HHP) on the structural and rheological properties of sugar beet pectin (SBP) at pH 3, 7 and 8. The weight-average molar mass (M) of SBP at pH 3, 7 and 8 decreased significantly with increased HHP. The degree of esterification (DE) of SBP at pH 3 and 7 was unchanged by treatment with HHP; the degree of acylation (DA) was a little higher compared to the controls. Both DE and DA of SBP at pH 8, however, decreased significantly after treatment with HHP. There was a marked peak at 1550 cm^{-1} in FT-IR spectrum for SBP at pH 3 and 7 after treatment with HHP. The shear rate versus shear stress curves of SBP fit well with a Herschel–Bulkley model ($R^2 \geq 0.99$) and showed shear-thinning behavior. The yield stress (σ_0) of SBP solutions at different pH values increased after treatment with HHP indicating the viscosity of SBP solutions was increased, perhaps because the protein in SBP was stretched.

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1. Introduction

Pectin is a heterogeneous polysaccharide component of the cell walls of higher plants. It is comprised of $\alpha(1 \rightarrow 4)$ linked D-galacturonic acid (the "smooth" region) and $\alpha(1 \rightarrow 4)$ D-galacturonic acid units interrupted by the insertion of $\alpha(1 \rightarrow 2)$ linked L-rhamnopyranosyl residues with a large number of neutral sugar side chains (the "hairy" region) (Morris, Ralet, Bonnin, Thibault, & Harding, 2010; Sila et al., 2009; Voragen, Coenen, Verhoef, & Schols, 2009). Usually, the galacturonic acid component of the pectin chain is partially methylesterified at C6 (methylation) or O-acetylated at C2 and/or C3 (acetylation), which is an important indicator for evaluating the functional properties and sources of pectin (Shpigelman, Kyomugasho, Christiaens, Loey, & Hendrickx, 2014). Pectin is often used in the food industry as a thickener and stabilizer, of which the viscosity is the primary judgment used to evaluate the effect of thickening or stability. The viscosity of a pectin solution is related to its physical and chemical characteristics (degree of esterification, molecular mass) and concentration (Mesbahi, Jamalian & Farahnaky, 2005; Villay et al., 2012) and is affected by pH, ionic strength, temperature and shear rate (Arslan & Kar, 1998; Kar & Arslan, 1999).

High hydrostatic pressure (HHP, 100–1000 MPa) is a non-

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thermal technology able to induce structural changes of biomacromolecules, including protein denaturation, enzyme inactivation and starch gelatinization, which affect their functional properties (Alexandrakis et al., 2014; Khan, Mu, Zhang, & Arogundade, 2014; Li, Zhu, Zhou, & Peng, 2012; Liu, Selomulyo, & Zhou, 2008). In addition, treatment with HHP does not damage the main chain of pectin and can increase the viscosity of a pectin solution (Kato, Teramoto, & Fuchigami, 1997; Michel et al., 1998). Hydrophobic groups in the pectin molecule are likely exposed during treatment with HHP, which can change the intermolecular hydrophobic effect and intermolecular interactions.

Sugar beet pectin (SBP) is a polysaccharide that can be extracted from sugar beet pulp, which is a kind of protein–polysaccharide compound containing large amounts of neutral sugars, acetyl sugars and 5%–9% of protein surrounded by or connected to the end of carbohydrate chains (Fishman, Chau, Cooke, & Hotchkiss, 2008; Kirby, Macdougall, & Morris, 2008; Kirby, MacDougall, & Morris, 2006; Nunez, Fishman, Fortis, Cooke & Hotchkiss, 2009). SBP has poor gelling and thickening properties compared to other polysaccharides (Kuuva, Lantto, Reinikainen, Buchert, & Autio, 2003; Levigne, Ralet, & Thibault, 2002). Funami et al. (2008) used maturation treatment to form polysaccharide chains crosslinked with protein to generate SBP with a high weight-average molar mass (*M*). Peroxidase or laccase was used to catalyze the ferulic acid components in SBP, which induced the formation of oxidative crosslinks to increase *M* and improve the rheological properties (Zaidel, Chronakis, & Meyer, 2012; Zaidel, Chronakis, & Meyer, 2013). Earlier, we showed the potential of SBP extracted by an ultrasound/microwave-assisted acidic method as a functional additive during food product development (Peng et al., 2015). Hence, the appropriate structure modification of SBP by HHPs might lead to enhanced functionality, which could increase the applications of SBP in food industries.

The objective of this study was to investigate the effects of HHP treatment on structural and rheological properties of SBP at pH 3, 7 and 8 for better understanding SBP as a functional agent in the food industry.

