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Sugar beet pectin fractionated using isopropanol differs in galacturonic acid, protein, ferulic acid and surface hydrophobicity

Deepika Karnik [a], Jiyoung Jung [a, 1], Samatha Hawking [b], Louise Wicker [a, c, *]

[a] Department of Food Science and Technology, The University of Georgia, 100 Cedar St, Athens, 30602, United States
[b] Richard B. Russell Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, 950 College Station Rd, Athens, GA, 30605, United States
[c] Department of Home Economics Education, Korea University, Seoul, 136-701, Republic of Korea

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ABSTRACT

Isopropanol was used to sequentially precipitate sugar beet pectin (SBP) and generate six fractions that varied in galacturonic acid, protein, ferulic acid and other physic–chemical characteristics. Protein was high in Fractions 1 and 2 at 209 and 154 μg/mg alcohol insoluble solids (AIS), respectively. Fractions 3, 4, and 5 had lower protein values of 77–106 μg/mg AIS; the protein content was 87 μg/mg AIS in unfractionated control. Ferulic acid content in Fraction 1 was 93 μg/mg AIS and at least three times higher than measured in other fractions. The degree of esterification in Fraction 1 was 11% and ranged from 46 to 74% in later precipitating fractions. The relative fluorescence with an external probe for hydrophobicity ranged from 159 to 254 in F1, F2 and F3 compared to negligible values in later fractions. The ζ-potential ranged from −25 to −33 mV and was not different between fractions or control. Molecular weight analysis indicated highly heterogenous pectin (Mw/Mn greater than 2.5) with MW values of 398,630, 543,900, and 442,330 g/mol for F1, F4 and control, respectively. FTIR analysis confirmed that F1 and F2 were richer in protein. SBP that precipitated at the lowest isopropanol addition had the greatest protein, ferulic acid content, particle size, relative fluorescence intensity and lowest uronic acid content and degree of esterification. The type and/or nature of protein in F1 and F4 is likely different. Isopropanol fractionation resulted in SBP fractions with unique physico–chemical properties.

1. Introduction

Pectins are complex hetero-polysaccharides which are typically used as gelling and thickening agents in the food industry, but sugar beet pectin (SBP) shows good emulsifying properties (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). The homogalacturonan (HG) backbone or smooth regions of pectin may be methyl esterified; the rhamnogalacturonan (RGI) region is rich in neutral sugars; other side chains and functional groups on pectin include xylogalacturonans, arabinogalactan I and II and the highly conserved rhamnogalacturonan RGII region. Compared to apple or citrus, SBP pectin is a poor gelling pectin, attributed to the presence of acetyl groups (Michel, Thibault, Mercier, Heitz, & Pouillaude, 1985). SBP also contains ferulic acid, which is the site for oxidative crosslinking (Oosterveld, Grabber, Beldman, Ralph, & Voragen, 1997); protein is present in amounts greater than typically found in polysaccharides (Michel et al. 1985). The emulsifying properties of SBP are comparable to gum acacia and structural domains, such as degree of acetylation, ferulic acid, protein and GalA/protein ratio, influence emulsification (Leroux et al. 2003). The hydrophobic moieties of SBP, adsorb and anchor to the oil droplet surface and reduce the interfacial tension while the carbohydrate moiety stabilizes by steric and viscosity effects in the aqueous phase (Funami, Zhang, Hiroe, Noda, Nakauma, Asai, et al., 2007).

The amount of protein reported in SBP varies according to extraction and detection methods and reported values are 8.6% (Kirby, MacDougall, & Morris, 2008); 5.38% (Funami et al., 2011); 10.4% (Thibault, 1988); 2% (Leroux et al. 2003); 3.7% (Williams, Sayers, Viebke, & Senan, 2005); and 5.2% (Mesbahi, Jamalian, & Farahnaky, 2005). However, the quantity of protein is not a good indicator of emulsifying properties; protein rich and protein depleted fractions obtained from hydrophobic chromatography

* Corresponding author. Current address: School of Nutrition and Food Sciences, Louisiana State University AgCenter, Baton Rouge, LA, 70803, United States.
E-mail address: lwicker@agcenter.lsu.edu (L. Wicker).
[1] Department of Food Science and Technology, Sunchon National University, Seoul, Republic of Korea.

