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Total fractionation of green tea residue by microwave-assisted alkaline pretreatment and enzymatic hydrolysis

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HIGHLIGHTS

- Microwave-assisted alkaline pretreatment solubilized green tea residue by 64–74%.
- The alkali-soluble components showed 20.1 MJ/kg of higher heating value.
- Subsequent enzymatic hydrolysis converted cellulose into glucose.
- Final residue was predominantly constituted by aliphatic cuticular components.

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ABSTRACT

Total refinery of constituents of green tea residue was achieved by combination of microwave-assisted alkaline pretreatment and enzymatic hydrolysis. Alkaline pretreatment was effective at separating pectic polysaccharides, protein, phenolic compounds and aliphatic compounds (probably originating from cuticular components), and the solubilization rate was attained 64–74% by heating at 120–200 °C. The higher heating value (HHV) of alkali-soluble fraction attained 20.1 MJ/kg, indicating its usability as black-liquor-like biofuel. Successive cellulolytic enzymatic hydrolysis mainly converted cellulose into glucose and attained the maximum solubilization rate of 89%. Final residue was predominantly composed of aliphatic cuticular components with high proportion in 9,10,18-trihydroxyoctadecanoic acid (30.1–48.6%). These cuticular components are potential alternative feedstock for aliphatic compounds commonly found in oil plants.

1. Introduction

Recent increases in tea drink consumption around the world lead to produce large amounts of tea residues that require disposal. Tea residue has been utilized as animal feed (Kondo et al., 2004), as adsorbent for heavy metals (Amarasinghe and Williams, 2007) and in manufacture of particle board (Yalinkilic et al., 1998). Tea residues are predominantly composed of polysaccharides, proteins, phenolic compounds and cutin (a plant biopolyester from cuticular membrane, covering outer surface of leaves), and they have potential to be used as renewable functional materials alternative to petroleum chemicals (Tsubaki et al., 2008, 2010a).

Although biomass components are ideal chemical feedstock, its recalcitrant structure requires severe pretreatments (Sun and Cheng, 2002; Mosier et al., 2005; Hendriks and Zeeman, 2009). We have been working on the utilization of microwave irradiation technology to improve the reactivity of biomass for separation of useful chemical constituents from lignocellulose and food industrial residues (Azuma et al. 1984; Tsubaki et al., 2008). Microwaves are electromagnetic waves at frequencies between 300 MHz and 300 GHz, which are extensively used for telecommunication, radar as well as heating source for domestic cooking and industrial processes. Dipole molecules, conducting substances and electromagnetic materials can be effectively heated by microwave irradiation due to dielectric and induction heating. As an integration of these unique heating properties, microwaves have been shown to increase yield and selectivity of chemical reactions at reduced reaction time (Kappe, 2008). Microwave irradiation is also capable of direct and fast energy transfer into biomass substrate and catalyst, providing higher reaction efficiency. For example, addition of halide salts improves microwave absorption of the reaction system and accelerates hydrothermal hydrolysis of

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carbohydrates by improving frequency factor (Tsubaki et al., 2012a). Carbon materials can be a good microwave energy absorber, and reduces the energy demand for generating hydrothermal condition required for hydrolysis of polysaccharides (Matsumoto et al., 2011).

In our previous works, microwave irradiation of green tea residues under autohydrolytic conditions (180–230 °C without catalyst) produced carbohydrates, polyphenols and plant biopolyesters at very short duration of microwave irradiation (⩽2 min) (Tsubaki et al., 2008). However, the maximum liquefaction of components by microwave was as low as 40% even after irradiation generating 230 °C, which makes it difficult for practical utilization of overall green tea residue. Therefore, more powerful treatments such as alkaline pretreatment and successive enzymatic hydrolysis were expected to overcome this limitation. Alkaline pretreatment is generally applied in biorefinery of monocotyledonous plant biomass to separate hemicellulosic polysaccharides, lignin and protein (Hu et al., 2008; Janker-Obermeier et al., 2012; Chen et al., 2012). By alkaline pretreatment both lignin and hemicellulose were extracted and further decomposed to form black liquors. At the same time, alkaline pretreatment were expected to improve enzymatic susceptibility of cellulose and enhance the purity of cuticular components remained in the solid residue.

In this study, we have applied alkaline pretreatment with fast heating property of electromagnetic irradiation to achieve total refinery of components in green tea residue at very short duration of reaction with higher fractionation efficiency. Enzymatic hydrolysis of the residue remained after the treatment was also carried out for further downstream refining. Finally, the results were compared with those given by the autohydrolysis and weak acid pretreatments under microwave irradiation combined with enzymatic treatment using cellulase and protease.