2. Materials and methods

2.1. Materials

Dried SBP was provided by COFCO TUNHE Co., Ltd. (Tunhe, Xinjiang Uygur Autonomous Region, China) in March 2012. SBP contained (on a dry weight basis) 10.14% (w/w) protein (AOAC 955.04), 1.10% (w/w) fat (AOAC 992.6), 26.25% (w/w) crude fiber (AOAC 993.21) and 5.49% (w/w) ash (AOAC 923.03). All the reagents used in this study were of analytical grade.

2.2. Preparation of SBP

SBP was prepared as described by Peng et al. (2015). A 100 g sample of dry SBP was crushed through a 10 mesh sieve (pore size 2000 μm) then immersed in 400 mL of boiling anhydrous ethanol for 15 min. The residue was dried overnight at 50 °C and ground to a powder. A 2 g sample of the powdered SBP was mixed with 0.1 M hydrochloric acid (HCl) (1:30, w/v) pH 1 then placed into a microwave/ultrasound extraction/reaction apparatus (CW - 2000, Shanghai Xintuo Analysis Instrument Co., Ltd., Shanghai, China) of the pressure in the microwave cell was 1 bar with settings as follows: ultrasonic power 50 W at a frequency of 40 kHz; microwave power varied between 10 and 800 W at a frequency of 2450 MHz to keep the sample at 92 °C for 37 min. The mixture was filtered through filter paper; the filtrate was adjusted to pH 3.5 with 4 M NaOH, cooled and then subjected to ultrafiltration through a 10 kDa molecular mass cut-off membrane (DF − 4010, Shanghai Dongya Nuclear Grade Resin Co., Ltd., Shanghai, China) against deionized water. The concentrated filtrate was precipitated at room temperature overnight with anhydrous ethanol (1:2, v/v) and then centrifuged at 7000*g* for 40 min. The precipitate was recovered, washed successively with 75%, 80% and 85% (v/v) ethanol then centrifuged at 7000*g* for 10 min to remove the mono- and disaccharides. The SBP was dispersed in deionized water and freeze-dried for storage. The SBP contained 75.38% (w/w) galacturonic acid, 8.41% (w/w) protein and 3.24% (w/w) moisture. The weight-average molar mass was 559.51 kDa and the degree of esterification and acetylation was 81.86% and 34.93%, respectively.

2.3. Preparation of pectin solution

Three 1% (w/v) solutions of SBP were prepared; one in 0.1 M glycine–HCl buffer pH 3, one in 0.1 M Tris–HCl pH 7 and another in 0.1 M Tris–HCl pH 8. The solution was stored at 4 °C overnight, and then centrifuged at 968*g* for 10 min to sediment insoluble ingredients. A 50 mL sample of each solution was sealed in a polyethylene bag under vacuum and stored at 4 °C.

2.4. Treatment with HHP

Treatment with HHP was done with high-pressure equipment (model HHP.L3-600/0.6; Tianjin Huatai Senmiao Engineering and Technique Co. Ltd, Tianjin, China) equipped with a hydraulic-type cell (inner capacity 1 L) and a water jacket for temperature control. The bagged SBP solutions were treated at 25 °C for 30 min each time at a pressure of 250, 350, 450 or 550 MPa. The target pressure was attained at a rate of ~250 MPa/min and released at ~300 MPa/min.

2.5. Weight-average molar mass (M)

M refers to aggregates of pectin and it was determined as described by Funami et al. (2007). SBP was dissolved in 0.1 M $NaNO_3$ at a concentration of 0.5 mg/mL, filtered through a 0.22 μm pore size membrane and injected into a high-performance size-exclusion liquid chromatography (HPSEC) apparatus equipped with a TSKgel G-4000PWXL chromatography column (30 cm × 7.8 mm ID) and three detectors; a multi-angle laser detector (Dawn Heleos II), a UV detector (L-2400) and a differential refractometer (OptilabrEX; Wyatt Technology Corporation, Santa Barbara, CA, USA). The column was eluted with 0.1 M $NaNO_3$, 0.04% (w/v) NaN_3 at a flow rate of 0.5 mL/min. The refractive index increment dn/dc was taken to be 0.135 mL/g (Funami et al., 2011). When *M* was determined, the peak began with the differential peak starting and the end of the laser signal peak as the end. The earlier differential peak appeared and the sooner the laser signal peak ended, the greater the *M*.

2.6. Degree of esterification (DE) and degree of acetylation (DA)

DE of pectin was determined as described by Pinheiro et al. (2008) with slight modification. The pectin (0.1 g) was mixed with 50 mL deionized water on magnetic stirring machine for 2 h at room temperature, added six drops of phenol red indicator and titrated against 0.1 M NaOH until the color of indicator changed to pink and persisted for at least 30 s, record the volume of NaOH as V1. Then added 10 mL of 0.25 M NaOH to stir for 30 min then add 10 mL 0.25 M HCl and titrated with 0.1 M NaOH to the same end point as before, record the volume of NaOH as V2. The DE was calculated as follows:

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