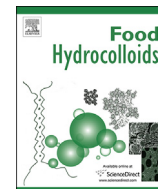




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# Ethanol precipitation of sugar beet pectins as affected by electrostatic interactions between counter ions and pectin chains

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## ABSTRACT

The ethanol precipitation of sugar beet pectins (SBPs) with and without counter ions was investigated. In aqueous solution, when cations bind to SBPs, the negative charges on the pectin chains are reduced and the hydrodynamic radius ( $R_h$ ) of the whole polymer is decreased. Cation-bound SBP is more prone to precipitation by ethanol because it is less solvated by water molecules. In the absence of cations, partially-ionized SBP interacts extensively with water molecules at the initial precipitation pH (I-pH) range of 3.26–2.83, which makes it difficult to precipitate the pectins by addition of ethanol. However, in the presence of cations, precipitation of SBPs is straightforward due to cation binding and ethanol dehydration. Protonated SBPs and SBP salts were prepared and their conformational features were analyzed by  $^{13}\text{C}$  solid-state NMR and X-ray diffraction spectroscopies. The results show that no conformational transition occurs in cation-bound SBP after ethanol precipitation, which indicates that the electrostatic nature of the cation–SBP interaction is important during the precipitation process. A schematic illustration of the cation–SBP electrostatic interaction during the precipitation process is proposed.

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## 1. Introduction

Pectins are a family of heterogeneous polysaccharides that are comprised of three types of structural regions: a homogalacturonan (HG), a type I rhamnogalacturonan (RG-I) and a type II rhamnogalacturonan (RG-II) (Voragen, Coenen, Verhoef, & Schols, 2009). Galacturonic acid (GalA) residues act as important constructing units in these regions, particularly in the HG and RG-I regions. Hence, the galacturonic acid residues are the most widely distributed and common structural unit among the diverse glycosyl residues. The carboxyl groups of the galacturonic acid residues can be partially esterified by methanol (Caffall & Mohnen, 2009) and the degree of methylation (DM) depends on the plant source and extraction conditions (Chan & Choo, 2013; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007). Pectins can be classified into two categories: high-methylated-pectins (DM > 50%) and low-methylated-pectins (DM < 50%) (Yapo, Robert, et al., 2007). Chemically, pectins are considered to be polyanions because the carboxyl groups along

the backbone can dissociate into ionic form, making pectins negatively charged. For this reason, pectins are electrostatically different from neutral polysaccharides, such as starch and hemicellulose.

The strength of ionization of the pectins is governed by intrinsic factors, such as the galacturonic acid content and the degree of methylation of the carboxyl groups (Ralet, Crépeau, Buchholt, & Thibault, 2003) and by extrinsic factors, such as pH and solvent (Faravash & Ashtiani, 2007; Kalapathy & Proctor, 2001; Smidsrød & Haug, 1967). Among these, the pH of the solution has the largest effect on the extent of ionization of the carboxyl groups. The carboxyl groups of pectin are fully deprotonated at  $\text{pH} \geq 4.5$  and fully protonated at  $\text{pH} \leq 2.0$  (Capel, Nicolai, Durand, Boulenguer, & Langendorff, 2006). The tunability of the electronic properties of the pectins by changing the pH of the solution suggests that varying the state of the pectins may result in distinct interactions (i.e. repulsion or aggregation) between the polymer chains. Furthermore, pectins with different electrostatics show behavior differently with cationic compounds under different pH conditions.

Pectins are hydrophilic polysaccharides and interact extensively with water molecules in aqueous solution. Organic precipitants

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may be used to interrupt these pectin-water interactions in order to separate the pectins from the solution. Ethanol and isopropanol are commonly used to purify pectins from an aqueous mixture (Yapo, Wathelet, & Paquot, 2007). Isopropanol is less polar than ethanol and is therefore used in large-scale production (May, 1990). A high alcohol concentration reduces the solubility of the polymer in solution. A 3:1 v/v ratio of alcohol to water gives the best pectin yield for the cost (Xu et al., 2014; Yapo, Wathelet, et al., 2007). It should be noted that the pectin precipitation process can vary largely based on how the pectin solution is mixed with the alcohol. When a pectin solution is added to a known volume of alcohol, the initial relative alcohol concentration is high. However, this relative alcohol concentration decreases upon mixing. Alternatively, when alcohol is added to a pectin solution, the relative alcohol concentration increases from 0 to a maximum concentration. The continuous rise in alcohol concentration, in this case, favors a sequential precipitation of pectin fractions with varying tolerances toward alcohol concentration (Guo, Meng, Zhu, et al., 2016; Karnik, Jung, Hawking, & Wicker, 2016).