2. Methods

2.1. Materials

Tea residues were provided from a local tea drink manufacturer in Wakayama Prefecture, Japan. The lithium aluminum hydride ($LiAlH_4$) was purchased from Wako Purechemical Industries, Ltd. Meicelase CEP 17320 and Proteinase K were supplied by Meiji Co., Ltd. and Merck KGaA, respectively. BSA (*N*,*O*-bis(trimethylsilyl)acetamide) and BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) were purchased from Tokyo Chemical Industry Co., Ltd. The other reagents used in this study were all analytical grades.

2.2. Microwave-assisted pretreatments and enzymatic treatments

Microwave-assisted alkaline pretreatments were performed in HPR-100 TFM reactor (100 mL closed reactor made of Teflon) by using multimode microwave ovens of MicroSYNTH (frequency; 2.45 GHz, max output; 1 kW, Milestone Inc.). One gram of green tea residue was suspended in 20 mL of aqueous 1% sodium hydroxide solution, and microwaved at temperatures in a range of 120–200 °C for 5 min with 4 min of come-up time to reach the desired temperatures. For autohydrolysis and weak acid treatments, the reaction media was replaced by distilled water and 4% acetic acid, respectively. Reaction temperature was controlled by PID (Proportional Integral Derivative) controller with direct temperature measurement of the reactant using thermocouple thermometer to trace the temperature program. Distribution of microwave and reactant were kept homogeneous by using a diffuser and stirrer bar. After the reaction, the reactor was immediately cooled in ice bath (cca. 15 min). The solid residues after microwave irradiation were neutralized by addition of acetic acid, separated by centrifugation and freeze dried.

Native green tea residue and residues remained after microwave reaction were hydrolyzed by commercial cellulase (Meicelase, 1.0%, w/w) in 50 mM sodium acetate buffer (pH 5.0) at 37 °C for 48 h. For protease treatment, the residue was treated with 0.05% (w/w) Proteinase K in 0.1 M sodium phosphate buffer (pH 7.4) at 37 °C for 48 h according to the method of Ookushi et al. (2008). Triplicate experiments were conducted for alkaline pretreatment and cellulase treatments. In the case of comparative experiments by autohydrolysis, weak acid and protease treatments, single experiment at consecutive different heating condition was conducted.

Solubilization rate after the reaction was calculated by following equation.

$$\text{Solubilization rate}(\%) = 100 \times \frac{W_i - W_r}{W_i} \quad (1)$$

where W_i and W_r indicate initial weight of green tea residue and weight after alkaline pretreatment or enzymatic hydrolysis, respectively.

2.3. Carbohydrate analysis

Each fraction after microwave-assisted pretreatment and enzymatic hydrolysis was hydrolyzed according to the method of Saeman et al. (1945) using sulfuric acid, and the monosaccharide composition was analyzed by high performance anion exchange chromatography (HPAEC) on a DIONEX DX-500 system (Sunnyvale, CA) equipped with a CarboPac PA-1 column (4 × 250 mm) and a pulsed amperometric detector (ED-40) using 1.0 mM NaOH as a mobile phase (Tsubaki et al., 2008). Neutral carbohydrate content was determined by the phenol–sulfuric acid method (Wrolstad et al., 2004) using a standard solution containing arabinose, rhamnose, galactose, glucose, xylose and mannose mixed together according to the relative monosaccharide composition determined by HPAEC.

2.4. Polyphenol analysis

Polyphenol content in alkali-soluble fraction was determined by the Folin-Denis method using 80% methanol and gallic acid as an extractant and standard, respectively (Tsubaki et al., 2008). The results were expressed as gallic acid equivalents (GAE).

2.5. Aliphatic compounds analysis

Compositions of aliphatic compounds in the alkali-soluble fraction were determined by GC/MS as described in the same method as our previous reports (Tsubaki et al., 2010a,b). The peaks in total ion chromatograms were identified by mass spectra library NIST05, together with mass spectra and retention times given from authentic compounds.

2.6. CHN analysis

Contents of carbon, hydrogen and nitrogen were determined by FlashEA 1112 CHNS analyzer (Thermo Fisher Scientific Inc., MA, USA) using freeze dried materials of each fraction after alkaline pretreatment and enzymatic hydrolysis. Protein content was estimated after multiplication of nitrogen content by 6.25 as a nitrogen-protein factor. Protein removal ratio was determined by following equation;

$$\text{Protein removal}(\%) = 100 - (100 - SR) \times \frac{P_r}{P_i} \quad (2)$$