Microwave Heating of Tea Residue Yields Polysaccharides, Polyphenols, and Plant Biopolyester

SHUNTARO TSUBAKI,* HIROYUKI IIDA, MASAHIRO SAKAMOTO, AND JUN-ICHI AZUMA

Division of Environmental Science and Technology, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606-8502, Japan

Microwave heating was used to produce aqueous-soluble components from green, oolong, and black tea residues. Heating at 200−230 °C for 2 min extracted 40−50% of polysaccharides and 60−70% of the polyphenols. Solubilization of arabinose and galactose by autohydrolysis occurred with heating above 170 °C, whereas heating above 200 °C was necessary to solubilize xylose. Catechins were soluble in water by heating at low temperature (110 °C); however, new polyphenols having strong antioxidant activity were produced above 200 °C. The amount of solubilized materials and antioxidant activity increased with increased fermentation of harvested tea leaves (green tea < oolong tea < black tea). Cutin, a plant biopolyester, remained in the residue after heating as did cellulose and lignin/tannin. The predominant cutin monomer that was recovered was 9,10-epoxy-18-hydroxyocta-decanoic acid, followed by dihydroxyhexadecanoic acid and 9,10,18-trihydroxyoctadecanoic acid.

KEYWORDS: Tea residue; microwave heating; autohydrolysis; cutin; plant biopolyester

INTRODUCTION

Many treatments have been tested to extract useful chemicals from plant biomass: physical (mechanical cmminution and pyrolysis), physicochemical (steam, ammonia and CO_2 explosions, and hot compressed water), chemical (ozonolysis, acid hydrolysis, alkaline hydrolysis, oxidative delignification, and organosolv process), and biological treatments (*1*). Hot compressed water is one of the more environmentally friendly physicochemical methods to hydrolyze biomass (*2*). An additional advantage to hot compressed water is that it has a lower dielectric constant and a higher ion product than water in ambient condition; furthermore, hydrolysis occurs without catalyst.

Microwave heating is now attractive as a new heating source (*3*) that may be used to produce hot compressed water. Microwave ovens, widely used in household kitchens, enable rapid and uniform sample heating by direct and internal heating generated by friction occurring with dipole rotations of water molecules. Microwave heating has been widely applied in organic chemistry (*4*), in an extraction method (microwave-assisted extraction) (*5*), and in pretreatment for lignocellulosic materials prior to enzymatic hydrolysis (*6*). Microwave heating is regarded as an alternative method for autohydrolysis that partially overcomes the deficiencies in previous autohydrolytic processes (*6*). A continuous microwave system (*7*) has the potential to be scaled up to a larger semi-industrial scale process.

Previously, we extracted β-glucans from the fruiting body of a mushroom (*Hericium erinaceum*) using microwave-assisted extraction and demonstrated the advantages in terms of extraction time compared to conventional external heating (*8*). Furthermore, Fischer and Bipp reported that microwaves have an advantage not only in reducing the reaction time but also in providing reagents required for the hydrolytic and oxidative transformation of carbohydrate-rich biomass (*9*).

Tea is one of the most consumed beverages in the world. With recent increases in tea consumption in Japan, a large amount of tea residue is produced (*10*). Most of the tea residues are burned or dumped into landfills, although alternative uses for tea residues have been reported, for example, as a feedstock (*10*), an adsorbent for heavy metals (*11*), or compost (*12*) and for particle board (*13*). Because tea leaves contain polyphenols such as catechins, amino acids such as theanine, and carbohydrates and minerals (*14*), tea residues are expected to retain these useful substances. Only a small number of research programs have reported on the utilization of chemical components found in tea residue. For example, Senol and Aydin reported the extraction of caffeine by a solid−liquid extraction method using water and chloroform (*15*).

We are interested in using microwave energy to extract useful constituents from natural resources. For this paper, we used microwave heating to extract polysaccharides and polyphenols from three kinds of tea residues (green, oolong, and black tea). We also report the presence of the plant biopolyester "cutin" in the residues after microwave irradiation. Cutin forms a three-dimensionally networked polyester of hydroxyl fatty acids

* Corresponding author (telephone +81-75-753-6465; fax +81-75-753-6471; e-mail stsubaki@kais.kyoto-u.ac.jp).

11294 *J. Agric. Food Chem.,* Vol. 56, No. 23, 2008

Tsubaki et al.

Table 1. Chemical Composition (Percent, w/w) of Native Green, Oolong, And Black Tea Residues

composition	green tea residue	oolong tea residue	black tea residue
neutral carbohydrate	36.7	30.5	29.6
uronic acid	0.7	0.8	0.8
protein	25.4	20.3	20.8
ash	3.1	4.3	3.3
lignin	30.8	40.1	35.4
alcohol benzene extract	10.4	11.6	12.4
total polyphenol	12.7	18.7	23.3
catechins	3.2	3.0	3.8
theaflavins	0	0	0.8
caffeine	0.1	0.3	0.4

constituting the cuticular membrane together with wax and cutan (*16*). The cuticular membrane covers the aerial parts of higher plants and protects the plant body from water loss or attacks from insects or pathogens. The polyfunctional fatty acids in cutin seem to have the potential to be biorefined.

