SEPARATION AND CHARACTERIZATION OF PECTIN FROM JUICE PROCESSING RESIDUE EXTRACTED BY SUB-CRITICAL WATER

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Abstract: Citrus Junos is an evergreen tree whose fruit processed into juice and often preferred to vinegar as an ingredient in sauces and salad dressing for its special flavor. Most food processing residues are disposed without being recycled. These residues are mainly composed of the part of peel, juice sack and seed. Especially, peel includes valuable hydrocarbons such as terpenoid and carbohydrate such as pectin and cellulose. Sub-critical water has solubility and reactive selectivity to polar substance carbohydrate such as pectin and cellulose based on pressure and temperature. Pectin is composed of an acidic polysaccharide and several types of neutral sugars. The main chain consists of α-1, 4-linked D-galacuronic acid, which is partly methyl esterified. Conventionally using mild acid or chelating agent, pectin can be extracted and separated from the waste of beet pulp and citrus fruits such as lemon and apple pomace, which is an effective utilization of industrial waste. In this work, we aimed to develop an effective separation method for pectin using subcritical water from the residue of Citrus junos and to examine the characteristics of pectin. Experimental apparatus we used was semi-continuous flow reactor. Experimental condition follows: at the temperature range of 110-160°C, at the pressure range of 4-30MPa. As a result, the yield of pectin was more than 75% at the temperature range from 120-140℃ and pressure range from 4-30MPa. And they lack the amount of impurities. Extracted at higher temperature 160℃, molecular weight of pectin was lower molecules. On the other hand, pectin extracted at 120℃ and 140℃ were almost the same molecular weight of commercial pectin. This result indicates that different molecular weight pectin was able to recover by changing the extraction temperature.

1. Introduction

Citrus fruits are grown in subtropical and tropical areas. The origin of citrus fruit is considered to be China. Since early 1900's, development of transportation have increased citrus product and processing in countries with suitable climate for citrus culture [1]. It has been important to deal with increase juice processing residue. These residues are mainly composed of the part of peel, juice sack and seed. Especially, peel includes valuable compounds such as terpenoid, pectin, and flavonoid. The traditional citrus by-product of cattle feed pellets and essential oil are still most important utilization of juice processing residue.

Pectin is composed of an acidic polysaccharide and several types of neutral sugars [2]. The main chain consists of α-1, 4-linked D-galacuronic acid, which is partly methyl esterified. It is impossible to extract free pectin with water from cell tissues because it exists in an insoluble form known as protopectin. Having this characteristic, conventionally using mild acid [3] or chelating agent, pectin can be extracted and separated from the waste of beet pulp and citrus fruits such as lemon and apple pomace, which is an effective utilization of industrial waste. Recently, pectic origosaccharide has become a more important material in the health food market, because it has many bioactivities, such as hypocholesterolemic activity and elevated blood pressure inhibitory effect.

Citrus Junos, called yuzu in Japan, is an evergreen tree harvested mainly in the southern part of Japan, whose fruit processed into juice and often preferred to vinegar as an ingredient in sauces and salad dressing for its special flavor. Most food processing residues are disposed without being recycled.

Recently, an environmentally-friendly extraction and fractionation technology for natural materials has been developed which uses sub-critical water. Sub-critical water has some unique properties. The hydrogen bond between water molecules weakens by raising the temperature, and the permittivity can be changed greatly. Ion product of water (K_w) is increased dramatically with the temperature rising to around 270℃. At these properties, sub-critical water has solubility and reactive selectivity to polar substance carbohydrate such as pectin and cellulose based on pressure and temperature. This technique has been applied to recover protein and amino acid[4], and phenolic compounds [5] from natural products. And also, the hydrothermal treatment has been demonstrated by several studies to effectively convert cellulosic [6] and lignocellulosic biomass [7] into useful products.

In this work, extraction and separation of pectin was carried out from Citrus junos juice processing residues using green solvent such as sub-critical water. Furthermore, the optimum extraction condition was investigated under the various temperature and pressure conditions, and effect of temperature and pressure were investigated on the molecular weight of pectin.

2. Experimental

For the yuzu flavedo starting material, the residue from supercritical CO_2 extraction at

60℃ and 20MPa was used. The residue was freeze dried, ground, and sieved. Flavedo with a particle size of 170–450μm was obtained as the raw material. This pretreatment of the raw material may eliminate the mass transfer limitation during the reactive extraction process, which help to understand dissolution behavior of materials in the subcritical water.

Reagents used for quantitative analysis of pectin were as follows: D-galacturonic acid, sodium chloride, sulfuric acid, and acetic acid (Wako Pure Chem. Ind. Ltd.,) and 3,5-dimethylphenol (Tokyo Kasei Kogyo Co., Ltd.).

