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Combined effects of independent variables on yield and protein content of pectin extracted from sugar beet pulp by citric acid

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ABSTRACT

The extraction of pectin from sugar beet pulp by citric acid was carried out under different conditions using Box–Behnken design for four independent variables (pH, temperature, time and liquid to solid ratio). The yield of sugar beet pulp pectin ranged from 6.3% to 23.0%, and the content of protein from 1.5% to 4.5%. All independent variables significantly affected the yield, and all variables except liquid to solid ratio significantly affected the protein content. The yield increased as decreasing pH of extracting solution, extending time and advancing temperature, and an opposite relationship of effects between variables and content of protein was obtained. The chemical composition of collected samples was determined. Moreover, from the results of emulsifying properties study, the extracted pectin from sugar beet pulp could prepare steady oil-in-water emulsions. Therefore, it was inferred that the extraction conditions could influence yield and protein content, resulting in different emulsifying property.

1. Introduction

Pectin substances are complex glycanogalacturonan polysaccharides in which the backbone consists of 1,4-linked α-D-galacturonic acid residues (Westereng, Michaelsen, Samuelsen, & Knutsen, 2008). Due to the emulsifying, thickening and gelling properties, pectin was used in food industry for a long history. In the past decades, pectin was obtained form sugar beet pulp (Ma et al., 2013; Turquois, Rinaudo, Taravel, & Heyraud, 1999; Yapo, Robert, Etienne, Wathelet, & Paquot, 2007), apple pomace (Wang, Chen, & Lü, 2014), passion fruit peel (Kulkarni & Vijayanand, 2010; Seixas et al., 2014), lemon by-product (Masmoudi et al., 2008; Masmoudi et al., 2012), cacao pod husks (Chan & Choo, 2013; Vriesmann, Teófilo, & Lúcia de Oliveira Petkowicz, 2012), green tea (Ele-Ekouna, Pau-Roblot, Courtois, & Courtois, 2011), *etc*. Commercial pectins are usually extracted from apple and citrus by-products, and have been reported to contain relatively low level of hydroxyproline-rich protein (Kravtchenko, Voragen, & Pilnik, 1992) which was proposed correlated positively with emulsion-stabilizing ability (Dalev & Simeonova, 1995; Kirby, MacDougall, & Morris, 2008). The pectin–protein complexes consist of pectin molecules with protein attached to one end of the pectin chain, and associate by covalent linkage (Kirby, MacDougall, & Morris, 2006). Pectin could also be obtained from sugar beet pulp which contains higher content of neutral lateral chains and protein composition (Kirby et al., 2006; Nuñez, Fishman, Fortis, Cooke, & Hotchkiss, 2009; Williams et al., 2005).

Based on the known data, different agents such as organic acid, mineral acid, alkali, enzymes were used for pectin extraction under the condition of traditional water-bath heating, microwave-assisted, ultrasonic-assisted heating, high hydrostatic pressure treatment and other methods (Guo et al., 2014; Košťálová, Hromádková, & Ebringerová, 2013; Ma et al., 2013; Methacanon, Krongsin, & Gamonpilas, 2014; Seixas et al., 2014; Xu et al., 2014). All the variables obviously affected the structural features and functional properties of pectin. The studies (Kurita, Fujiwara, & Yamazaki, 2008; Yapo, 2009) showed that citric acid had less damaging effects on the extracted pectin. Organic acid such as citric acid, malic acid and lactic acid were employed to extract sugar beet pulp pectin (SBPP) under different pH (1.5 and 2.0) and time (1 and 2 h). The value of protein contents in turn was as follows: lactic acid, malic acid, citric acid and the emulsifying activity and emulsion stability was significantly positively correlated with protein contents (Ma et al., 2013). Similar result was obtained under different conditions (Funami et al., 2007; Yapo et al., 2007). However, few if any of these studies directly understood the combined effects of processing variables on the protein content, say nothing of emulsifying activity and emulsion stability.

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In the present works, citric acid was employed to extract SBPP, and the pH of citric acid, time, temperature and liquid to solid ratio (LSR) were chosen as the variables of extraction process. The main objectives of this work were to quantify yield, protein contents, emulsifying activity and emulsion stability of SBPP, and then analyze the combined effects of processing variables on the above-mentioned responses by using response surface methodology (RSM).

2. Material and methods

2.1. Materials and chemicals

Sugar beet pulp was obtained from LüYuan Sugar Industry Co., Ltd. (Hejing, Xinjiang Uygur Autonomous Region, China). The sugar beet pulp was pulverized into powder with granularity of 60 meshes, and the powder was bleached at 95 °C for decreasing the enzyme activities and dried in an air convection oven at 45 °C. All the chemical reagents were of analytical grade without further treatment.

