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New insights into the functionality of protein to the emulsifying properties of sugar beet pectin

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ABSTRACT

Protein plays an important role in the emulsifying properties of sugar beet pectin (SBP). The present work was carried out to investigate the role of protein content and the combination mode between proteins and pectins on the emulsifying properties of SBP. It was found that protein contents and structural differences of SBPs affected the stability of emulsion. As the protein content increased from 0.5 to 3.0%, droplet size of emulsion stabilized by SBP decreased sharply. It turned to be stable when the protein content increased to a high level (from 3.0 to 6.0%). Thermal gravimetric analysis confirmed the structural differences among SBP samples. In order to study the functionality of protein/pectin combination mode to emulsifying properties, modification of SBP was conducted by depleting a portion of proteins, and electrostaticly or covalently binding BSA to the de-proteinized SBP. Results showed that Maillard induced conjugates exhibited the best emulsifying stability, as compared with original SBP, de-proteinized SBP and the electrostatic bonding complex. These results suggested that both protein content and the combination mode between protein and pectin could affect the emulsifying properties of SBP.

1. Introduction

Pectins are ionic plant polysaccharides and widely used in the food industry utilizing because of its gelling, thickening, and stabilizing properties. The common feature of pectins is the backbone of α-(1–4)-linked D-galacturonic acid (GalA) units. Pectin It must consist of at least 65% GalA as stipulated by the Food and Agricultural Organization (FAO) and the European Union (EU) (Willats, McCartney, Mackie, & Knox, 2001). These "smooth" homogalacturonic regions are interrupted by "hairy" rhamnogalacturonic regions. Some rhamnosyl residues are substituted by arabinose, galactose, rhamnose and 13 other monosaccharides (Willats, Knox, & Mikkelsen, 2006).

Sugar beet pectin (SBP) as an emerging potential pectin is obtained extracted from sugar beet pulp. Structural differences between SBP and other common pectins include a higher content of acetyl groups (4–5%) (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003) at O-2 and O-3 positions within the galacturonic backbone which could be additional anchors for the oil droplet (Nakauma et al., 2008), higher content of phenolic esters (0.7–0.9%) in the lateral chains (Michel, Thibault, Mercier, Heitz, & Pouillaude, 1985), protruding lateral chains, and higher content amount of proteins bound to the lateral chains through covalent linkages (Funami et al., 2011; Williams et al., 2005).

Due to the unique structural characteristics, SBP does not have the capability of forming gels like conventional pectin but possessing excellent emulsifying properties (Gülseren & Corredig, 2014; Williams et al., 2005). It has been confirmed that SBPs effectively stabilize emulsion by adsorbing on the oil droplet surface, then forming a thick hydrated layer. The SBP-layer could prevent droplets from flocculation and coalescence through electrostatic and steric repulsive force (Nakauma et al., 2008). SBP is superior to other food hydrocolloids as it requires relatively less amounts to be active at the oil-in-water interface. Its working concentration as an emulsifier is tremendously low (about 1.5%), compared with soybean soluble polysaccharides (4%) and gum arabic (as high as 10%) (Funami et al., 2011; Nakauma et al., 2008). Good emulsifying properties of SBP could be attributed to many aspects (protein moiety, acetyl groups and highly branched polysaccharides structures), in which protein plays a predominant role

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(Chanamai & McClements, 2001; Funami et al., 2007; Zhang et al., 2014). Funami et al. (2011) found that de-proteinized SBP (protein content decreased from 5 to 0.5%) showed worse emulsifying properties than the untreated SBP. Siew, Williams, Cui, and Wang (2008) reported that the fractions of SBP with higher protein content (12% higher than the average) would be more prone to adsorb to the oil droplet.

It has been well recognized that impressive improvements in emulsion stability and functional properties can be achieved via the complex between combination of proteins and polysaccharides., which It could combines the superiority of both proteins and polysaccharides each of them: proteins prior to preferentially attach to O/W interface (Dickinson & McClements, 1996; McClements, 2004) and polysaccharides enable to could form a thicker stabilizing layer that which is capable of protecting droplets against aggregation (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012). In addition, emulsion stabilized by protein/polysaccharide complex interface have has been acknowledged known to improve chemical stability against oxidation reactions (Nagasawa, Takahashi, & Hattori, 1996),.e.g. For example, interactions between lipids and metal ions can be minimized by controlling the interfacial charge, thickness and density (Berendsen, Güell, Henry, & Ferrando, 2014). There are three classes of protein/polysaccharide combinations namely modes: naturally-occurring complexes in which protein residues are covalently attached to the polysaccharide chains; electrostatic complexes formed between polysaccharides and proteins with opposite net charge (Evans, Ratcliffe, & Williams, 2013), including alternative layers of oppositely charged biopolymers can be formed around the oil globules to obtain multi-layered membranes. These layers provide both electrostatic and steric stabilizations thus improving thermal stability and resistance to external treatment; artificially-covalent cross-linking which is mainly divided into including enzymatic modification (Littoz & McClements, 2008) and Maillard reaction (Akhtar & Dickinson, 2007; Xu et al., 2014). The use utilization of polysaccharides and proteins to stabilize oil in water emulsion has been undertaken studied for many years (Akhtar & Dickinson, 2007; Li, Fang, Al-Assaf, Phillips,& Jiang, 2012; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2003). However, comparison and complex combination of these binding modes have rarely not been studied reported.