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(Williams et al. 2005) or selective extraction (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007 both show good emulsifying activity. Nevertheless, the preponderance of evidence suggests that protein is essential to emulsification. Enzymatic proteolysis of SPB reduces the emulsifying activity (Funami et al. 2007, 2011). The SBP adsorbed to the oil interface is consistently richer in protein (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002; Leroux et al. 2003; Siew, Williams, Cui, & Wang, 2008; Williams et al. 2005; Yapo et al., 2007.

The structural features responsible for good emulsification by SBP remain elusive and emulsification properties are influenced by the accessibility of protein and ferulic acid to the surface of the oil droplets, the proportion of ester groups and molecular mass distribution of the fractions (Williams et al. 2005). The adsorption of protein moiety on the oil droplet is retarded by the carbohydrate portion of the hydrocolloid (Castellani, Al-Assaf, Axelos, Phillips, & Anton, 2010). Notably, protein associated pectin fractions tend to have lower molar mass of less than 70 kDa, compared to protein poor pectin fractions (Fishman, Chau, Qi, Hotchkiss, & Yadav, 2013). From selective enzymatic fractionation studies with protease, polygalacturonase and/or arabinanase/galactanase, protein and ferulic acid were predicted to be attached to the neutral sugar rich region of pectin dispersions (Funami et al. 2011). Based on atomic force microscopy (AFM) images, SBP is portrayed as tadpole-like structures with the globular protein moiety attached to the end of a rigid, unbranched pectin chain (Kirby et al. 2008). AFM also revealed that spherical structures are embedded in a stranded network that dissociated into component parts of varying shapes when SBP concentration was reduced from 12.5 to 6.25 μg/mL (Fishman, Cooke, & Hotchkiss, 2008). Tryptic digests of SBP revealed that the protein moiety of SBP was homologous with arabinogalactan extensin protein; the protein was likely embedded in rod like structures, potentially in a protein–pectin complex (Nunez, Fishman, Fortis, Cooke, & Hotchkiss, 2009). Consecutive and/or independent enzymatic degradation of SBP by esterases, proteases, pectinases, arabinases, and galactanases showed that protein, ferulic acid and ferulic acid-arabino-galactan protein conjugates contributed to emulsifying activity (Chen, Fu, & Luo, 2016), confirming earlier findings that fractions of SBP adsorbed to oil droplet surface were richer in protein and ferulic acid (Siew & Williams, 2008).

The hydrophobic nature of SBP can be attributed to several structural features. The methyl and acetyl ester groups on galacturonan residues provide hydrophobic character to pectin (Burapapadh, Kumpugdee-Vollrath, Chantasart, & Sriamornsak, 2010; Dea & Madden, 1986). Ferulic acid also contributes to the hydrophobic nature of SBP and is esterified to C-2 of the arabinose residues and C-6 of the galactose residues in SBP (Colquhoun, Ralet, Thibault, Faulds, & Williamson, 1994); about 30% of the ferulic groups are carried by the arabinose side chains (Guillon & Thibault, 1990). The SBP protein moiety is considered to be the principal parameter affecting the hydrophobic nature and emulsifying capability based on adsorption (Leroux et al. 2003; Siew & Williams, 2008), enzymatic degradation (Funami et al. 2011), and membrane extraction (Yapo, Wathelet, & Paquot, 2007).

