



## Stepwise ethanolic precipitation of sugar beet pectins from the acidic extract



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### ABSTRACT

A stepwise ethanol-precipitation (SEP) procedure was developed for the purification of sugar beet pectins (SBP) from a pectin-containing aqueous extract. Five fractions of different chemical and molecular characteristics were produced by stepwise elevating the alcohol concentration of the precipitation medium from 50% to 80% v/v. Comparison of chemical and macromolecular features between the obtained fractions indirectly suggested that the ability of pectin to solubilize in the ethanol-water binary mixture depended greatly on the polymer structure. Fractions rich in neutral sugars were precipitated at relatively high ethanol concentrations, probably due to the enhanced interactions generated between pectin side chains and solvent molecules. Furthermore, the obtained fractions displayed different surface activities. Results obtained in this work indicate that the SEP procedure is more selective with respect to pectin structural features and surface properties than the one-step ethanolic precipitation.

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### 1. Introduction

Pectins are a family of heterogeneous polysaccharides located in higher plant cell walls. Chemically, pectins are mainly characterized by a linear backbone comprised of repeating (1 → 4)-linked- $\alpha$ -D-galacturonic acid units (Yapo, 2011). Galacturonic acids among the backbone can be substituted with 2-O-linked- $\alpha$ -L-rhamnose which can bear neutral side-chains (Schols & Voragen, 1994). According to the cell wall models, pectins may interact with protein, cellulose and hemicellulose via complex covalent bonds to form network, which enables cell wall to maintain the entirety and stability of the tissue (Cassab, 1998; Zykwsinska, Ralet, Garnier, & Thibault, 2005). In view of that, the liberation of pectins from cell wall materials is usually based on destructing the compact network using a variety of extraction procedures, e.g. enzymatic and chemical means (May, 1990; Zykwsinska et al., 2008).

Acid extraction is frequently used to solubilize pectins from plant materials into the extractant, and it is usually carried out in the acidic pH range (1.0–1.3) under varying time (20–360 min) and

temperature (60–100 °C) conditions (Yapo, 2009b). This conventional extraction used for pectin production, however, will provoke undesired degradation of pectin chains, particularly the side-chains (Yapo, Robert, Etienne, Wathelet, & Paquot, 2007; Zykwsinska et al., 2008). Consequently, the resulting pectin-containing aqueous extract is most likely to consist of both branched pectin molecules, as well as the partially degraded pectin components (less branched).

Pectins can be purified from aqueous solution by many methods, such as metal precipitation, alcohol precipitation and electrostatic precipitation of pectin–protein complexes (Garna, Emaga, Robert, & Paquot, 2011; Hwang, Roshdy, Kontominas, & Kokini, 1992; Yapo et al., 2007). Among these methods, alcohol precipitation is used on both laboratory and industrial scale as it is advantageous for obtaining a satisfactory pectin yield, and avoiding generating hazardous effluents. However, this commonly-used method has some limitations. It tends to co-precipitate some non-pectic polysaccharides which may include arabinan and galactan (Yapo, 2009b), thereby being a source of error in the assessment of pectin yield (Mañas & Saura-Calixto, 1993). Moreover, due to its insufficient selectivity, alcohol precipitation with a subsequent alcohol washing leads to a heterogenous product consisting of fractions with different structural characteristics (Yapo et al., 2007). These disadvantages with alcohol precipitation, to some extent, do not permit to clearly unveil the pectin structure–function relationship. Procedures that

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are more selective toward precipitating specific fractions, are therefore of considerable practical interest.

Effects of precipitation conditions on pectin precipitation have been examined. Previous studies demonstrated that the yield and chemical characteristics of pectins were greatly susceptible to many factors such as conditions of post alcohol washing, the final ethanol concentration and precipitation pH of the precipitation medium (Faravash & Ashtiani, 2007; Kalapathy & Proctor, 2001; Mañas & Saura-Calixto, 1993). Among them, the ethanol concentration used for pectin precipitation is responsible for interrupting the interactions between pectins and solvent molecules. For obtaining a complete precipitation of pectins, in most cases, an alcohol-to-extract volume ratio of 3:1 is used to give a final ethanol concentration above 70%, which agrees well with the literature data (70–80%) commonly-used for precipitating natural polysaccharides (Xu et al., 2014).

Recently, by using dextrans and pullulans as reference polysaccharides, Xu et al. (2014) stated that the alcoholic-precipitation effect depended not only on ethanol concentration, but also on macromolecular characteristics of the examined polymer. These results suggest the possibility of separating fractions of different structures from the starting mixture using appropriate ethanol concentration. Following this idea, a stepwise alcoholic precipitation procedure, which was conducted by stepwise elevating the ethanol concentration from 50% to 80% v/v, was used for recovering SBP from the acidic extract. Purposes of this work were therefore to assess the feasibility of SEP for pectin precipitation, and to examine how precipitation modes affect structural features of the resulting SBP. Also, correlations between structural characteristics and pectin precipitation behaviors under various ethanol concentrations were discussed.

