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Emulsion stability of sugar beet pectin fractions obtained by isopropanol fractionation

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ABSTRACT

Protein rich (F1) and protein poor (F4) fractions of sugar beet pectin (SBP). Protein rich F1, protein poor F4, and control SBP were used to make oil-in-water emulsions with 0.5% or 1.5% SBP (F1_0.5, F1_1.5, F4_0.5, F4_1.5 or C_1.5) and 15% (medium chain triglyceride) MCT oil and emulsifying activity was determined. Changes in D[4,3], light microscopy and rheological parameters during storage at 32 °C for 10 days indicated that SBP fraction and concentration influenced emulsion stability. Emulsions prepared with 0.5% fraction F4_0.5, with lower protein and surface hydrophobicity, had a D[4,3] values less than 1 μm, which remained the same during storage. D[4,3] values of emulsions, prepared with the 1.5% fraction F1_1.5 with higher protein and surface hydrophobicity, increased from 1.42 to 2.09 μm at 10 d. Emulsions prepared with unfractionated control SBP were intermediate in particle size. Emulsions prepared with F4_1.5 and F1_0.5 had higher D[4,3] values of 3.45 and 4.18 μm after 10 d, respectively. There were no significant changes in viscosities of individual emulsions over 10 days. Emulsions were shear thinning or near Newtonian. Protein poor Fraction F4 with greater hydrophile/lipophile ratio formed more stable emulsions at 0.5%, but not at 1.5%, compared to protein rich Fraction F1 at 1.5%.

1. Introduction

Sugar beet pectin (SBP) contains higher amounts of hydrophobic acetyl groups on the galacturonic acid chain than apple or citrus pectin (Michel, Thibault, Mercier, Heitz, & Pouillaude, 1985), greater neutral sugar side chains, phenolic esters of ferulic acid and protein in the neutral sugar region (Kirby, MacDougall, & Morris, 2008). The fraction of sugar beet pectin adsorbed on the oil phase of an emulsion is enriched in hydrophobic groups like protein (Siew, Williams, Cui, & Wang, 2008; Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Akhtar, Dickinson, Mazoyer, & Langendorff, 2002; Funami et al., 2007) and ferulic acid (Siew et al., 2008; Funami et al., 2007). Also, the ratio of galacturonic acid to side chains is low in the adsorbed pectin fraction. It is proposed that the positively charged protein moieties interact with the negatively charged galacturonic acid and form multi-layers at the surface of the oil droplets (Siew et al., 2008). Many studies show that protein is an important factor for emulsification, but there is no simple relationship (Siew et al., 2008; Williams, Sayers, Viebke, & Senan, 2005). Emulsification properties are influenced by the accessibility of protein and ferulic acid to the surface of the oil droplets, proportion of ester groups and molecular mass distribution of the fractions (Williams et al., 2005). Larger MW fractions (306 kDa-562 kDa) gave larger droplet sizes and less stable emulsions (Williams et al., 2005). The higher amount of acetyl groups confer emulsifying properties instead of gelling (Leroux et al., 2003), although, treatment with hydrogen peroxide/peroxidase and ammonium persulfate causes gelling in SBP (Oosterveld, Grabber, Beldman, Ralph, & Voragen, 1997).

The proposed mechanism by which SBP acts as an emulsifying agent is that the hydrophobic moieties like protein and ferulic acid adsorb and anchor pectin to the oil droplet surface and reduce the interfacial tension between the oil and water interface (Funami et al., 2007; Leroux et al., 2003); Chen, Fu, & Luo, 2016. The carbohydrate moiety stabilizes by steric and viscosity effects in the aqueous phase (Leroux et al., 2003).

SBP may be a naturally sourced alternative to gum arabic, a very efficient food emulsifying agent. SBP has more protein than gum arabic and can be used in smaller quantities of ~1.5%–3%, compared

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to 10–20%. Also, SBP has an extended configuration and a semi-flexible chain with radius of gyration of 39 nm, whereas gum arabic has a globular, coil configuration with radius of gyration of 28 nm (Castellani, Al-Assaf, Axelos, Phillips, & Anton, 2010). SBP produces the same reduction in interfacial tension as 15% gum arabic (Akhtar et al., 2002; Leroux et al., 2003).

SBP emulsifying activity was enhanced using a patented maturation process that included heating sugar beet pectin powder at 50–150 °C at relative humidity of 20%–90% for 1–48 h. The modified pectin formed a bulky carbohydrate layer and improved steric stabilization around the oil droplets (Funami et al., 2008). The enhanced SBP also produced orange oil and lime oil emulsions that were stable to thermal treatment, unlike the untreated control SBP.

Nevertheless, the emulsifying nature of SBP is elusive. Hydrophobic affinity chromatography was used to fractionate SBP into fractions that differed in protein, ferulic acid and molecular weight (Williams et al., 2005). Emulsions made with the low molecular weight fractions had smaller droplet sizes and were stable for a longer time as compared to the high molecular weight fractions. However, Williams et al. (2005) did not observe a correlation between protein or ferulic acid content and emulsification property; some protein or ferulic acid rich fractions formed stable emulsions, but others did not. Likewise protein or ferulic acid depleted fractions formed stable emulsions. The contribution of protein was shown by poor emulsifying activity of protease treated SBP (Funami et al., 2007; Chen et al., 2016). The modified SBP with reduced protein content, molecular weight and radius of gyration gave emulsions with increased droplet size (from 0.56 μm to 3 μm), increased creaming and lower adsorption to the oil phase. Enzymatic degradation of enhanced or non-enhanced SPB that cleaved protein, neutral sugar side chains or the polygalacturonic backbone, respectively, resulted in greater loss of emulsification ability Funami et al. (2011). Cleaving the side chains reduced both the protein and ferulic acid content. These results indicated that the protein moiety in SBP contributed significantly to the emulsifying property. More recently, the impact of enzymatic degradation of SBP with esterases, proteases, arabinases, galactanases, pectinases, yielded fractions that varied in EA. The major contributors to EA was ferulic acid, conjugates of ferulic acid, arabino-galactan-protein and protein (Chen et al., 2016).