MATERIALS AND METHODS

Samples. Tea residues (green, oolong, and black teas) were supplied from a tea drink manufacturer in Wakayama Prefecture, Japan, and were lyophilized and powdered before experiments. Antioxidant activity was measured by using a kit (Radical Catch) supplied from Aloka Co., Ltd. (Tokyo, Japan). Pullulans and a nonasaccharide from xyloglucan used as molecular weight standards were purchased from Showa Denko K.K. (Tokyo, Japan) and Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), respectively. Standards of (−)-catechin (C), (−)-catechin gallate (CG), (−)-gallocatechin (GC), (−)-gallocatechin gallate (GCG), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin gallate (EGCG), caffeine (CAF), theaflavin (TF), theaflavin 3-gallate (TF3G), theaflavin 3′-gallate (TF3′G), and theaflavin 3,3′-digallate (TF3,3′diG) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The solvents for HPLC were of HPLC grade, and all other reagents used were of analytical grade.

Microwave Irradiation. Microwave irradiation was performed with a MicroSYNTH Labstation (maximum output, 1 kW, 2.45 GHz; Milestone Inc., Shelton, CT) using an HPR 100 (high-pressure 100 mL) reactor. The MicroSYNTH Labstation is a multimode microwave oven in which the real-time temperature inside the reactor is monitored with a thermometer. Heating temperature is controlled precisely with a PID (Proportional, Integral, Derivative) algorithm by changing the power of microwave irradiation. The reactant in the reactor is agitated with a stir bar that facilitates homogeneous microwave heating. The HPR 100 reactor is a high-pressure reactor made of TFM [polytetrafluoroethylene (PTFE) containing very little (<1%) perfluoropropyl vinyl ether (PPVE) modifier] that can endure temperatures up to 250 °C and pressure up to 5.5 MPa. Microwave energy is transmitted through the reactor and directly heats the reactant inside.

One gram of each tea residue was suspended in 20 mL of water in an HPR 100 reactor at a liquid/solid ratio of 20:1. Because of the high viscosity of the reactant, this liquid/solid ratio was the minimum to maintain homogeneous conditions by mixing with a stir rod. Microwave power was adjusted to attain the desired temperature (110, 140, 170, 200, or 230 °C) in 2 min. The reactor was cooled in an ice bath immediately after irradiation and centrifuged to separate water-soluble and insoluble fractions.

General Analytical Methods. Uronic acid contents was determined according to the the method given in the *Handbook of Analytical Chemistry* (*17*). Protein content was determined from elemental analysis and calibrated with the N content of caffeine according to AOAC official method 920.103. Ash content of raw materials was determined according to AOAC official method 920.100. Alcohol−benzene extract and acid-insoluble lignin contents were determined according to TAPPI test methods T 204 om-88 and T222 om-88, respectively. The solubilization rate was calculated by using the following equation:

$$\text{solubilization rate (\%)} = 100 \times (\text{initial wt of tea residue} - \text{wt of residue after microwave heating})/\text{initial wt of tea residue}$$

Each fraction after microwave heating was hydrolyzed according to the method of Saeman (*18*), and the monosaccharide composition was analyzed by high-performance anion exchange chromatography (*19*) (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. Neutral carbohydrate content was determined by using the phenol−sulfuric acid method (*17*) and a standard solution containing arabinose, rhamnose, galactose, glucose, xylose, and mannose mixed together according to the relative monosaccharide composition determined by HPAEC. Molecular weight distribution was analyzed by size exclusion chromatography (SEC) on a column of Asahipak GS 220 (7.6 × 500 mm, Showa Denko K.K.) at 65 °C with a refractive index detector (RI-8, Tosoh Co., Tokyo, Japan). Molecular weight distribution was determined using pullulan as the standard (*20*). Polyphenol content was determined according to the Folin−Denis method (*21*) using 80% methanol as an extractant and gallic acid as standard and expressed as gallic acid equivalents (GAE). Antioxidant activity was measured by a chemiluminescence method based on the Fenton reaction in which antioxidants are trapped by luminol, resulting in light emission (*22*); 50 μL of cobalt solution and 50 μL of luminol solution were mixed with 20 μL of sample solution and incubated for 5 min at 37 °C. Generation of hydroxyl radical was started by the addition of 50 μL of H_2O_2 solution. Light emission at 430 nm was measured for 120 s immediately after initiation. Ultrapure water (Simplicity, Millipore) was used as a control. Light emissions from 80 to 120 s were integrated. The rate of decrease in light emission compared to the control was expressed as the antioxidant activity.

Catechin and caffeine contents were determined according to the modified method of Khokhar and Magnusdottir (*23*). Lyophilized water-soluble fractions were solubilized in mobile phase A (8% acetonitrile in 0.025 M phosphate buffer, pH 2.4) and analyzed by HPLC on a Gulliver (Jasco, Tokyo, Japan) system equipped with a YMC-Pack ODS-AM-303 column (4.6 × 250 mm, YMC Co., Ltd., Kyoto, Japan), and the absorbance at 231 nm was monitored with a UV−vis detector (UV-970). The mobile phase gradient elution system was carried out as follows: 0−5 min, 100% mobile phase A; 5−25 min, a linear gradient of 15−100% to mobile phase B (25% acetonitrile in 0.025 M phosphate buffer, pH 2.4); 25−30 min, 100% mobile phase B. Column temperature was 40 °C, flow rate was 1 mL/min, and injection volume was 5 μL. Theaflavin content was determined with the modified method of Nishimura et al. (*24*); lyophilized water-soluble fractions were solubilized in mobile phase A (15% acetonitrile containing 0.5% acetic acid) and analyzed with the same HPLC system as described above. The absorbance at 280 nm was monitored. The mobile phase gradient elution system was carried out as follows: 0−8 min, a linear gradient of 0−20% to mobile phase B (80% acetonitrile containing 0.5% acetic acid), and held at this ratio for 12 min. Column temperature was 40 °C, flow rate was 0.8 mL/min, and injection volume was 5 μL.

