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Extraction, characterization and spontaneous emulsifying properties of pectin from sugar beet pulp

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ABSTRACT

The effects of organic acid extractants on the yield and characteristics of pectin from sugar beet pulp were investigated with citric acid, malic acid and lactic acid at different pH (1.5 and 2.0) and time (1 h and 2 h). The results demonstrated that the yields of pectins were directly correlated with the decrease of pH and reaction time, and the optimum yield of 17.2% was obtained at pH 1.5 and 2 h. Furthermore, the acid type also affected the physicochemical characteristics of pectin, especially on the esterification degree (42–71), galacturonic acid content (60.2–77.8%), emulsion activity (35.2–40.1%) and emulsion stability (62.1–79.4%), and a relatively single pectin mainly consisted of homogalacturonan could be obtained under a suitable reaction condition, which was an excellent crude material for the production of emulsion activity.

1. Introduction

Sugar beet (*Beta vulgaris*) pulp (SBP) is a byproduct of the sugar industry abundant in China. It is mainly employed for feed formulation, which developed few commercial uses and the high energy costs required for drying SBP present a burning problem. However, SBP has high pectin content on dry weight basis, and beet pectin exhibits superior emulsifying properties than pectin extracted from others (Drusch, 2007).

Pectins are natural polysaccharides from higher plants, consisting mainly of D-galacturonic acid (GalA), and monosaccharides, such as L-rhamnose (Rha), L-arabinose (Ara), D-galactose (Gal) and D-xylose (Xyl) (Vidal, Williams, O'Neill, & Pellerin, 2001). A common feature of pectin molecules is that a backbone composed of (1–4)-linked α-D-Gal units, which can bear neutral sugar side chains (Mohnen, 2008). Moreover, the GalA residues could be partly methyl-esterified at C-6 and acetyl-esterified at position O-2 and O-3. There was broad agreement that the main structure of pectin are homogalacturonan (HG), rhamnogalacturonan (RG) I and II (Kar & Arslan, 1999; Limberg et al., 2000; Vidal et al., 2001).

The structural features and functional properties of pectin might be influenced by extraction methods. Extraction agents such as mineral acid, sodium hydroxide as well as enzymes have generally been used (Kar & Arslan, 1999; Limberg et al., 2000; Vidal et al., 2001). Although various alternative methods have been reported in recent years, the process extracted by dilute mineral acid was mainly used in commerce. The acid extraction of pectin is advantageous, apart from high extraction yield, the pectin obtained is generally enriched in galacturonic acid, which may derive more HG type than RG I (Kurita, Fujiwara, & Yamazaki, 2008; Yapo, 2009).

Various studies have shown the effect of acid extractants on pectin yield, chemical composition, structure and properties. In contrast, a few available reports are somewhat contradictory on the kind of acid extractants (Fissore et al., 2010; O'Donoghue & Somerfield, 2008; Vriesmann, Teofilo, & Petkowicz, 2011). Earlier studies indeed reported no significant effects of extracting acid type (hydrochloric against nitric acids) on the characteristics of SBP pectins (Levigne, Ralet, & Thibault, 2002). However, the latter studies showed that citric acid had more positive effects on the yield and less damaging effects on the degree of esterification of extracted pectin compared to nitric acid (Kurita et al., 2008; Yapo, 2009). It seemed the characterization of pectin was depended on the difference between mineral and organic acids.

As it is known, nitric and sulfuric acid are corrosive, which may be a potential threat to health, the waste liquor and gas generated from the industrial processes may lead burden on the environment. In contrast, citric acid is economical and better from an

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environment point of view. Although great efforts had been made to extract pectin with the mineral acid, there are relatively few studies on the effect of organic acids on the properties of pectin and it is not yet known whether the difference of organic acids has different effects on the properties of beet pectin. Therefore, the goal of this study was to examine the influence of three common organic acids (citric acid, malic acid and lactic acid) on the yield, purity, biochemical features and emulsify properties of pectin from SBP.

2. Materials and methods

2.1. Sample preparation

SBP was offered by Luyuan Sugar Industry Co., Ltd (Xinjiang, China). The pulp was washed twice with distilled water, dried (50 °C, 24 h) in a convection oven, milled and sieved for obtaining beet powders in the size range 0.425–0.710 mm. The moisture content of powders obtained was 7.6%. It was stored at room temperature (23 °C) until used.

2.2. Pectin production

SBP pectins were extracted with hot distilled water acidified with citric acid, malic acid and lactic acid under ultrasonic irritation (150 W, 25 min). Extraction agent hydrochloric acid was used as reference. The experimental design used considered two variables: pH at two levels (1.5, 2.0) and time at two levels (1 and 2 h); the powders were treated under stirring in different acid solution of pH previously mentioned for 1 or 2 h at 80 °C. The resulting slurries were centrifuged at 13,000 × *g* for 20 min in a Beckman J2-M1 centrifuge (Beckman Instruments, Inc., Fullerton, CA, USA). The supernatants were separated through filtration with glass fiber filter, followed by dispersing into 4 volumes of 95% ethanol at 4 °C for 3 h. The pectin precipitate was washed twice with 70% ethanol followed by 95% ethanol, and then oven-dried at 40 °C for 36 h and weighed for yield assessment. The experiments performed were briefly summarized in Fig. 1.