## 2. Materials and methods

### 2.1. Materials

Fresh sugar beet pulp with sugar removed was a gift from Lvxiang Beet Sugar Company (Xinjiang, China). Sugar beet pulp was air-dried at 45 °C for 48 h, and then ground to pass through a 20-mesh screen. It was stored at –20 °C until used for extraction. A liquid enzyme preparation, namely Viscozyme L (Novozymes, Tianjin, China), was used to decompose SBP because of its activity against branched pectin-like carbohydrates. Before being used, 1 mL of the enzyme preparation was purified through dialysis (6000–8000 g/mol, molecular weight cut off) against acetate buffer (20 mM, pH 5.0) for 48 h at 4 °C to remove small molecular weight sugars. The retentate solution was made up to 250 mL with the same buffer.

### 2.2. Acidic extraction

Dried sugar beet pulp was dispersed in water (solid–liquid ratio 1:20, w/v) and set to pH 1.5 with 6 M HCl. Extractions were performed by heating the suspension at 80 °C under stirring (250 rpm) for 1 h. The resulting slurries were then filtered through nylon filter cloth of approximately 23 μm, and centrifuged (10,000 × g, 20 min) to remove the residue. After centrifugation, the supernatant was pooled, prepared for precipitation. Acidic extraction was performed in two independent runs.

### 2.3. Stepwise ethanolic precipitation (SEP)

The acidic extract from each extraction was divided into two equal parts. The first half of the pectin containing extract was used for stepwise precipitation. Fig. 1 shows the scheme for precipitation of SBP by using the SEP procedure. Equal volume of ethanol

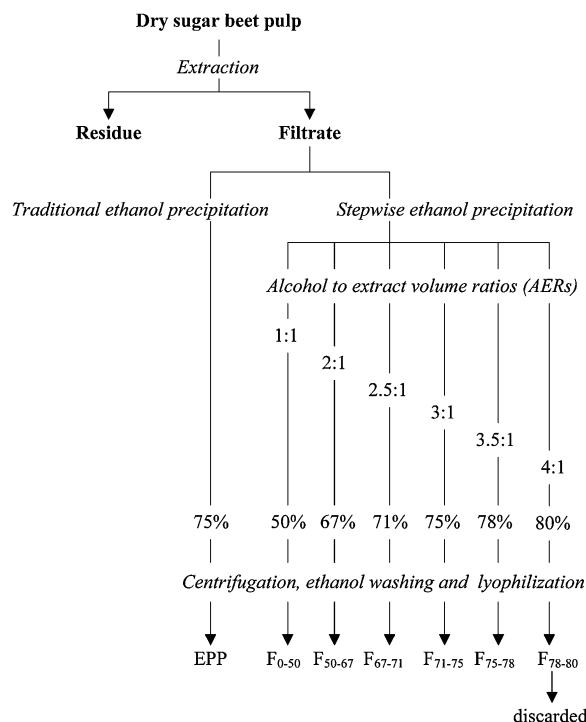


Fig. 1. Scheme for stepwise ethanolic precipitation of fractions from the aqueous extract.

was added to a 500 mL aqueous extract under stirring (350 rpm) to obtain a final concentration of 50%. The flow rate of ethanol addition was 10 mL/min controlled by a constant flow pump (Longer Pump Ltd., Hebei, China) (Fig. 2). The mixture was then allowed to stand for 3 h at 25 °C. Pectin precipitates were recovered via centrifugation (12,000 × g × 20 min), and subsequently washed with 75% and 95% ethanol (each twice, solvent–gel ratio = 1:1, w/w) to eliminate some inorganic salts and free sugars. After evaporating ethanol for 10 h under vacuum conditions, these washed-precipitates were recovered in water, freeze-dried and air-dried at 45 °C until constant weight. This fraction was referred to as F<sub>0-50</sub> (the subscript refers to the ethanol concentration range in which pectins were precipitated). The ethanol concentration of the supernatant was stepwise increased to 67, 71, 75, 78 and 80%, respectively, by adding different volumes of ethanol in a similar manner. The precipitated fractions, referred to as fractions F<sub>50-67</sub>, F<sub>67-71</sub>, F<sub>71-75</sub> and F<sub>75-78</sub> were recovered as described above, except that F<sub>75-78</sub> was only washed with 95% ethanol. The fraction F<sub>78-80</sub> only yielded negligible amounts (<0.1 g/100 g sugar beet pulp), therefore was not studied. SEP experiments were carried out in two independent runs. The obtained fractions were stored in a desiccator at 25 °C until used for various determinations.

### 2.4. One-step ethanolic precipitation

The other part of the pectin-containing extract was used for the preparation of ethanolic precipitated pectins (EPP). The one-step ethanolic precipitation was conducted by drop-by-drop addition of 3 volumes of ethanol to acidic extract under magnetic stirring (350 rpm). The flow rate of ethanol addition was 10 mL/min, controlled by the constant flow pump. The precipitation medium was allowed to stand for 3 h at 25 °C. The pectin precipitates were centrifuged, washed and dried as described above. The resulting pectins were kept in a desiccator at 25 °C, and referred to as EPP. The one-step precipitation was carried out in two independent runs.

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