Solid-state CP/MAS ^{13}C NMR spectra were obtained on a Chemagnetics CMX-300 spectrometer (JEOL Ltd., Tokyo, Japan), operating at 74.7 MHz. The pulse repetition time was 5 s, the cross-polarization contact time was 2 ms, the sweep width was 30 kHz, and the acquisition time was 34 ms. Magic angle spinning was performed at 3.5 kHz in a Zirconia rotor with Teflon caps. Chemical shifts were referenced to the methyl-carbon signal (17.3 ppm) of hexamethylbenzene.

Morphological properties of the residues after microwave heating were analyzed with a low-voltage scanning electron microscope (LV-SEM, VE-8800, Keyence Co., Osaka, Japan) at 1.7 kV.

Analysis of Cutin. Residues were hydrolyzed with enzymes (1.0% w/w cellulase from *Aspergillus niger*, MP Biochemicals, Inc., and 2.0% w/w pectinase from *A. niger*, Sigma-Aldrich) at 37 °C for 2 days with the addition of a few drops of toluene to prevent microbial contamination. The residues were recovered by centrifugation at 8000 rpm for 20 min. Waxes were removed by overnight extraction with chloroform/methanol (1:1, v/v), and the remaining cellulose was removed by treatment by $ZnCl_2$−HCl according to the method

Products from Microwaved Tea Residues

J. Agric. Food Chem., Vol. 56, No. 23, 2008   **11295**
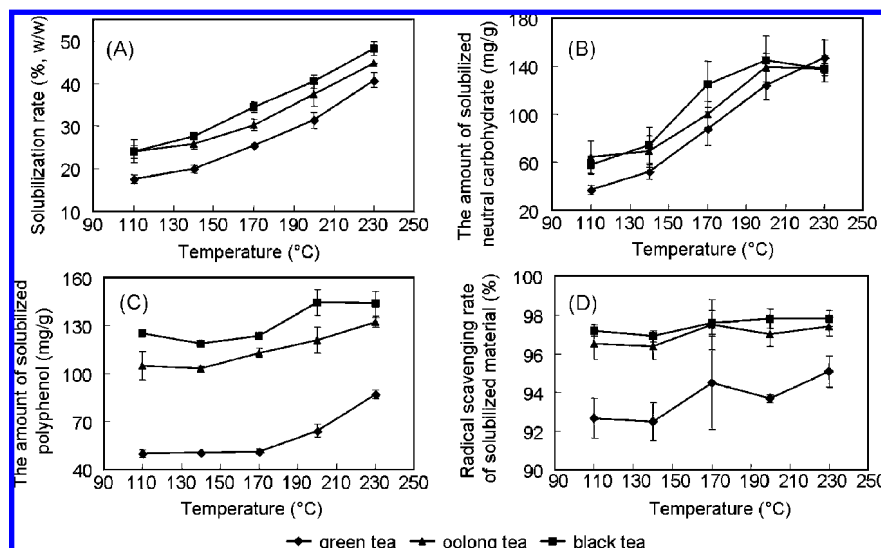
Figure 1. Effects of heating temperature on solubilization rate (**A**), amounts of neutral carbohydrates (**B**) and polyphenols (**C**) solubilized by microwave heating in water, and antioxidant activity of solubilized materials (**D**). Error bars indicate standard deviations ($n = 3$).
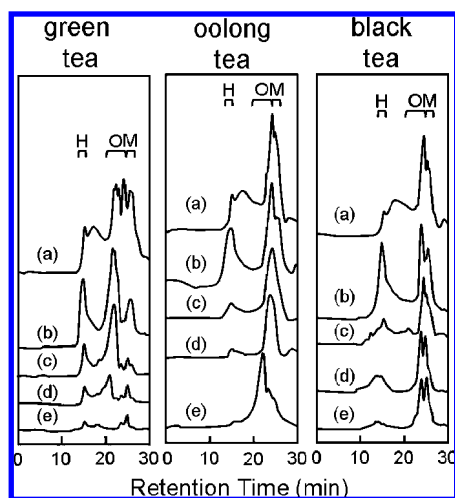
Figure 2. Size exclusion chromatograms of solubilized material obtained from green, oolong, and black tea residues by microwave heating. (a), (b), (c), (d), and (e) represent heating temperatures of 230, 200, 170, 140, and 110 °C, respectively. H, O, and M describe fractions having molecular weights equivalent to the standards >12 kDa (pullulan), oligosaccharides, and monosaccharide, respectively.

of Holloway and Baker (*25*). Monomers of cutin were obtained by reduction with LiAlH$_4$ in THF for 2 days according to the method of Walton and Kolattukudy (*26*). Reduction was also carried out with LiAlD$_4$ to label carboxyl and epoxy groups in cutin monomers with deuterium. Excess reagents were degraded by the addition of distilled water, the solution was then acidified with HCl, and the hydrogenolysate and deuteriolysate were extracted with diethyl ether. The extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The trimethylsilyl ethers of cutin monomers were obtained by treatment with BSA (*N*,*O*-bis(trimethylsilyl)-acetamide) and analyzed by GC-MS (Shimadzu GC-2010/PAR-VUM2 system, EI; 70 eV, Shimadzu Co., Kyoto, Japan) equipped with a DB-1 capillary column (J&W Scientific, 0.25 mm × 30 m, df = 0.25 μm, Agilent Technologies, Inc., Santa Clara, CA). The oven temperature was programmed from 195 to 240 °C at 2 °C/min and held at this temperature for 10 min. For analysis of cutin by solid-state CP/MAS ^{13}C NMR, cuticular membrane was isolated from raw green leaves of a tea plant (*Camellia sinensis*) by the enzymatic treatment as described above.

