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Extraction of Reducing Sugar with Anti-Oxidative Scavengers from Peels of *Carya cathayensis* Sarg.: Use of Subcritical Water

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Abstract

The peels of *Carya cathayensis* Sarg. (PCCS) were treated under subcritical water conditions (130°C to 280 °C for 0 to 120 min). The extract from PCCS included reducing sugar, proteins, and compounds with radical scavenging activity. Addressing the reducing sugar that is a resource of bioethanol, we could maximize the reducing sugar under the subcritical water (190°C for 60 min) and obtain 0.24 g/g-sample together with 9.7 units/mg-sample of radical scavenging activity. The obtained extract was estimated to correspond to 1 L of bioethanol/100 g-sample. It was therefore considered that the treatment by subcritical water could yield reducing sugar and natural compounds with radical scavenging activity.

Keywords: Anti-oxidative scavenger, Biomass, Extraction, Subcritical water

1. Introduction

Biomass is the largest and promising renewable energy source and has the advantages on both low sulphur content and carbon dioxide capture capability. In particular, wood biomass has therefore been utilized for its conversion to biofuel [1]. It has been reported that biofuels could be extracted from peels, juice, fruit and seed, together with a variety of natural compounds with radical scavenging activity [2]. The extraction of biofuel from wood biomass would therefore be a promising method, because the biofuels from peels might show the anti-oxidation of materials.

In China, *Carya cathayensis* Sarg. is used as food. Meanwhile, an annual 7,000 ton of peels of *Carya cathayensis* Sarg. (PCCS) is generated without any treatment. To treat PCCS as wood biomass has been expected to obtain biofuels.

Subcritical water is one of the possible media to treat the biomass in a green and environmentally friendly manner [3]. Subcritical water is water that maintains its liquid state under pressurized conditions in the temperature range of 100°C to 374°C. Subcritical water has unique characteristics, such as a low relative dielectric constant [4-6] and a high ion product [7], which is advantageous for the extraction of compounds that are a resource of biofuels. Actually, it has been demonstrated that the treatment of biomass by subcritical water could yield reducing sugar that can be converted to a biofuel [8]. In addition, the treatment of wood biomass by subcritical water favors the yield of natural compounds with anti-oxidant activity; i.e., phenol derivatives, furfural, and hydroxymethylfurfural [3, 9]. Therefore, it is considered that treatment of PCCS by subcritical water is a possible method to obtain a biofuel that is resistant to oxidation.

In this study, we treated PCCS by subcritical water. We investigated the optimal condition to acquire the reducing sugar involving the compounds with radical scavenging activity.

2. Materials and Methods

2.1. Materials

The PCCS were produced in Lin'an, China. D-glucose, phenol, Folin-Ciocalteu phenol reagent, bovine serum albumin (BSA, molecular weight 66000), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. The other chemicals were of analytical grade.

2.2. Preparation of Powdered PCCS

PCCS was used as raw material in all the experiments. We analyzed the content of PCCS and the contents of cellulose, hemicelluloses, lignin, and others (such as proteins and decomposed compounds) included in PCCS were 13%, 22%, 56%, and

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9%, respectively. PCCS were milled and screened with a diameter under 0.3 mm using a milling machine (WB-1; Osaka Chemical Corporation, Osaka, Japan) and the powder was dried at 105°C for 24 hr before the experiment.

2.3. Subcritical Water Treatment of PCCS

The 10 mL in volume of the powdered PCCS and distilled water were loaded into the batch reactor cell. This batch reactor cell was placed in an oil bath, to elevate the temperature to 130°C–280°C. The pressure in the reactor cell was approximately 8 MPa. The treatment time was 0–240 min. After the treatment, the extract obtained from PCCS was separated from the residues by filtration. These procedures are shown in Fig. 1.

2.4. Measurement of Reduced Sugar

The reducing sugar obtained was measured by using a phenol-H_2SO_4 method [10]. In short, 1 mL of a diluted sample was mixed with 25 L of 80% phenol solution. Thereafter, this mixture was vigorously mixed with 2.5 mL of H_2SO_4 solution and incubated for 30 min. The absorbance of the mixture was measured at 485 nm by using a UV-Vis spectrophotometer (Hitachi U-2000; Hitachi, Tokyo, Japan). Instead of a sample, the absorbance of D-glucose solution was measured in the same manner to acquire the calibration line of concentration of D-glucose, and its absorbance at 485 nm. By using the calibration line, the obtained absorbance of samples was converted to the concentration of reducing sugar.