2.2. RSM design

Response Surface Methodology is a statistical approach which uses multivariate quadratic regression equation to study the combined effects between independent variables and the response, and obtain the optimal condition. This experimental methodology is an effective tool that clarifies the chemical or biochemical processes by using shortest time with the least number of experiments. Meanwhile, it is a good facility to analyze the influences among different variables (Baş & Boyacı, 2007).

In the present study, variables were determined from the published data (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Lv, Wang, Wang L.-j. Li, & Adhikari, 2013; Ma et al., 2013; Prakash Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013; Yapo et al., 2007). The effects of extraction time (X_1), pH (X_2), extraction temperature (X_3) and LSR (X_4) were chosen in the pectin isolation process. According to the results from preliminary experiments, a three level four factors Box–Behnken design (BBD) was employed to investigate the individual and interactive effects of four variables on pectin yield and content of protein from sugar beet pulp, the factors under investigation, levels and the values of responses were indicated in Table 1.

2.3. Pectin extraction by citric acid

The sugar beet pulp powder was marinated in distilled water with LSR of 1:10, 1:15, 1:20, and the pH value of mixture was adjusted to three levels of 1, 1.5 and 2.0 by adding citric acid. Then the mixtures were heated to different temperature (80, 90 and 100 °C) for three times (120, 150 and 180 min). The mixtures need to squeeze by a moisture expeller when the process was over. The mixed liquid was cooled down to room temperature and centrifuged. Then, twice volume of absolute alcohol was added to supernatant in order to float pectin. The pectin was dried to a constant weight at 45 °C after rinsing three times with 95% ethanol.

2.4. Yield

After drying the pectin to constant weight, the yield was calculated as follows:

$$\text{Pectin yield}(\%) = \frac{m_0}{m} \times 100\% \quad (1)$$

where m_0 (g) is the weight of dried pectin; m (g) is the weight of dried sugar beet pulp powder.

2.5. Characterization

The total carbohydrate (Total CH) were determined by Dubois, Gilles, Hamilton, Rebers, and Smith (1956) which named as the phenol–sulfuric acid photometric method, using D-glucose as standard. The GalA content was determined by the method of *m*-hydroxydiphenyl, using D-galacturonic acid as standard (Garna, Emaga, Robert, & Paquot, 2011). The monosaccharide composition was determined by aldononitrile acetate precolumn-derivatization gas chromatography method (Guerrant & Moss, 1984). Protein content of pectin was determined by Lowry, Rosebrough, Farr, and Randall (1951) procedure, using BSA as standard.

2.6. Emulsifying properties

Emulsifying activity and emulsion stability were assessed using Yapo et al. (2007) procedure which was developed from Dalev and Simeonova (1995) procedure. To prepare the oil-in-water (O/W) emulsions, *n*-dodecane (3 mL, 43 wt% final oil level) was added to pectin solutions (3 mL, 0.5%, w/w) which containing 0.02% sodium azide in 15 mL graduated transparent centrifuge tubes, and then treating the mixtures in an ultrasonic instrument for 5 min. The pre-emulsions were spun vigorously and centrifuged to achieve a good emulsion level at room temperature. The emulsifying activity was calculated as follows:

$$\text{Emulsifying activity}(\%) = \frac{ELV}{W_v} \times 100\% \quad (2)$$

where ELV and W_v are the volume of emulsified layer and whole system.

Another four systems were prepared as above for studying emulsion stability. Two tubes were cooled to 4 °C, centrifuged for 5 min, and stored for 1 and 30 days, respectively. The other tubes were treated as the same way but the temperature changed to room temperature. The emulsion stability was calculated as follows:

$$\text{Emulsion stability}(\%) = \frac{ELV_r}{ELV_i} \times 100\% \quad (3)$$

where ELV_r and ELV_i are the initial and remaining volume of emulsifies layer, respectively.

3. Results and discussion

3.1. Yield

3.1.1. Response measurements

The values of SBPP yield were set out in Table 1. We could see that the yield ranged from 6.3% to 23%. Compared with the published data (Ma et al., 2013), the results was lower if the process was taken with the temperature of 80 °C, the main matter is caused by process of solid–liquid separation which was squeezed once in order to study the influence of the LSR effectively. The values were also significantly lower than that extracted by H_2SO_4 (Li, Jia, Wei, & Liu, 2012). The main reason for this complexion was that proper extraction method could effectively hydrolyze the insoluble pectin to soluble.

3.1.2. Fitting model

In order to find the optimum conditions that maximize the yield of SBPP and study the combined effects between independent variables and the response, a second order polynomial equation was built. The fitting model in term of coded variable exhibit below by