RESULTS

Effects of Microwave Heating on Solubilization of Tea Residues. Effects of microwave heating were investigated for three kinds of tea residues from green, oolong, and black teas. Chemical compositions of these tea residues are summarized in **Table 1**. The amount of carbohydrate decreased with fermentation, whereas the polyphenolic and caffeine contents showed inverse relationships. **Figure 1** shows the dependence of heating temperature on solubilization rate, amounts of neutral carbohydrates and polyphenols solubilized in water, and antioxidant capacity of the solubilized materials. Increased microwave heating temperatures were effective for solubilizing tea residues (**Figure 1A**), neutral carbohydrates (**Figure 1B**), and polyphenols (**Figure 1C**). The antioxidant activity (**Figure 1D**) showed good correlation with the amount of polyphenols solubilized. Forty to 50% of polysaccharides and 60−70% of polyphenols were extracted in water by heating at 200−230 °C for 2 min.

Figure 2 shows the molecular weight distributions of solubilized materials. In the case of green tea, the amount of a component (fraction H) having a molecular weight higher than 12 kDa increased as heating temperature increased above 170 °C (**Figure 2A**). In the cases of oolong and black teas, the appearance of low molecular weight components was evident, indicating production of these components during the process of tea fermentation [**Figure 2B**(e) and **C**(e)]. High molecular weight components from all three tea residues were degraded by heating above 200 °C, and the peak intensities with retention times identical to those of monosaccharides (fraction M) and oligosaccharides (fraction O) increased.

Table 2 shows the amount of catechins and caffeine in the solubilized materials determined by HPLC equipped with an ODS column according to the modified method of Khokhar and Magnusdottir (*23*). EGCG was the major catechin in the solubilized materials from green tea and black tea residues; however, EGC predominated in oolong tea. Yields of EC, ECG, EGC, and EGCG decreased with increases in heating temperature, whereas an inverse relationship was observed in the amounts of C, CG, GC, and GCG, indicating catechin epimerization had occurred. Catechins were almost entirely decomposed above 200 °C, but caffeine was rather stable against heating and remained at 1.51 mg/g (green tea) to 10.64 mg/g (black tea) after microwave heating at 230 °C. Compared to

Table 2. Amount of Catechins and Caffeine (Milligrams per Gram) Solubilized by Microwave Heating[a]

temperature (°C)	catechins								CAF
	GC	EGC	C	EC	EGCG	GCG	ECG	CG	
	Green Tea Residue								
110	0.48 ± 0.04	3.78 ± 0.50	0.44 ± 0.02	2.16 ± 0.18	6.11 ± 0.83	0.49 ± 0.02	1.70 ± 0.05	0.28 ± 0.03	2.55 ± 0.07
140	1.00 ± 0.03	3.82 ± 0.69	0.77 ± 0.17	0.69 ± 0.02	3.04 ± 0.34	0.47 ± 0.01	0.75 ± 0.05	0.19 ± 0.03	2.91 ± 0.36
170	2.21 ± 0.09	1.70 ± 0.11	1.28 ± 0.04	0.49 ± 0.12	1.91 ± 0.36	1.32 ± 0.07	0.63 ± 0.02	0.36 ± 0.11	2.38 ± 0.07
200	1.66 ± 0.05	1.21 ± 0.25	1.21 ± 0.03	0.27 ± 0.03	0.84 ± 0.09	0.98 ± 0.10	0.42 ± 0.03	0.38 ± 0.01	2.52 ± 0.10
230	0.45 ± 0.25	0	0	0	0	0	0	0	1.51 ± 1.09
	Oolong Tea Residue								
110	1.76 ± 0.44	7.18 ± 0.58	1.44 ± 0.98	0	1.98 ± 0.49	0	0	0	7.72 ± 1.27
140	1.68 ± 0.23	2.58 ± 0.23	1.02 ± 0.14	0.78 ± 0.21	3.70 ± 0.93	1.00 ± 0.33	1.10 ± 0.14	0	7.49 ± 1.23
170	2.52 ± 0.27	1.58 ± 0.20	1.91 ± 0.46	0.60 ± 0.27	2.65 ± 0.98	2.44 ± 0.43	0.91 ± 0.16	0.56 ± 0.28	7.64 ± 1.03
200	1.33 ± 0.11	0.92 ± 0.10	1.29 ± 0.07	0.25 ± 0.15	0.92 ± 0.30	1.29 ± 0.43	0.62 ± 0.19	0.65 ± 0.12	6.38 ± 0.25
230	2.39 ± 0.20	0	0	0	0	0	0	0	6.73 ± 0.24
	Black Tea Residue								
110	0.60 ± 0.08	5.84 ± 0.97	0.72 ± 0.19	5.16 ± 3.16	6.91 ± 1.09	0	2.57 ± 0.39	0.25 ± 0.22	9.51 ± 2.32
140	0.95 ± 0.09	5.58 ± 0.33	0.93 ± 0.15	2.15 ± 0.13	6.52 ± 0.14	0.46 ± 0.06	1.75 ± 1.17	0.08 ± 0.13	9.04 ± 0.55
170	2.63 ± 0.15	2.81 ± 0.13	2.66 ± 0.09	1.12 ± 0.19	3.47 ± 0.47	2.56 ± 0.19	1.72 ± 0.34	1.08 ± 0.08	8.57 ± 1.68
200	2.98 ± 0.20	1.81 ± 0.14	3.58 ± 0.27	0.75 ± 0.10	2.29 ± 0.31	2.78 ± 0.38	1.40 ± 0.30	1.59 ± 0.10	10.00 ± 1.27
230	2.52 ± 0.41	0.49 ± 0.42	0.31 ± 0.35	0	0	0	0	0	10.64 ± 1.58

[a] Values are expressed as mean ± SD (*n* = 3).