2.5. Measurement of Protein

The concentration of protein included in extracts obtained from PCCS was estimated by the Lowry-Folin method. First, 0.1 mol/L of NaOH (0.4 g) was added to 100 mL of 2 w/v% Na_2CO_3 solution (solution I). Citric acid (0.1 g) was added to 10 mL of 0.5 w/v% $CuSO_4$ $5H_2O$ solution (solution II). The 100 mL of solution I was thereafter mixed with 2 mL of solution II (solution III). Solution IV was prepared by mixing 10 mL distilled water with 10 mL Folin-phenol reagent. Next, 0.6 mL of samples or BSA solution was mixed with 3.0 mL of solution III to incubate for 10 min. The 0.3 mL of solution IV was thereafter mixed with them to incubate for 30 min and its absorbance was measured at 750 nm by using a UV-Vis spectrophotometer (Hitachi U-2000). The protein concentration of the sample was estimated, by converting its absorbance at 750 nm based on the calibration line obtained from BSA.

2.6. Measurement of Scavenger Activity

The radical scavenging activity was determined by using DPPH, according to the method in [11]. In short, 80 L of extract was added with 320 L distilled water and 2 mL of 0.12 mM DPPH in methanol. The mixtures were then mixed vigorously and allowed to stand at room temperature in the dark for 30 min. The absorbance of the sample mixture was then measured at 517 nm, using the UV-Vis spectrophotometer (Hitachi U-2000). The control sample was prepared in the same manner as the preparation of sample mixtures, except that deionised water was used instead of the samples. The blank sample was handled in the same manner, but deionised water was used instead of DPPH solution. The percentage of DPPH radical scavenging activity was defined as:

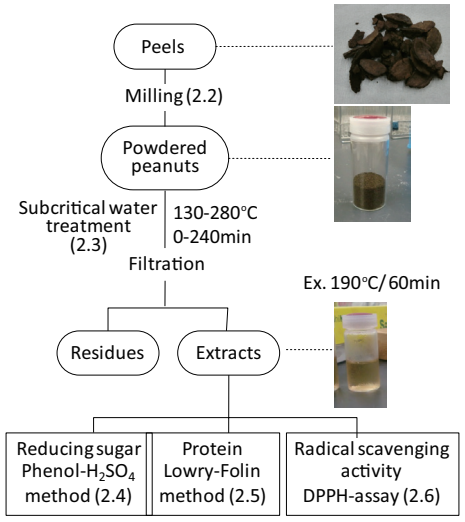

Fig. 1. Flowchart of extracts from peels of *Carya cathayensis* Sarg.

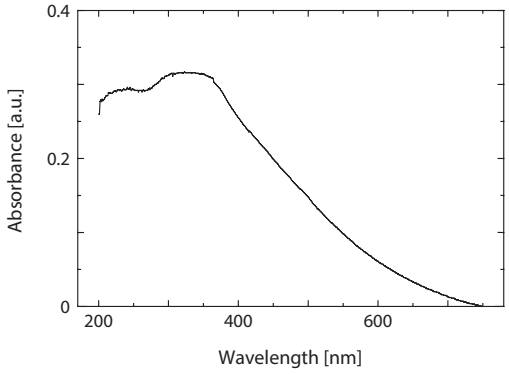

Fig. 2. Absorption spectrum for extracts from peels of *Carya cathayensis* Sarg. by subcritical water treatment.

$$1 - 100\, A_{blank} / A_{sample}\ (\%)$$

where A_{blank} and A_{sample} stands for the absorbance of blank and samples, respectively.

3. Results and Discussion

3.1. Absorption Characteristic of Extract

In the first series of experiments, we spectrophotometrically investigated the extract. The absorption spectrum for extracts was measured to investigate which compounds were involved. The absorption was observed in the wavelength range between 200 and 240 nm (Fig. 2), which resulted from the $\pi \rightarrow \pi^*$ transition of benzene derivatives in the extract [12]. The broad absorption observed in the range between 300 and 400 nm was considered to result from the presence of carbonyl or aldehyde derivative [13]. Those results suggested that the extract included the compounds with benzene ring, carbonyl or aldehyde.

It is therefore considered that PCCS might include reduc-

ing sugars, proteins, and compounds with scavenging activity, together with cellulose/hemicelluloses/lignin. In the following section, we quantified the amounts of those compounds extracted during the treatment of subcritical water.

3.2. Extraction of Reducing Sugar, Protein, and Compounds with Scavenging Activity from PCCS

Of sugars obtained from biomass, glucose is one of the major monosaccharides to be converted to bioethanol. Glucose that is an aldose shows reducing activity. The reducing sugar from PCCS was then measured by using a phenol-H_2SO_4 method advantageous for the detection of reducing sugar under a variety of temperature conditions.

