Short communication

Microwave-assisted extraction of phenolic compounds from tea residues under autohydrolytic conditions

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ABSTRACT

Phenolic compounds were extracted from three kinds of tea residues (green, oolong and black tea residues) by microwave-assisted extraction in water under autohydrolytic conditions without using any catalyst or organic solvent. Productions of phenolic compounds were enhanced by microwave heating at 230 °C. The main phenolic constituent in the extract from green tea residue was pyrogallol (24.6%) which was estimated to be originated from degradations of catechins. Derivatives of guaiacyl units of lignin such as dihydroconiferyl alcohol (10.3%) and vanillin (8.1%) were, however, the main constituents in oolong tea residue. In the case of black tea residue, derivatives of both catechins and lignin were comparably extracted. These phenolic compounds are interesting as a bio-based chemical feedstock such as phenolic precursors and antioxidants.

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1. Introduction

A large amount of vegetative by-products are emitted as a biomass from food industries. Chemical constituents of these by-products such as cellulose and hemicelluloses, lignin, proteins, lipids and lower molecular weight extractives are attractive as a renewable resource for chemical feedstock alternative to petroleum derived materials (Kamm & Kamm, 2004).

Microwave-assisted extraction (MAE) of refined chemicals under autohydrolytic condition does not require organic solvent or catalyst, thus its environmental-friendliness is noticeable for utilisation of plant biomass. Previously we have conducted MAE for extraction of polysaccharides, polyphenols (catechins and theaflavins) and caffeine from tea residues (Tsubaki, Iida, Sakamoto, & Azuma, 2008) which is one of the major by-products from food industries in Japan. Catechins were extracted in water at relatively low temperatures from tea residues. Although these compounds were fragile to heat treatment and degraded at autohydrolytic conditions (>180 °C), total phenol content increased under these conditions accompanied with an increase in antioxidant activity. This discrepancy indicated the production of new phenolic compounds by degradation of higher molecular weight phenolic compounds such as lignin by autohydrolysis.

Since these plant phenolic compounds are an attractive source for natural antioxidants (Garrote, Cruz, Moure, Domínguez, & Para-

jó, 2004) as well as renewable bio-based chemical feedstock alternative to petroleum derived chemicals (Kamm & Kamm, 2004), we have newly elucidated the composition of phenolic compounds produced from three kinds of tea residues (green, oolong and black) except compounds analysed previously (Tsubaki et al., 2008) by MAE under autohydrolytic condition in this study.

2. Materials and methods

2.1. Materials

Tea residues (green, oolong and black) were supplied from tea drink manufacturer in Wakayama prefecture in Japan. These residues were lyophilised and powdered. *N,O*-Bis(trimethylsilyl) trifluoroacetamide (BSTFA) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). All the authentic reagents and solvents used were analytical grade.

2.2. Microwave-assisted extraction

Powdered tea residues were suspended in 20 ml of water in the high-pressure reactor HPR 100 (Milestone Inc., Shelton, CT, USA). The reactor was placed at the centre of the microwave apparatus (MicroSYNTH, max output; 1 kW, 2.45 GHz, Milestone Inc.) and heated to desired temperature (110–230 °C) in 2 min. The temperature in the reactor was directly measured using thermocouple-type thermometer and controlled automatically by PID. The reactant was stirred with magnetic stirrer bar for homogeneous

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heating. After microwave heating, the reactor was immediately cooled in ice bath. The extracted liquors from tea residues were recovered by filtration through no. 3 filter paper.

2.3. GC/MS analysis

Compositions of phenolic compounds were determined by using GC/MS. Firstly, the extracted liquor was acidified to pH 1.0 by addition of concentrated hydrogen chloride, and extracted with chloroform three times. The chloroform layer was dried with anhydrous sodium sulphate and evaporated to dryness, trimethylsilylated by BSTFA at 70 °C for 30 min and analysed by using GC/MS analysis (Shimadzu GC-2010/PURVUM 2 system, EI; 70 eV, Shimadzu Co., Kyoto, Japan) equipped with a DB-5 MS capillary column (J&W Scientific, 0.25 mm × 30 m, df = 0.25 μm, Agilent Technologies Inc., Santa Clara, CA, USA). The chromatographic condition was set according to the method of Esteves, Graça, and Pereira (2008); the oven temperature was programmed from 100 °C to 320 °C at 2 °C/min and held at this temperature for 15 min. The peaks were identified using a mass spectra library NIST05, together with mass spectra and retention times given from authentic compounds. The composition of the chloroform-soluble fraction was expressed as the ratio of peak area/total peak area of identified four categories of compounds (phenolic compounds, 5-hydroxymethylfurfural, monocarboxylic acids and di-carboxylic acid). The data were obtained in duplicate experiments and expressed as mean value ± standard deviation.