Table 3. Amount of Solubilized Theaflavins (Milligrams per Gram) from Black Tea Residue by Microwave Heating[a]

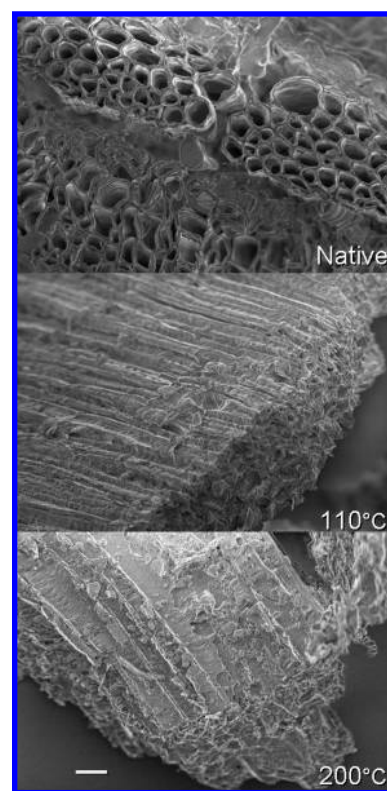| temperature (°C)	TF	TF3G	TF3′G	TF3,3′diG
110	0.35 ± 0.05	0.17 ± 0.03	0.05 ± 0	0.18 ± 0.02
140	0.15 ± 0.09	0.07 ± 0.05	0.02 ± 0.01	0.05 ± 0.03
170	0.17 ± 0.03	0.09 ± 0.01	0.02 ± 0.01	0.08 ± 0.03

[a] Values are expressed as the mean ± SD (*n* = 3).

Table 4. Relative Monosaccharide Composition (Percent, w/w) of the Residues Remaining after Microwave Heating

temperature (°C)	Ara	Rha	Gal	Glc	Xyl	Man
	Green Tea Residue					
110	13.6	3.4	14.2	49.9	16.1	2.8
140	16.0	3.6	16.4	45.5	15.9	2.6
170	13.5	1.2	13.1	50.7	19.1	2.3
200	9.3	0.8	10.2	60.0	19.0	0.8
230	3.5	0	6.3	74.8	10.9	4.4
	Oolong Tea Residue					
110	15.7	1.5	13.4	51.2	14.2	4.0
140	13.5	1.1	11.4	56.1	14.5	3.3
170	12.2	2.5	12.0	57.8	14.3	1.2
200	5.8	0	6.5	65.2	17.0	5.5
230	2.0	0	5.9	82.0	6.9	3.2
	Black Tea Residue					
110	19.5	2.2	17.9	40.6	14.4	5.4
140	17.2	1.2	17.4	48.4	13.4	2.5
170	10.3	0.8	11.6	53.6	18.8	4.9
200	7.0	0	8.4	66.3	16.0	2.3
230	2.7	0	6.0	74.6	7.2	9.6

Figure 3. Typical LV-SEM images of the residues remaining after microwave heating (green tea). White bar indicates 10.0 μm.

the amount of solubilized catechins, theaflavins were detected only in the solubilized materials given by heating at 110−170 °C from black tea residue (**Table 3**). TF was the most abundant theaflavin in the solubilized material, with the amount of TF decreasing with increases in heating temperature; however, the concentration of TF was much lower than the concentration of catechins.

Properties of Residues after Microwave Heating. Table 4 shows the relative monosaccharide compositions of the residue remaining after microwave heating. Arabinose and galactose were released by microwave heating above 170 °C, whereas xylose was solubilized in water only above 200 °C. Glucose was the main neutral carbohydrate in the final residue after

microwave heating at 230 °C. **Figure 3** shows typical LV-SEM images of the residue obtained from green tea after microwave heating. Native green tea leaves have thick cell walls that became thinner and sharper after microwave heating at 200 °C, indicating that cell wall components were solubilized in water. Similar observations were made with oolong and black tea residues.

Solid-state CP/MAS ^{13}C NMR was used to characterize the chemical components remaining in the residues. Typical spectra from green tea are shown in **Figure 4**. The spectra were simply assigned to carbonyl (160−200 ppm), lignin (around 130−160 ppm), lignin and protein (around 110−130 ppm), carbohydrate (60−110 ppm), OCH_3 (around 56 ppm), and lipid (10−45)

Products from Microwaved Tea Residues

J. Agric. Food Chem., Vol. 56, No. 23, 2008 **11297**
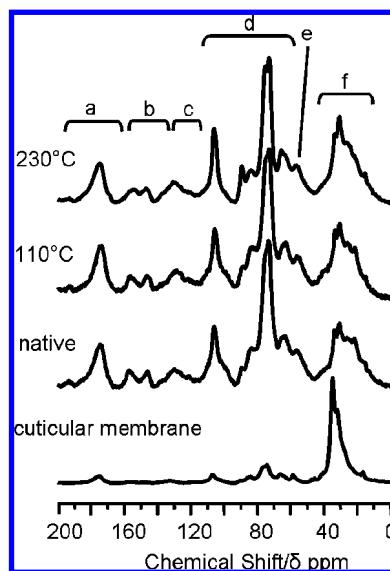
Figure 4. Solid-state CP/MAS ^{13}C NMR spectra of the residues obtained from green tea residue. Native indicates native green tea residue. Cuticular membrane was isolated from raw green tea leaves by enzymatic treatment. Signals: a, carbonyl-C; b, aromatic-C (lignin/tannin); c, aromatic-C (lignin/tannin or protein); d, carbohydrates; e, methoxyl- and *N*-amino-C; f, alkyl-C.
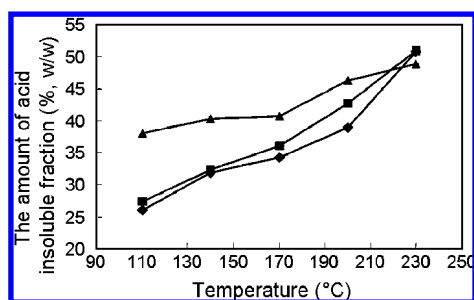
Figure 5. Effects of heating temperature on the amount of acid-insoluble fraction (percent, w/w) containing cutin obtained from residues after microwave heating. See **Figure 1** for symbols.