Although some studies have reported on the emulsifying properties of sugar beet pectins use of polysaccharides and proteins to stabilize oil in water emulsion has been undertaken for many years, few researches focused on literature are available with regard understanding the impact of protein content and combination mode between protein and pectin polysaccharide on the emulsifying properties of SBP. In the present work, SBPs were extracted under different conditions. Protein, ferulic acid, galacturonic acid content, and pyrolysis characteristics of SBPs were analyzed. Correlation between SBP protein content and particle size of emulsions stabilized by SBPs was carried out. SBP extracted at 85 °C, pH 1.4, for 2 h with protein content of 3.18% was selected to further illustrate the role of protein on SBPs' emulsifying properties. It was treated by pepsin to deplete proteins. Supplementary amount of bovine serum album (BSA) was added to the de-proteinized SBP through electrostatic bonding or covalent binding to confirm the functionality of protein/pectin combination mode on emulsifying properties. Various environmental stresses including thermal treatment, ionic strengths and pH were employed to evaluate emulsifying stability of those complexes or SBP alone to further clarify the role of protein on emulsifying properties of SBP.

2. Material and methods

2.1. Materials and chemicals

Sugar beet pulp was supplied by Actis trade Co., LTD (Xinjiang Province, China). Soybean oil was purchased from local supermarket. Pepsin (806.3 U/mg, Lot No. P7000, Sigma–Aldrich Co., USA) from porcine gastric mucosa was applied to deplete proteins. BSA (Lot No. JM 1042) was purchased from Roche LTD (USA). Ferulic acid and GalA were supplied by State Center for Standard Matter (China). Potassium bromide was obtained from Sigma–Aldrich Co. (USA).

2.2. Samples preparation

2.2.1. Extraction of sugar beet pectin (SBP)

SBPs were extracted from sugar beet pulp with aqueous hydrochloric acid (1:30, w/v) under different extraction conditions, mainly including three important parameters: temperature (75, 85, 95 °C), pH (1, 1.4, 1.8) and duration (2, 3, 4 h) as listed in Table 1 (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). The resulting slurries were cooled to room temperature before the pH was adjusted to 4.5 using 1 M NaOH/1 M HCl. The suspension was filtrated with gauze to eliminate residues. Then it was centrifuged at 25 °C for 5 min at 8000 rpm using centrifuge (GL-20G-II, Anke, Shanghai, China). Two volumes of ethanol were added to the supernatant in order to precipitate pectin. The obtained mixture was kept at room temperature for 4 h to precipitate pectin completely. Then pectin was filtered with 250 mesh filter cloth, then washed by acetone, and 85% ethanol separately (Levigne, Ralet, & Thibault, 2002). Finally, the obtained pectin was freeze-dried and stored in the desiccator for further analysis.

2.2.2. De-proteinization of SBP (DSBP)

The de-proteinization of SBP was conducted by enzyme treatment. Our previous study showed that only 50% protein content could be depleted by the pepsin treatment (data not shown). DSBP originated from SBP with too high protein content may still on the right dashed line (Fig. 1) which was not sensitive to protein content and keep good emulsifying activity. As a result, it would not reveal the impact of protein content. However, emulsifying capacity of SBPs close to the dashed line in Fig. 1, was sensitive to changes in protein content. Decreasing in protein content could affect emulsifying properties significantly. Therefore, SBP extracted under 85 °C, pH 1.4, 2 h with protein content of 3.18%, corresponding to SBP-L in Fig. 1 was used to prepare the de-proteinzed SBP, and then form DSBP/BSA hybrids.

One gram of SBP was dispersed in 100 mL distilled water (1 g 100 mL^{-1}), with magnetic stirring for 12 h. Pepsin (800 U/g SBP) was added into the dispersions and then incubated at 40 °C for 12 h to decompose protein completely. The mixture was heated at 90 °C for 5 min using water bath to terminate the enzymatic reaction. It was dialyzed against distilled water at room temperature using a dialysis membrane with a 10 kDa molecular weight cut off. The dialyzed sample was lyophilized for following measurement (Funami et al., 2011).

2.2.3. Preparation of the hybrids of DSBP and BSA (DSBP/BSA complex and DSBP/BSA conjugate)

Certain amounts of BSA was added to DSBP sample, enabling it to achieve the equivalent protein content (3.18%) of the original SBP. One gram of the mixture was dispersed into 100 mL distilled water with magnetic stirring for 12 h to ensure complete dissolution to obtain SBP/BSA complex. The pH value of the mixture was 3.8, at which BSA and de-proteinized SBP were oppositely charged to