3. Results

3.1. Composition of phenolic compounds produced by MAE of green tea residue

Water-soluble components were extracted by MAE at 110–230 °C for 2 min. To analyse the constituents of phenolic compounds, extracted liquors were further extracted by chloroform and its compositions were subsequently analysed by using GC/MS. Phenolic compounds as well as 5-hydroxymethylfurfural, monocarboxylic acid and di-carboxylic acids were identified in the chloroform-soluble fraction.

The proportion of the phenolic compounds increased with an increase in temperature from 25.3% (110 °C) to 74.4% (230 °C) (Fig. 1A). The widest variation of compounds was observed in the chloroform-soluble fraction given at 230 °C (Table 1). The proportion of pyrogallol (24.6%) was greatly pronounced at this temperature. Dihydroconiferyl alcohol (7.0%) and catechol (6.7%) were also produced rich in the extracted liquor. As minor constituents, benzoic acid, p-hydroxybenzaldehyde, vanillin, cinnamic acid, p-coumaric acid p-hydroxyacetophenone and syringaldehyde were found over 2% in the chloroform-soluble fraction given at 230 °C.

3.2. Composition of phenolic compounds produced by MAE of oolong tea residue

The ratio of phenolic compounds in oolong tea residue exceed 50% constantly at all ranges of temperature tested (110–230 °C), and attained a maximum value of 74.0% at 170 °C (Fig. 1B). Proportion of 5-hydroxymethylfurfural increased above 200 °C attaining 19.7% at 230 °C. The most abundant phenolic compound produced at 230 °C was dihydroconiferyl alcohol (10.3%) followed by vanillin (8.1%) (Table 1). The production of catechol (6.4%) and pyrogallol (5.9%) were enhanced significantly at 230 °C. In the same extracted liquor, benzoic acid, cinnamic acid, p-hydroxybenzaldehyde, salicylic acid, syringaldehyde, p-hydroxyacetophenone, phenylpyruvic acid and p-coumaric acid were also detected in the chloroform-sol-
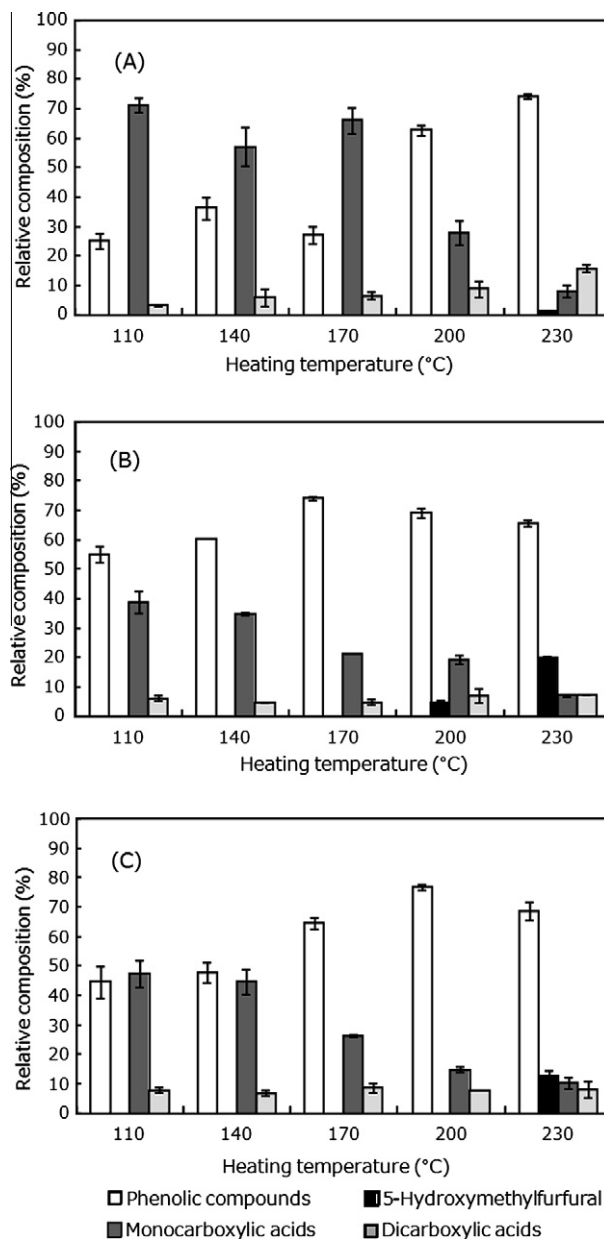