according to the method of Himmelsbach (*27*). The spectra revealed increases in signals around 10−45 ppm, a region assigned to alkyl carbons, indicating that aliphatic components of tea remained in the residue. A sharp signal centered at 33 ppm corresponded to the cuticular membrane isolated from native tea leaves (**Figure 4**). The area of 10−45 ppm grew to 25.1% of the whole NMR spectrum at 230 °C. The spectrum showed the presence of cellulose (60−110 ppm), lignin/tannin (130−160 ppm), and lignin/tannin or protein (110−130 ppm) in the residue. Similar results were obtained for oolong and black tea residues.

Because aliphatic components may be mainly derived from cutin, a constituent of tea leaf cuticular membranes, we next analyzed the composition of cutin monomers in the residues after microwave heating. Cutin can be recovered from dewaxed residues as an acid-insoluble residue after hydrolysis with cellulase and $ZnCl_2$−HCl. The amount of acid-insoluble components including cutin is shown in **Figure 5**. The concentration of acid-insoluble components increased with increased heating temperature. Condensed tannins contained in tea leaves may be included in the acid-insoluble components (*26*).

The positions of hydroxyl groups, epoxy groups, and carbonyl groups of cutin monomers were determined by comparing the MS spectra obtained from hydrogenolysate and deuteriolysate according to the method of Walton and Kolattukudy (*26*). **Table 5** shows the identified cutin monomer composition obtained from the acid-insoluble components. The most abundant cutin monomer was 9,10-epoxy-18-hydroxyoctadecanoic acid, followed by dihydroxyhexadecanoic acid and 9,10,18-trihydroxy-octadecanoic acid that were detected in almost equal ratios in all tea residues. The MS spectrum indicated that dihydroxy-hexadecanoic acid was recovered as an equal mixture of 9,16- and 10,16-isomers. Hexadecanoic acid, octadecenoic acid, octadecanoic acid, 16-hydroxyhexadecanoic acid, 18-hydroxyo-tadecanoic acid, and 10,18-dihydroxyoctadecanoic acid were also detected in the acid-insoluble components in amounts as low as a trace to 8%. No significant compositional changes were seen in the three tea residues, indicating the stability of cutin against both fermentation and microwave heating up to 230 °C.

DISCUSSION

In this study, three kinds of tea residues (green, oolong, and black teas) were subjected to microwave heating for 2 min. Water was selected as the extraction solvent because it is a good absorbent of microwave energy and an environmentally friendly solvent.

Solubilization of neutral carbohydrates from green tea residue attained maximum value at 230 °C; however, corresponding values for oolong and black tea residues were reached at 200 °C and decreased slightly at 230 °C. This result indicates that the destruction of solubilized carbohydrates occurred at 230 °C. Similar temperature dependencies of carbohydrate solubilization were observed in lignocellulosic wastes (*6*), starch (*28*), and mushrooms (*8*). Generally, hemicelluloses are hydrolyzed in water above 180 °C; however, solubilized carbohydrates are susceptible to further decomposition (*2*). Precise control of heating time is crucial at high-temperature treatments. We, therefore, limited microwave irradiation to 2 min followed by rapid cooling in an ice bath to minimize secondary destruction of solubilized materials. Neutral carbohydrate compositions of the residues showed solubilization of arabinose and galactose

Table 5. Monomer Composition of Cutin Recovered from the Residues Obtained after Microwave Heating (Relative Percent)

cutin monomer	green tea residue, heating temperature					oolong tea residue, heating temperature					black tea residue, heating temperature				
	110°C	140°C	170°C	200°C	230°C	110°C	140°C	170°C	200°C	230°C	110°C	140°C	170°C	200°C	230°C
hexadecanoic acid[a]	2.9	2.6	4.3	6.6	2.9	0.4	3.9	7.0	6.1	6.3	1.8	2.9	2.5	5.6	0.2
octadecenoic acid	tr	tr	tr	1.8	0.2	tr	tr	tr	tr	tr	tr	tr	tr	0.8	0.5
octadecanoic acid	tr	tr	1.1	tr	0.6	tr	tr	2.2	tr	1.5	tr	tr	1.1	0.8	0.5
16-hydroxyhexadecanoic acid	3.2	3.1	2.7	4.0	2.9	3.1	2.4	6.6	8.0	3.5	1.4	1.6	1.6	1.4	1.3
18-hydroxyoctadecanoic acid	0.9	1.0	2.4	2.4	2.4	2.8	1.6	1.6	0.9	2.1	1.9	1.9	3.2	3.0	2.7
dihydroxyhexadecanoic acid	26.4	25.3	27.7	29.5	26.2	26.4	25.6	30.4	27.9	32.3	24.0	21.6	24.6	24.9	23.5
10,18-dihydroxyoctaoctadecenoic acid	0.8	1.1	tr	1.2	0.4	tr	tr	tr	tr	tr	1.3	1.1	1.4	tr	tr
9,10-epoxy-18-hydroxyoctadecanoic acid	44.8	45.2	57.0	49.8	42.6	43.7	41.8	45.0	40.5	40.0	46.7	45.2	42.0	42.3	40.9
9,10,18-trihydroxyoctadecanoic acid	21.0	21.8	5.9	6.5	22.6	23.6	24.8	9.5	16.6	15.8	22.8	25.6	24.8	23.0	31.4

[a] Including 9,16- and 10,16-isomers.