A simpler means to fractionate SBP according to polarity and solubility in non-polar solvents was previously described and the fractions varied in protein content, surface hydrophobicity, ferulic acid and other physico-chemical characteristics (Karnik, Jung, Hawkins and Wicker, 2016). Isopropanol precipitation offers a low technology alternative fractionate SBP by surface activity. In our earlier study, pectins fractionated at lower isopropanol concentration (F1) had higher protein, higher surface hydrophobicity, higher ferulic acid, higher particle size than pectins fractionated at higher isopropanol concentration (F4) or control, unfractionated pectin. In this study, the objective was to determine the EA and stability in an accelerated shelf life study of protein rich and protein depleted SBP fractions derived from isopropanol precipitation. Additionally, the effect of concentration of emulsifier on emulsion droplet size and consistency was determined.

2. Materials and methods

2.1. Materials

Sugar beet pectin- GENU® pectin type BETA was donated by CPKelco (Copenhagen, Denmark, Batch no. GR91400). Isopropanol and sodium benzoate were obtained from J.T Baker (Phillipsburg, NJ). Medium chain triglyceride was obtained from (Now Sports MCT oil, Catalog no. 02211) from Now Foods, Bloomingdale, IL.)

2.2. Methods

2.2.1. Fractionation

A total volume of 3 L of 2% SBP was made in deionized Type II water. After removal of 300 mL of pectin for use as control, pectin was sequentially fractionated by adding 300 mL aliquots of isopropanol as described previously by Karnik et al., 2016. Briefly, 300 mL isopropanol to 2700 mL SBP while stirring on magnetic stirrer. With each fractionation step, the precipitated material was recovered by centrifugation and an additional 300 mL isopropanol was added to the supernatant until four fractions were obtained. The pellets were dried under a fume hood, ground using a Satake AC 100 grinder (Stafford, TX) and stored at 4 °C. The alcohol insoluble solids (AIS) collected at successive alcohol additions were denoted as Fractions F1, F2, F3 and F4, respectively. Based on preliminary data, in addition to the control, Fractions F1 and F4 were selected for further analysis. Results from three replications were reported.

2.2.2. Emulsion preparation

Fractions F1, F4 and unfractionated control SBP were used to make emulsions at 0.5% or 1.5% pectin and labeled F1_1.5, F1_0.5, F4_1.5, F4_0.5 and C_1.5, respectively. Sugar beet pectin or fractions (1.5 g or 0.5 g) were dissolved in 80 g DI water and hydrated overnight at 4 °C. The SBP dispersion was homogenized at speed 4 for 2 min at 4 °C using a PRO Scientific Inc. PRO300A homogenizer (Oxford, CT). A stock solution of 10% was made by dissolving 5 g sodium benzoate in 50 mL Type II deionized water. A 1 g aliquot of 10% sodium benzoate stock and 15 g MCT oil was added to the homogenized solution and the final weight was made to 100 g using DI water. The mixture was homogenized again at 24,000 rpm for 2 min. This crude emulsion was immediately homogenized at 35 MPa (Avestin EmulsiFlex-C5 from Avestin Inc., Ottawa, Canada) for a total of three passes; emulsions were collected on ice between passes. The preparation of the five emulsions is summarized in Table 1. The emulsions were stored 10 days in glass jars with screw caps at 32 °C (THELCO Model 4, Thermo Scientific, Asheville, NC).

2.2.3. Emulsion characterization

The particle size was measured using a Malvern Mastersizer 2000 (Worcestershire, UK). The emulsion was diluted in the dispersion unit by stirring at 2000 rpm until an obscuration value between 10 and 30% was obtained. The droplet diameters were reported on a volume basis D[4,3] and surface area basis D[3,2]. The relative span value $(d_{90} - d_{10})/d_{50}$ (calculated by the software), was used to report the distribution width of the emulsions. Relative span is given by $(d_{90} - d_{10})/d_{50,}$ where d_{90} is the diameter below which 90% of the droplets can be placed, d_{10} the diameter below which 10% of the droplets can be placed, and d_{50} the diameter below which 50% of the droplets can be placed. The droplet size and distribution width were measured each day of storage.

Steady stress controlled tests were done on the emulsions on day 0 and day 10 of storage using a Rheometric Scientific SR-5000 stress rheometer (Thermo Scientific, Ashville, NC) with concentric cylinder geometry. The gap between the cylinder and cup was 0.5 mm. An aliquot of 20 mL of sample was kept on ice for an hour to equilibrate to 4 °C, and was filled into the cup and allowed to relax for 5 min with the cylinder inside the cup (Meriem-Benziane, Abdul-Wahab, Benaicha, & Belhadri, 2012). The sample was then subjected to steady stress sweep test. The range of stress applied on the sample was 0.1 Pa–100 Pa. The rheological parameters tested were viscosity, yield stress and consistency.

Dark field microscopy was done on the emulsions on days 0, 5 and 10. The sample was put on a glass slide and covered gently with a coverslip. It was viewed with 100× oil immersion lens. Spot