Pectins are usually precipitated from water/extract mixtures that contain inorganic sodium, potassium, magnesium and calcium salts (Kamnev, Colina, Rodriguez, Ptitchkina, & Ignatov, 1998). These cations may contribute to the precipitation process by forming ion pairs with the pectins (Smidsrød, & Haug, 1967). This is likely, as the precipitation process is conducted in solutions with pH 1.5–3.5 (Faravash, & Ashtiani, 2007; Kalapathy, & Proctor, 2001) where polyanionic pectin chains may bind cations. Since there are cations present in mixtures of pectin extract and water, it is crucial to investigate how the cations impact the alcoholic-precipitation of pectins. The relationship between cations and pectins during the process of alcohol precipitation has, to our knowledge, never been explored.

The aims of the present work are (i) to elucidate the role of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  in the precipitation of sugar beet pectins in ethanol-water mixtures and (ii) to study the effects of the binding of these cations on the hydrodynamic properties of SBP. A modified precipitation approach was utilized in which ethanol was gradually added to a pectin solution while stirring. Ethanol was chosen as the precipitation solvent because it is highly miscible with salt solutions. Based on the results, possible precipitation behaviors of the protonated-SBPs and deprotonated-SBPs in the presence or absence of cations are proposed.

## 2. Materials and methods

### 2.1. Materials

Sugar beet pectins were extracted from sugar beet pulp (Lvxiang sugar factory) using a heat-acid extraction described in the literature (Guo, Meng, Tang, et al., 2016). Glucan with a molecular weight of 200 kDa was purchased from Sigma-Aldrich Corp. (MO, United States). HPLC-grade chemicals were used in chromatography and reagents for chemical analysis were analytical grade.

### 2.2. Purification of SBP

Fig. 1 shows the scheme for pectin purification and the preparation of different samples. Generally, 10 g of SBP powder was stirred in 1 L Milli-Q water for 12 h at room temperature. The supernatant was collected by centrifugation (15 min at 5000xg), and subsequently purified by precipitating free proteins by addition of  $\text{Cu}^{2+}$  (Guo, Meng, Zhu, Zhang, & Yu, 2015). The resulting pectin solution was passed through a cation-exchange column (500 mm × 30 mm, 001 × 7 resin, Zhengguang Ltd., Zhejiang, China) at a flow rate of 300 mL/h in order to remove any bound

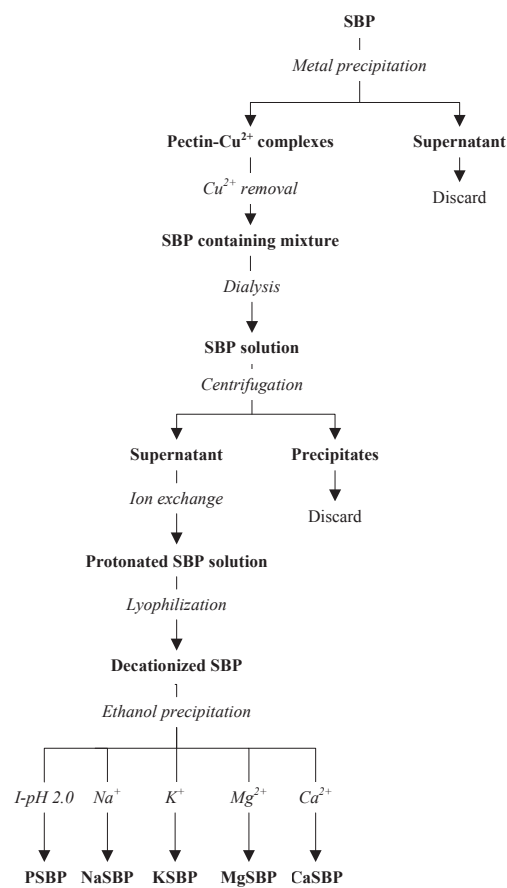


Fig. 1. Scheme for the ethanol precipitation of protonated SBP and SBP salts.

metal ions. Then, the decationized SBP solution was freeze-dried and then air-dried at 45 °C until it was a constant weight. After this purification, a negligible amount of metal ions were present according to high performance cation exchange chromatography (HPCEC) analysis (Supplementary Information Fig. 1). Based on the GalA content (65.8 wt %) and the DM (34.8%), the purified SBPs had an effective formula weight of 422 per carboxyl group.

### 2.3. Ethanolic precipitation

#### 2.3.1. Effects of cations

The polymer concentration used in all precipitation experiments was 5 mequiv · L<sup>-1</sup>. The SBP solutions were precipitated with ethanol in the presence of counter ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ). Each counter ion was added at a known equivalent ratio of cation to carboxyl group in the SBPs before precipitation of the SBPs with ethanol. Ethanol was gradually added to the SBP solution while stirring, and the flow rate of the added ethanol (10 mL/min) was controlled by a constant pump (Longerpump Ltd, Hebei, China). The effect of ethanol on SBP precipitation was evaluated at two concentrations (50% and 67% v/v). The precipitation mixtures were allowed to stand for 6 h to reach an equilibrium state of precipitation, and then centrifuged for 10 min at 12000×g to separate the precipitated pectin gels. After centrifugation, the remaining polysaccharides in the supernatant were analyzed by the phenol-sulfuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Ethanol precipitation experiments were carried out at -25 °C and effects from temperature variation were not taken into account.

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