11298 *J. Agric. Food Chem.*, Vol. 56, No. 23, 2008

Tsubaki et al.

above 170 °C and of xylose above 200 °C. These carbohydrates probably originated from pectic and hemicellulosic polysaccharides. By LV-SEM analysis (**Figure 3**) destruction of cell wall structure was evident with increases in heating temperature, in accordance with changes in the chemical composition analyses. Recently, Monobe et al. reported that a crude polysaccharide from green tea extract showed immunostimulating activity (*29*). Although further study is necessary, the solubilized materials from tea residues should be an interesting source of this kind of polysaccharide in the future.

Catechins were easily solubilized in water by microwave heating at 110 °C, and the amount of solubilized catechins decreased with increased heating temperature. Because catechins are the predominant flavonoid in tea leaves, much attention has been directed toward these compounds for their health-promoting properties. Because of the heat lability of catechins in contrast to caffeine, a lower heating temperature is desired to extract catechins from tea residues. In comparison with the total polyphenol content, the levels of catechins and theaflavins in residues were remarkably small, indicating the other phenolic oligomers were solubilized. The total amount of polyphenols in the solubilized materials increased slightly with increased heating temperature above 200 °C, contrary to the behavior of catechin solubilization. This result indicates that microwave heating at temperatures >200 °C could be used to generate new polyphenols. Azuma et al. reported that lignocellulosic materials are partly delignified above 180 °C by microwave heating, and the molecular weight of the solubilized materials decreased with increased temperature (*6*). The newly produced polyphenols might originate from lignin or condensed tannins. Because the solubilized materials showed strong antioxidant activity without catechins, these polyphenols were expected to possess antioxidant activity.

The degree of fermentation also affected the solubilization of tea residues in addition to the heating temperature. Oolong tea is the result of partial oxidation by endogenous oxidases present in tea leaves, whereas black teas are fully oxidized (*14*). Both oolong and black tea residues gave higher solubilization rates, amounts of solubilized polyphenols, and materials that had antioxidant activity than the green tea residue. In addition, both fermented tea residues solubilized more neutral carbohydrates than green tea residue, indicating that the polysaccharides portion of tea leaves was partly degraded by fermentation; thus, the peaks of low molecular weight components increased in height in SEC chromatograms (**Figure 2**).

By solid-state CP/MAS ^{13}C NMR, tea residues were revealed as a cutin-rich biomass (**Figure 4**). The presence of cutin in the residues was demonstrated by detection of three kinds of monomers: 9,10-epoxy-18-hydroxyoctadecanoic acid, dihydroxyhexadecanoic acid, and 9,10,18-trihydroxyoctadecanoic acid (**Table 5**) that are commonly seen in many plants (*26*) and sewage sludge (*30*). This is the first paper reporting the presence of cutin in tea residues. The present results indicate stability and durability of cutin against microwave heating or the tea manufacturing process. Because cutin is attractive as a "weather-resistant" and "durable" biodegradable polymer, tea residues are an interesting resource for production of cutin monomers, as well as polysaccharides and catechins.

In summary, three kinds of tea residues from green, oolong, and black teas were treated with microwave heating. By heating at 200−230 °C for 2 min, 40−50% of the polysaccharides and 60−70% of polyphenols were found to be extractable in water. Catechins and theaflavins were also solubilized in water by microwave heating. Cutin, as well as cellulose and lignin/tannin,

however, remained in the residue. Monomeric units of tea residues were similar in all tea residues and predominately recovered as 9,10-epoxy-18-hydroxyoctadecanoic acid, followed by dihydroxyhexadecanoic acid and 9,10,18-trihydroxyoctadecanoic acid. Microwave heating contributed to the concentration of cutin present in tea residues.

ABBREVIATIONS USED

GC, (−)-gallocatechin; EGC, (−)-epigallocatechin; C, (−)-catechin; EC, (−)-epicatechin; EGCG, (−)-epigallocatechin gallate; GCG, (−)-gallocatechin gallate; ECG, (−)-epicatechin gallate; CG, (−)-catechin gallate; CAF, caffeine; TF, theaflavin; TF3G, theaflavin 3-gallate; TF3′G, theaflavin 3′-gallate; TF3,3′diG, theaflavin 3,3′-digallate.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Sun, Y.; Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour. Technol.* **2002**, *83*, 1–11.

(2) Yu, Y.; Lou, X.; Wu, H. Some recent advances in hydrolysis of biomass in hot-compressed water and its comparisons with other hydrolysis methods. *Energy Fuels* **2008**, *22*, 46–60.

(3) Adam, D. Out of the kitchen. *Nature* **2003**, *421*, 571–572.

(4) Perreux, L.; Loupy, A. A tentative rationalization of microwave effects in organic synthesis according to the reaction medium, and mechanistic considerations. *Tetrahedron* **2001**, *57*, 9199–9223.

(5) Pan, X.; Niu, G.; Liu, H. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem. Eng. Process.* **2003**, *42*, 129–133.

(6) Azuma, J.; Tanaka, F.; Koshijima, T. Enhancement of enzymatic susceptibility of lignocellulosic wastes by microwave irradiation. *J. Ferment. Technol.* **1984**, *62*, 377–384.