As shown in Fig. 3(a), no extraction of reducing sugar was observed at 25°C. Overall, more than 0.1 g/g-sample of reducing sugar could be obtained in temperature conditions other than 25°C. Previously, a small amount of carbohydrates (0.02 g/g-sample [14]) was obtained by hot water for 30 min. It was considered that the treatment by subcritical water was more efficient in extracting the reducing sugar than the ordinary hot water. In the temperature range between 130°C and 170°C, the extraction of reducing sugar was observed in the manner of first-order kinetic. Meanwhile, maximal value of extracted reducing sugar was observed at the temperature condition of more than 190°C in Fig. 3(a) and (b). In our series of studies, polysaccharides were favored to be decomposed to monosaccharides under the subcritical water [15], and further decomposition of monosaccharides, such as alsode and ketose, yields furfural or 5-hydroxymethylfurfural [1], which would result in the intermediate-like production behavior of reducing sugar in the temperature range of more than 190°C.

Alternatively, the extraction of protein from PCCS was monitored under various temperature conditions. Overall, the time-course of extracted protein was similar to the reducing sugar, as shown in Fig. 4(a) and (b). Meanwhile, the extracted amounts of proteins increased with elevated temperature. A small amount of protein was extracted from PCCS at 25°C. Such extraction behavior was often observed in other fruit and leaves [14]. This is because part of the proteins might be dispersed on the powdered peels.

To estimate the scavenging activity involved in the extraction from PCCS, the DPPH-assay was examined. The time-course of radical scavenging activity in Fig. 5(a) and (b) was similar to those of proteins in Fig. 4(a) and (b). In the high temperature range (more than 200°C), the radical scavenging activity was increased. Such rough correspondence between protein and DPPH-assay is consistent with the previous finding that the proteins showed anti-oxidative activity [10].

3.3. Optimization for Reducing Sugar Extracts with Radical Scavenging Activity

To determine the optimal condition for extraction of reducing sugar including compounds with radical scavenging activity, the concentration of reducing sugar was plotted against the corresponding radical scavenging activity. A bell-typed curve was observed, as shown in Fig. 6. At the lower temperature range, both reducing sugar and scavenging activity were small. At 190°C, the maximum value of reducing sugar (0.24 mg/mg-sample) was observed at 9.7 units/mg-sample of radical scavenging activity. With elevated temperature up to 280°C, the concentration of re-

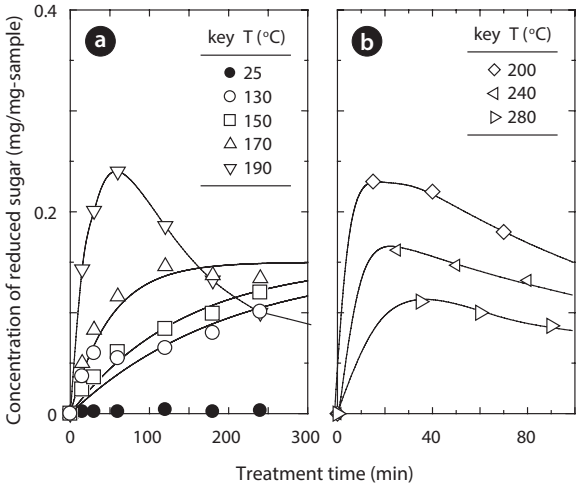

Fig. 3. Extraction of reduced sugar from peels of *Carya cathayensis* Sarg. by subcritical water treatment.

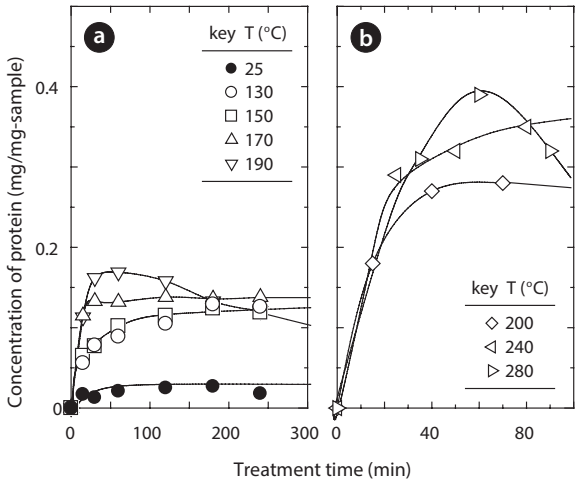

Fig. 4. Extraction of proteins from peels of *Carya cathayensis* Sarg. by subcritical water treatment.

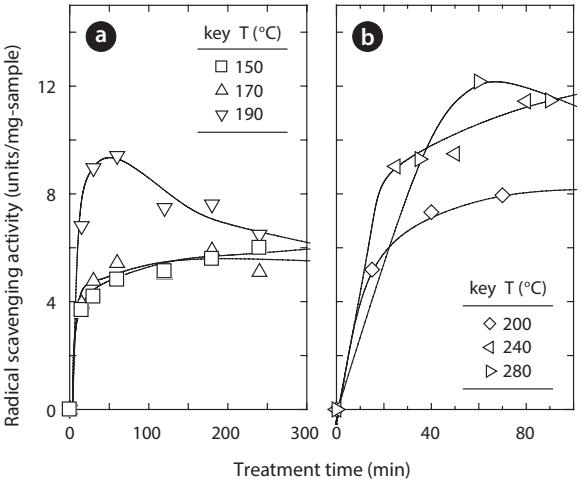

Fig. 5. Extraction of compounds with radical scavenging activity from peels of *Carya cathayensis* Sarg. by subcritical water treatment.