The pectin was quantitatively analyzed in terms of its galacturonic acid content according to the dimethylphenol method [8]. The extraction sample (125μL) was mixed with 5% NaCl (125μL) and then 2mL of H_2SO_4 was added to the mixture in an ice bath. The mixture was mixed well and placed in a water bath at 70℃ for 10minuites. 3,5-dimethylphenol–acetic acid reagent (0.1%, 100μL) was added and the reaction was stirred at room temperature for 10minuites. Absorbance of the mixture at 400 and 450nm was measured. The absorbance of the galacturonic acid and its methyl ester were calculated from the difference in the absorbance at 400nm subtracted from the absorbance at 450nm.

The molecular weight distribution were determined by gel filtration chromatography using OHpak SB-805HQ column (8.0×300mm, Showa Denko K.K) maintained 30℃ The eluent was 0.1M NaCl solution at a flow rate of 1.0mL /min, and monitored by a differential refractometer (Jasco Co., Ltd., Model RI-930)

A schematic diagram of the semi-continuous flow extractor is shown in Fig.1. The maximum working conditions of the apparatus are 450℃ and 45MPa.

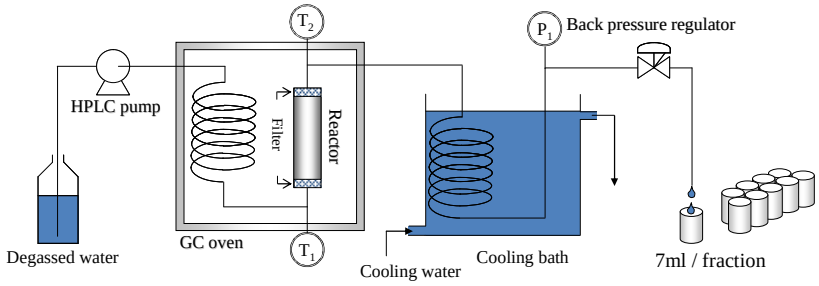

Fig.1 Schematic diagram of semi continuous sub-critical water flow reactor

A 0.5 g sample of dried flavedo was charged in the extractor (SUS 316; 8.7mm i.d.×118mm length, 7.0mL), and the extractor was capped with gasket filters (average pore size 20μm) to prevent sample particles from flowing out. Distilled water was degassed and continuously delivered through a heating coil placed in an oven into the extractor by a high-pressure pump (Jusco Co., Ltd., Model PU-980) at water flow rates of 0.5, 1.0, and 2.0mL/min. The pressure in the extractor was controlled by a back-pressure regulator (AKICO Co., Ltd., Model HBP-450) at 4, 10, 20, and 30MPa. The extraction temperature was represented by the temperature measured at the inlet and outlet of the extractor. Prior to

extraction, degassed water (60mL) was delivered to the extractor at the temperature of 80°C to remove the air and water soluble substance. At first, one fraction was collected. And then, the reaction temperature was immediately raised from 80℃ to various temperatures such as 110, 120, 130, 140, 150, and 160℃, respectively. Its reactive solutions were fractionated each 7ml volume. Ten fractions were collected at each temperature and pressure conditions.

3. Results and discussion

Fig. 2 shows the effect of temperature on the extraction behaviour at the pressure of 4MPa and the water flow rate of 0.5mL/min. At the highest temperature of 160°C, pectin extraction ended fastest. As the extraction temperature was lower, pectin was extracted more gently. This result indicated that the temperature accelerate separation of pectin from cell wall under the hydrothermal condition. Pectin functions as adhesive material to bond adjacent cell walls on contact with hydrogen bond [2]. Dielectric constant of water, ε, drastically decreases with increase in temperature.In other words, it was thought that dielectric constant of water is concerned to dissolve pectin in water.

Fig. 3 shows effect of pressure on the pectin yield at various temperatures. At lower temperature of 120℃, the pectin yield was higher as the pressure was lower. Though, the extraction pressure did not influence the yield of pectin at the higher temperature region. Moreover, the yield of pectin was decreased over the temperature of 140℃. This indicates that pectin molecules begin to be decomposed at the temperature higher than 140°C. As a result, highest yield was obtained under the temperature of 140℃ at each pressure. It is thought that the important factor was not pressure but temperature when extraction and separation of polar substance with hydrogen bond is carried out from natural product using sub-critical water.

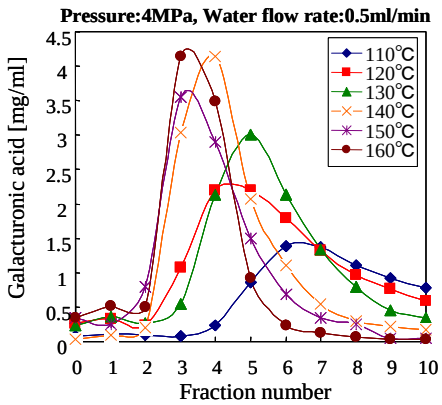

Fig.2 Effect of temperature on the extraction behavior

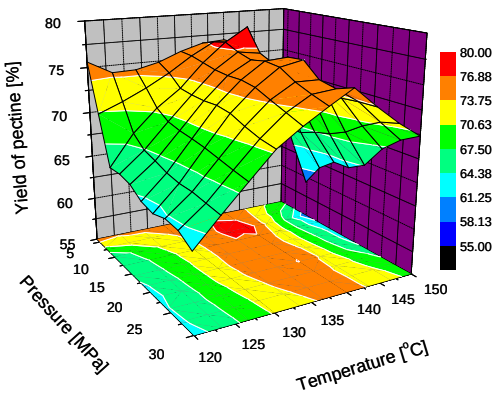

Fig.3 Effect of pressure on the pectin yield at various temperatures

Fig. 4 shows effect of water flow rate on the yield of pectin under the temperature of 140

℃ and pressure of 4 MPa. The water flow rate did not influence the cumulative yield of eleven fractions. Those yields were approximately 76%. Though, the pectin concentration per one fraction that was beginning of pectin elution was higher as the water flow rate was smaller.