(7) Magara, K.; Ueki, S.; Azuma, J.; Koshijima, T. Microwave irradiation of lignocellulosic materials IX. Conversion of microwave-irradiated lignocellulose into ethanol. *Mokuzai Gakkaishi* **1988**, *34*, 462–468.

(8) Ookushi, Y.; Sakamoto, M.; Azuma, J. Optimization of microwave-assisted extraction of polysaccharides from fruiting body of mushrooms. *J. Appl. Glycosci.* **2006**, *53*, 267–272.

(9) Fischer, K.; Bipp, H. P. Generation of organic acids and monosaccharides by hydrolytic and oxidative transformation of food processing residues. *Bioresour. Technol.* **2005**, *96*, 831–842.

(10) Kondo, M.; Kita, K.; Yokota, H. Effects of tea leaf waste of green tea, oolong tea, and black tea addition on sudangrass silage quality and in vitro gas production. *J. Sci. Food Agric.* **2004**, *84*, 721–727.

(11) Amarasinghe, B. M. W. P. K.; Williams, R. A. Tea waste as a low cost adsorbent for the removal of Cu and Pb from wastewater. *Chem. Eng. J.* **2007**, *132*, 299–309.

(12) Toker, H.; Baysal, E.; Yigitbasi, O. N.; Colak, M.; Peker, H.; Simsek, H.; Yilmaz, F. Cultivation of *Agaricus bisporus* on wheat straw and waste tea leaves based composts using poplar leaves as activator material. *Afr. J. Biotechnol* **2007**, *6*, 204–212.

(13) Yalinkilic, M. K.; Imamura, Y.; Takahashi, M.; Kalaycioglu, H.; Nemli, G.; Demirci, Z.; Ozdemir, T. Biological, physical and mechanical properties of particleboard manufactured from waste tea leaves. *Int. Biodeterior. Biodegradation.* **1998**, *41*, 75–84.

(14) Harold, N.; Graham, P. D. Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.* **1992**, *21*, 334–350.

Products from Microwaved Tea Residues

J. Agric. Food Chem., Vol. 56, No. 23, 2008 **11299**

(15) Senol, A.; Aydin, A. Solid−liquid extraction of caffeine from tea waste using battery type extracter: process optimization. *J. Food Eng.* **2006**, *75*, 565–573.

(16) Heredia, A. Biophysical and biochemical characteristics of cutin, a plant barrier biopolyester. *Biochim. Biophys. Acta* **2003**, *1620*, 1–7.

(17) Wrolstad, R. E.; Acree, T. E.; Decker, E. A.; Penner, M. H.; Reid, D. S.; Schwartz, S. J.; Shoemaker, C. F.; Smith, D. M.; Sporns, P. *Handbook of Analytical Chemistry*; Wiley: Hoboken, NJ, 2005; pp 647−756.

(18) Saeman, J. F.; Bubl, J. L.; Harris, E. E. Quantitative saccharification of wood and cellulose. *Ind. Eng. Chem., Anal. Ed.* **1945**, *17*, 35–37.

(19) Suzuki, M.; Sakamoto, R.; Aoyagi, T. Rapid carbohydrate analysis of wood pulps by ion chromatography. *Tappi J.* **1995**, *78*, 174–177.

(20) Kawahara, K.; Ohta, K. Preparation and solution properties of pullulan fractions as standard samples for water-soluble polymers. *Carbohydr. Polym.* **1984**, *4*, 335–356.

(21) Folin, O.; Denis, W. A colorimetric method for the determination of phenols (and phenol derivatives) in urine. *J. Biol. Chem.* **1915**, *22*, 305–308.

(22) Parejo, I.; Petrakis, C.; Kefalas, P. A transition metal enhanced luminol chemiluminescence in the presence of a chelator. *J. Pharmacol. Toxicol. Methods* **2000**, *43*, 183–190.

(23) Khokhar, S.; Magnusdottir, S. G. M. Total phenol, catechin, and caffeine contents of teas commonly consumed in the United Kingdom. *J. Agric. Food Chem.* **2002**, *50*, 565–570.

(24) Nishimura, M.; Ishiyama, K.; Watanabe, A.; Kawano, S.; Miyase, T.; Sano, M. Determination of theaflavins including methylated theaflavins in black tea leaves by solid-phase extraction and HPLC analysis. *J. Agric. Food Chem.* **2007**, *55*, 7252–7257.

(25) Holloway, P. J.; Baker, E. A. Isolation of plant cuticles with zinc chloride-hydrochloric acid solution. *Plant Physiol.* **1968**, *43*, 1878–1879.

(26) Walton, T. J.; Kolattukudy, P. E. Determination of structures of cutin monomers by a novel depolymerization procedure and combined gas chromatography and mass spectrometry. *Biochemistry* **1972**, *11*, 1885–1897.

(27) Himmelsbach, D. S. Structure of forage cell walls−session synopsis. In *Forage Cell Wall Structure and Digestibility*; Jung, H. G., Buxton, D. R., Hatfield, R. D., Ralph, J., Eds.; ASA-CSSA-SSSA: Madison, WI, 1993; pp 271−283.

(28) Khan, A. R.; Johnson, J. A.; Robinson, R. J. Degradation of starch polymers by microwave energy. *Cereal Chem.* **1979**, *56*, 303–304.

(29) Monobe, M.; Ema, K.; Kato, F.; Maeda-Yamamoto, M. Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *J. Agric. Food Chem.* **2008**, *56*, 1423–1427.

(30) Kolattukudy, P. E.; Purdy, R. E. Identification of cutin, a lipid biopolymer, as significant component of sewage sludge. *Environ. Sci. Technol.* **1973**, *7*, 619–622.