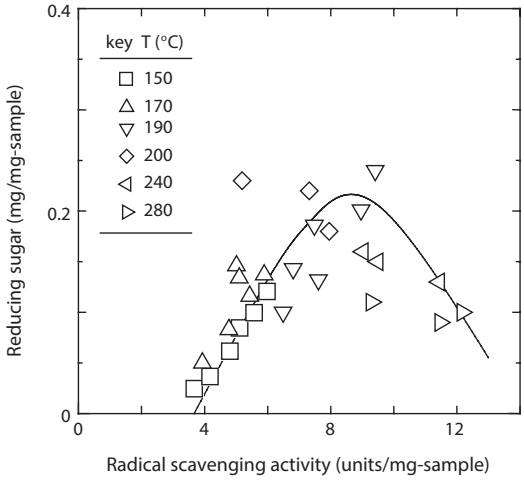

Fig. 6. Relationship between the concentration of reducing sugar and the corresponding scavenger activity.

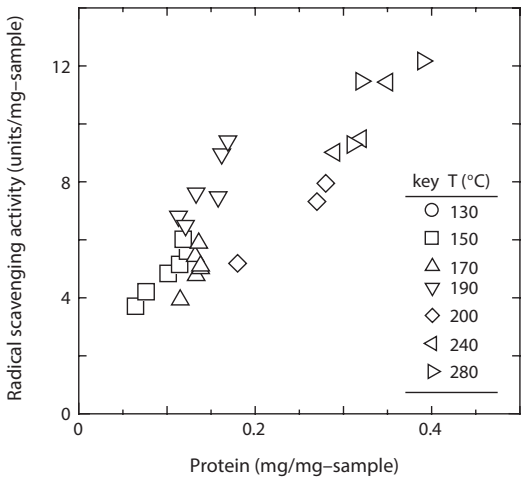

Fig. 7. Relationship between the scavenger activity of extracts and the corresponding protein concentration.

ducing sugar was reduced, whereas the corresponding radical scavenging activity increased. This indicated the instability of reducing sugar under high temperatures, relative to the compound with radical scavenging activity.

We estimated the potential conversion of obtained extracts to bioethanol. In general, 2 mol of bioethanol can be obtained from 1 mol of glucose. The production of 1 L in volume for 15% bioethanol requires 1.29 mol (232.4 g) of glucose. It is therefore considered that 100 g of samples including 0.24 g/g-sample of reducing sugar would yield 1 L of bioethanol. This estimation implies that the present method might be considerably effective for acquiring the bioethanol.

3.4. Implications of Compounds with Scavenging Activity

To clarify the radical scavenging activity of extracts, we contrasted them with the corresponding protein concentration. It was found from Fig. 7 that the radical scavenging activity discontinuously increased with increase in the protein concentration. This discontinuously increasing trend was observed between two temperature ranges: 130°C–190°C and 200°C–280°C.

In temperature range (130°C–190°C), the possible correlated line could be extrapolated into zero protein concentration suggesting that the radical scavenging activity might result from extracted proteins themselves. In contrast to the range (130°C–190°C), it was considered that the correlation in the high temperature range (200°C–280°C) related to aromatic amino acids rather than proteins, because proteins are decomposed into amino acids under high temperatures [10]. According to the principle of the Lowry method (that quantifies the aromatic amino acids), the decomposition of proteins should increase the apparent protein concentration, which might be a possible reason for the discontinuous increase observed in Fig. 7. Besides, the extracted carbohydrates, such as glucose and fructose, yield the phenol derivatives, furfural, or hydroxylmethylfurfural [3, 9, 16]. These compounds have been reported to show anti-oxidative activity [3, 9]. The decomposition of carbohydrates might contribute to the increase in scavenging activity in the high temperature range (200°C–280°C). Thus, the discontinuous correlation against the temperature might result from the contribution of compounds other than proteins.

4. Conclusions

The reducing sugar and protein were extracted above 130°C, whereas they were not extracted at 25°C. The optimal condition for the treatment of PCCS (190°C for 60 min) could yield 0.24 g/g-sample of reducing sugar and 9.7 units/mg-sample of radical scavenging activity. It was roughly estimated that 100 g of extracts obtained under optimal condition could be converted to 1 L of 15% bioethanol.

Acknowledgments

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