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Effect of molecular structure on emulsifying properties of sugar beet pulp pectin

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ABSTRACT

To investigate the impact of each functional group on the emulsifying properties of sugar beet pulp pectin (SBPP), seven enzymes were studied in a particular order. Compositions of SBPP and enzymatically modified SBPPs were determined, and the structures of SBPPs were characterized by FT-IR. In addition, the contribution of each functional group was evaluated based on the variation in emulsion characteristics. The results showed that protein, ferulic acid-araban/galactan-protein complexes and ferulic acid played important roles in improving the surface activity, emulsifying capacity and emulsifying stability of SBPP and the extent of the decrease in the emulsifying activity followed the order: ferulic acid > ferulic acid-arabinogalactan-protein complexes > protein. The decrease of methyl ester groups mainly affected the particle sizes of the emulsion. In addition to particle sizes, the cream index of the emulsion increased with the hydrolysis of acetyl groups. Arabinose and galactose less affected emulsifying properties than other functional groups.

1. Introduction

Pectin is widely used in the food industry for its gelling, thickening, and stabilizing properties (Funami et al., 2007; Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). Commercial pectins are extracted from citrus peel and apple pomace in most instances (Funami et al., 2011). A relatively new type of pectin, sugar beet pulp pectin (SBPP), has recently received much attention (Fissore, Rojas, Gerschenson, & Williams, 2013; Siew & Williams, 2008).

As shown in Fig. 1, SBPP is a heteropolysaccharide with a chain structure of (1 → 4)-linked α-D-galacturonic acid (GalA) units interrupted by the insertion of (1 → 2)-linked L-rhamnopyranosyl residues. Rhamnosyl residues (20–80%) can be substituted with side chains ('hairy' region) consisting of neutral sugars, such as D-galactose, L-arabinose, D-xylose, D-glucose, D-mannose, L-fucose and D-glucuronic acid. In addition, lateral chains contain phenolic acids such as ferulic acid, which are linked to the arabinose and galactose residues via ester linkages (Fry, 1983). In addition, there is a higher concentration of the proteinaceous materials bound to the side chains through covalent linkages (Williams et al., 2005). Some of the acid groups of the GalA in the linear chain structure ('smooth' region) can be partially methyl-esterified and O-acetylated at the C-2 and/or C-3 positions (Siew & Williams, 2008). Compared to other conventional pectins, SBPP tends to exhibit a higher degree of acetylation (DA) and a greater number of neutral sugar side chains (rich in hairy regions) (Siew & Williams, 2008). In addition, SBPP has a greater number of feruloyl groups attached to the galactose and arabinose side chains (Colquhoun, Ralet, Thibault, Faulds, & Williamson, 1994; Guillon, Thibault, Rombouts, Voragen, & Pilnik, 1989; Ralet, Thibault, Faulds, & Williamson, 1994; Rombouts & Thibault, 1986) and a greater amount of proteinaceous material bound to the lateral chains through covalent linkages (Funami et al., 2007; Williams et al., 2005). Because of these differences in structural characteristics, SBPP does not have the capability to form gels like conventional pectins, but it possesses excellent emulsifying properties (Funami et al., 2007; Li et al., 2013; Voragen et al., 1995; Williams et al., 2005). According to Endreß and Rentschler (1999), the emulsifying ability of beet pectin can be explained by the high percentage of acetyl groups in its chemical structure.

Abbreviations: SBPP, sugar beet pulp pectin; FT-IR, Fourier Transform Infrared Spectroscopy; GalA, galacturonic acid units; PG, polygalacturonase; ABN, arabinanase; GAL, galactanase; PE, protease (combination of pepsin and food-grade acid protease); FAE, feruloyl esterase; PME, pectin methyl esterase; PAE, pectin acetyl esterase; MCT, medium-chain triglyceride; Rha, rhamnose; Ara, arabinose; Xyl, xylose; Gal, galactose; Glc, glucose; GalA, galacturonic acid; FA, ferulic acid; DM, degree of methylation; DA, degree of acetylation; CI, cream index.

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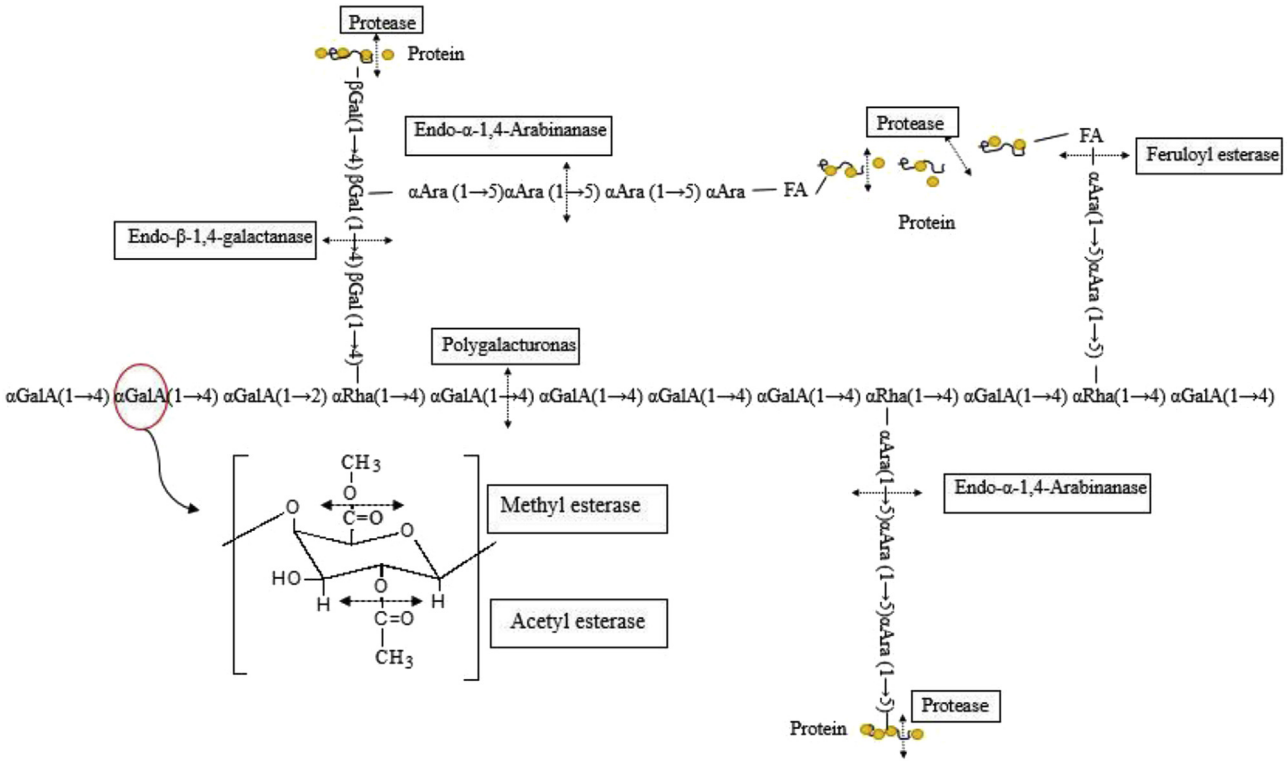