In protein chemistry, salting out proteins with salts like ammonium sulfate or selective solvent precipitation of surface active proteins by decreasing the dielectric constant are commonly used techniques to partially isolate and concentrate proteins. Alcohol precipitation of pectin is routinely used in commercial processing of pectin. Furthermore, in pectin extraction from the cell wall, differential fractionation from other cell wall polysaccharides and fractionation of pectin with different molecular weight values varies with the amount and type of alcohol (Kertesz, 1953). More recently, step precipitation of SBP fractions with incremental increases from 50% to 80% ethanol (Guo, Meng, Zhu, Tang, Pan & Yu, 2016) or at different pH values (Guo, Meng, Tang, Pan, Zhu, & Yu, 2016) resulted in pectin fractions of different yield and physico–chemical characteristics. It is likely that SBP of different amphiphilic nature may precipitate at different concentrations of alcohol depending on the surface activity. In this study, isopropanol was used to obtain fractions from sugar beet pectin dispersions and fractions were characterized for protein, ferulic acid, surface hydrophobicity, galacturonic acid, particle size, zeta potential and degree of esterification. The results may be useful to understand the nature of the emulsifying nature and other functional attributes of SBP.

2. Materials and methods

2.1. Materials

Sugar beet pectin- GENU® pectin type was donated by CPKelco, Denmark (Batch no. GR91400), isopropanol was obtained from J.T Baker (Phillipsburg, NJ). The standards, ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid) and D-galacturonic acid were obtained from Sigma Aldrich (St. Louis, MO). The fluorescent probe, 8-anilino-1-naphthalene-sulfonic acid (ANS), was obtained from Invitrogen Molecular Probes (Eugene, OR); m-hydroxydiphenyl was obtained from Eastman Kodak Company (Rochester, NY). Other chemicals were reagent grade and obtained from Fisher Chemical (Atlanta GA) or J.T Baker, (Phillipsburg, NJ). Type I (ultrapure) or Type II (pure) water was used as described; Type I and II water is defined according to ASTM standards (www.merckmillipore.com).

2.2. Methods

2.2.1. Fractionation

A dispersion of 20 mg/mL SBP was made by slowly adding 20 g of SBP to 1000 mL of Type II water and hydrated at 4 °C overnight. An aliquot of 100 mL SBP dispersion was removed and 100 mL isopropanol was added to the remaining 900 mL of the 20 mg/mL SBP dispersion. Isopropanol was slowly added while pectin was mixed on a magnetic stirrer, followed by centrifugation at 8,000x g, 4 °C for 20 min (Thermo Electron Corporation Sorvall RC 6 Plus High Speed Centrifuge (Ashville, NC)). The supernatant was filtered using Miracloth™ (Calbiochem, La Jolla, CA). The pellet was spread evenly on a petri dish and placed in a fume hood to evaporate residual isopropanol. The dried pellet was ground using a Satake AC 100 grinder (Stafford, TX) and stored at 4 °C until analysis. Additional aliquots of 100 mL isopropanol were sequentially added to the supernatant obtained in the previous step, mixed, centrifuged, dried and stored as stated earlier, for a total of six fractionations. At the end of six fractionations, no further pellet was obtained and the amount obtained in Fraction 6 was variable. Fractions were denoted as F1, F2, F3, F4, F5 and F6 respectively. The initial dry powder or 100 mL control was used directly in subsequent analysis.

2.2.2. Protein, ferulic and galacturonic acid (GalA)

Protein contents of unfractionated SBP (control) and fractions F1–F6 were determined using the Pierce® BCA Protein Assay Kit (Bicinchoninic acid, BCA, method) with bovine serum albumin as standard. The microplate was read at 560 nm using a BIO-RAD iMark microplate reader from Bio-Rad Laboratories (Hercules, CA).

Ferulic acid was estimated at 310 nm using a Shimadzu UV-Pharmaspec 1700 UV-Visible spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). A standard curve between 0 and 12.5 μg ferulic acid was constructed using standard trans-ferulic acid (trans-4-hydroxy-3-methoxycinnamic acid) as described (Siew & Williams, 2008). Dispersions of 0.05 mg AIS/mL