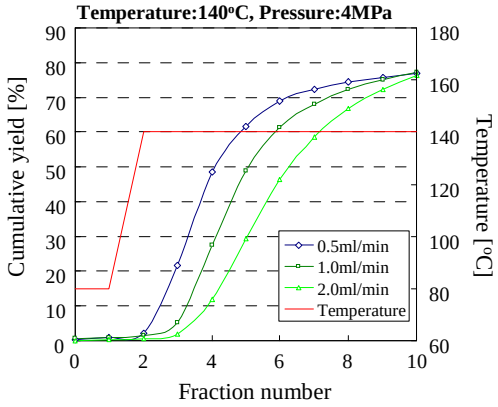

Fig.4 Effect of water flow rate on the yield of pectin

Fig. 5 shows the gel filtration chromatogram of pectin extracted under the various conditions. The average molecular weight of pectin extracted under the mild acidic condition was approximately 5.7×10^4. On the other hand, molecular weight of pectin extracted under the hydrothermal conditions were distributed mainly into three size which were approximately 63.5×10^4, 3.5×10^4, and 1.5×10^4, respectively. Under the hydrothermal condition, the pectin molecule was smaller as the extraction temperature was higher. This result indicates that different molecular weight pectin was able to recover by changing the extraction temperature. Some researchers have reported the decomposition of acidic polysaccharide. Miyazawa et al. have reported recovery of mono and origomers from poly galacturonic acid under the hydrothermal condition [9] .Matsushima et al. successfully separate alginic acid constituent sugar using quick-heating quick-cooling method under the sub-critical and supercritical water [10]. As prospects, recovery of pectin origosaccharide is expected changing the reaction conditions.

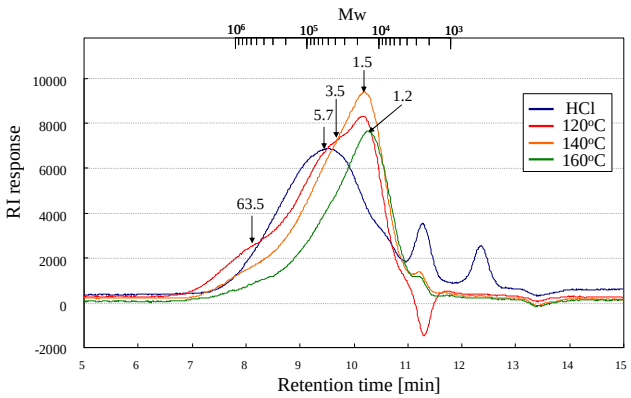

Fig.5 Gel filtration of the pectin extract under the various conditions

4. Conclusions

A sub-critical water extraction enabled the separation of pectin effectively without using chemicals such as acid. And, the yield of pectin was more than 75%. Different molecular weight pectin was able to recover by changing the extraction temperature.

References

[1] R. J. Braddock, Handbook of Citrus By-products and Processing Technology, *Willy Inter Science, New York*, **1999**.

[2] F. A. Henglein, Handbuch der Pflanzenphysiologie, *SpringerVerlag, Berlin*, **1958**, *6*, 407-478.

[3] C. D. May, *Carbohydr. Polym.*, **1990**, *12*, 79-99.

[4] I. Sereewatthanawut, S. Prapintip, K. Watchiraruji, M. Goto, M. Sasaki, A. Shotipruk, *Bioresource Technology*, **2008**, *99*, 555-561.

[5] A. T. Quitain, S. Katoh, T. Moriyoshi, *Ind. Eng. Chem. Res.*, **2004**, *43*, 1056.

[6] M. Sasaki, T. Adschiri, K. Arai, *Bioresource Technology*, **2003**, *86*, 301-304.

[7] Wahyudiono, T. Kanetake, M. Sasaki, M. Goto, *Chem. Eng. Technol.*, **2007**, *30*, 1113.

[8] R.W. Scott, *Anal. Chem.*, **1936**. *51*, 936-941.

[9] T. Miyzawa, T. Funazukuri, *Ind. Eng. Chem. Res.*, **2004**, *43*, 2310-2314.

[10] K. Matsushima, H. Kawanami, Y. Ikushima, M. Nishizawa, A. Kawamukai, K. Hara, *Ind. Eng. Chem. Res.*, **2005**, *44*, 9626-9630.