Fig. 1. Schematic figure of SBPP and enzymes used in the experiments. GalA: Galacturonic acid; Rha: Rhamnose; Gal: Galactose; Ara: Arabinose; FA: Ferulic acid.

Nevertheless, Leroux, Langendorff, Schick, Vaishnav, and Mazoyer (2003) studied the emulsifying ability of sugar beet pectin in relation to its chemical structure and concluded that there was no evidence that its emulsifying ability correlates with the number of acetyl groups in its structure but, rather, is related to its high concentration of proteinaceous components (Williams et al., 2005). Siew and Williams (2008) suggested that proteins and/or ferulic acid groups adsorb onto the surfaces of the oil droplets and stabilize the emulsions. Leroux et al., (2003) concluded that a highly methylesterified pectin is able to reduce the interfacial tension between the water and the oil phases. It is likely its hydrophobicity (due to its $COOCH_3$-groups) that gives pectin its emulsifying properties. SBPP was fractionated using hydrophobic affinity chromatography, and three fractions with different proportions of protein, ferulic acid and weight-average molecular mass were obtained (Williams et al., 2005). They concluded that the emulsification properties of SBPP were influenced by the protein, ferulic acid groups, proportion of ester groups, and molecular mass distribution of the fractions. SBPPs were structurally modified using protease to degrade the proteinaceous moiety, polygalacturonase (PG) to cleave the carbohydrate backbone, and a arabinanase/galactanase (ABN/GAL) combination to cleave the lateral chains (Funami et al., 2011). Methyl and acetyl groups were removed with galacturonic acid (GalA) via the modification of PG. In addition, protein and ferulic acid exist in the side chain (mainly covalently bonding with arabinose and galactose); therefore, the combination of ABN/GAL can alter four factors (arabinose, galactose, protein and ferulic acid) at the same time. None of the previous papers provided a clear explanation for the relationship between each factor and the emulsification ability of pectins.

The objective of the present study was to investigate the emulsifying properties of SBPP in relation to its structural characteristics. SBPPs were structurally modified using protease (combination of pepsin and food-grade acid protease, PE), endo-α-1,4-polygalacturonase (PG), endo-β-1,5-arabinanase (ABN), endo-β-1, 4-galactanase (GAL), feruloyl esterase (FAE), pectin methyl esterase (PME) and pectin acetyl esterase (PAE) to degrade the proteinaceous moiety, GalA unit, arabinose, galactose, ferulic acid, DM and DA subunits, respectively. The contribution of each of these structural units to the emulsification of SBPP was assessed through enzymatic modification in a particular order.

2. Materials and methods

2.1. Materials and chemicals

Sugar beet pulp pectin was purchased from CP Kelco (Lille Skensved, Denmark). Medium-chain triglyceride (MCT) was purchased from the Nisshin Oillio Group (Tokyo, Japan). GalA and bovine serum albumin were purchased from Sigma–Aldrich Chemical Co. (Milwaukee, WI, USA). PG (EC 3.2.1.15), ABN (EC 3.2.1.99), GAL (EC 3.2.1.89), and FAE (EC 3.1.1.73) were obtained from Megazyme International Ireland Ltd (Bray, Ireland). PME (E.C. 3.1.1.11) and PAE (E.C. 3.1.1.6) were purchased from Sigma–Aldrich Chemical Co. (Milwaukee, WI, USA). Pepsin (E.C. 3.4.23.1) and food-grade acid protease were purchased from Aladdin reagents Co., Ltd (Shanghai, China). The Viscozyme L9 enzyme was a commercial preparation obtained from Novo Nordisk (Copenhagen, Denmark), and because it was a multienzyme complex, purification was required before use (Garna, Mabon, Wathelet, & Paquot, 2004). 2-Deoxy-D-glucose, myo-inositol, L-rhamnose (Rha), L-arabinose (Ara), D-xylose (Xyl), D-galactose (Gal), D-glucose (Glc), D-galacturonic acid (GalA), ferulic acid (FA), succinic acid, glacial acetic acid and methanol were purchased from Sigma–Aldrich Chemical Co. (Milwaukee, WI, USA). All other chemicals were of analytical grade unless otherwise noted.