2.3. Pectin characterization

Moisture content of pectins was determined by drying pectin samples in an air-circulated oven at 105 °C, for 24 h. All values were calculated on a dry-weight basis. Total carbohydrates were determined by the phenol-sulfuric acid photometric method, using D-glucose as standard (DuBois, Gilles, Hamilton, Rebers, & Smith, 1956). The GalA content was titrimetrically determined by the 3,5-dimethylphenol colorimetric method, using D-galacturonic acid as standard (Scott, 1979). Protein content of pectin-enriched fractions was determined by Lowry, Rosebrough, Farr, and Randall (1951), using BSA as standard.

Individual neutral sugars were released from pectin molecules by acid hydrolysis with 1 M H_2SO_4 at 100 °C for 4 h and converted to a complete hydrolysis of non-starchy polysaccharides to monomers, which were assayed by HPAEC-PAD using a Dionex instrument (Martínez, Gullón, Schols, Alonso, & Parajó, 2009). Degrees of esterification (DE) were determined by titrimetric method (Yapo, 2009).

All these determinations were performed three times on each sample.

2.4. Emulsifying properties

Emulsifying activity and emulsion stability were assessed using Dalev and Simeonova (1995) procedure with a slight modification. Briefly, oil-in-water (O/W) emulsions were prepared by adding 3 ml of n-dodecane (43 wt% final oil level) to 3 ml pectin solutions (0.5%, w/w) containing 0.02% sodium azide as a bacteriocide in 15 ml graduated polypropylene transparent centrifuge tubes, and then treated with ultrasound for 5 min. The pre-emulsions were spun vigorously and centrifuged to achieve a good emulsion level. Emulsifying activity (*EA*) was calculated as follows:

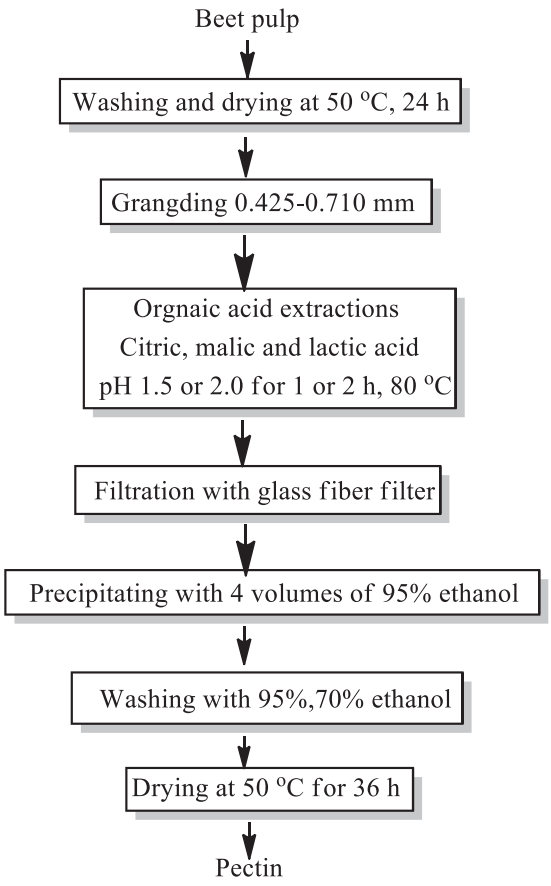

Fig. 1. Flow chart for preparation of pectin from beet pulp.

$$EA(\%) = \frac{ELV}{W_v} \times 100 \quad (1)$$

where *Wv* was the whole volume of the system and *ELV* was the emulsified layer volume.

To study the emulsion stability (*ES*), another four tubes were treated as above. Two tubes of four were centrifuged and stored at 4 °C for 1 and 30 days. The other two tubes were treated the same way but at room temperature and stored at that temperature. The emulsion stability was calculated by the following equation:

$$ES(\%) = \frac{ELV_r}{ELV_i} \times 100 \quad (2)$$

where ELV_r was the initial volume of emulsified layer, and ELV_i was the remaining volume of emulsified layer.

3. Results and discussion

3.1. Effect of organic acids on the pectin yield and DE

The yield of pectins ranging from 5.5 to 17.2% (w/w) depended on the pH and type of extracting solvent (Fig. 2). The amounts of pectin were significantly higher at pH 1.5 than at pH 2.0 whatever the acid type. This is probably due to disruption of the cell walls as a result of strong acid conditions, for which pectin tend to release and dissolve in the acid solution. So, the yield increased with increasing acid strength of the extraction. At the same pH, the yield of