Fig. 1. Effect of heating temperature on constituents in the chloroform fraction of MAE extract from (A) green tea residue, (B) oolong tea residue, and (C) black tea residue. Data were expressed as relative percentage of each category of compounds as shown in Table 1. Error bars show standard deviations (n = 2).

uble fraction over 2%. Vanillin, salicylic acid, dihydroconiferyl alcohol, syringaldehyde, gallic acid, syringic acid and vanillic acid were also found in relatively large proportion at low temperature (110 °C).

3.3. Composition of phenolic compounds produced by MAE of black tea residue

The proportion of phenolic compounds in black tea residue increased with increase in heating temperature and attained a maximum value of 77.0% at 200 °C (Fig. 1C). The generation of 5-hydroxymethylfurfural by secondary degradation of hexoses was pronounced at 230 °C and reached 12.5% which was the most abundant compound in the extract (Table 1). The pronounced increases were also observed in vanillin (9.6%), pyrogallol (8.7%), and catechol (6.7%) at 230 °C. In the same extracted liquor, benzoic

Table 1
Composition (relative area %) of chloroform-soluble fraction obtained from extracted liquor of tea residues by MAE at 110 °C and 230 °C (autohydrolytic condition).[a]

Compounds	Retention time (min)	Heating temperature (°C)					
		Green tea residue		Oolong tea residue		Black tea residue	
		110	230	110	230	110	230
Enanthic acid (m)	6.055	0	0	3.0 ± 0	1.6 ± 0.4	tr[b]	tr[b]
Guaiacol (p)	7.729	0	0	0	1.2 ± 0.1	0	tr[b]
Benzoic acid (p)	8.284	17.6 ± 2.2	5.0 ± 0	10.0 ± 0.9	4.8 ± 0.3	24.8 ± 4.3	8.5 ± 1.9
Caprylic acid (m)	8.776	2.7 ± 0.4	0.6 ± 0.1	6.1 ± 0.7	1.5 ± 0.2	3.0 ± 0.5	0.8 ± 0.1
5-Hydroxymethylfurfural	10.231	0	1.5 ± 0.2	0	19.7 ± 0.5	0	12.5 ± 2.2
Succinic acid (d)	10.392	0	8.2 ± 1.1	tr[b]	2.7 ± 0.1	tr[b]	3.8 ± 1.8
Catechol (p)	10.473	0	6.7 ± 0.7	0	6.4 ± 0.2	0	6.7 ± 1.5
Fumaric acid (d)	11.352	0	1.5 ± 0.1	tr[b]	0.4 ± 0.1	0	0.4 ± 0.3
Pelargonic acid (m)	11.620	0	0	7.6 ± 1.9	1.4 ± 0	0	tr[b]
p-Hydroxybenzaldehyde (p)	11.901	0	5.0 ± 0.6	0	3.8 ± 0.4	0	4.8 ± 1.3
p-Hydroxyacetophenone (p)	14.603	0	2.1 ± 0.2	0	2.5 ± 0.1	0	2.7 ± 0.6
Malic acid (d)	15.533	0	0.4 ± 0.2	0	tr[b]	0	0
Salicylic acid (p)	15.850	0	0.6 ± 0.1	10.5 ± 1.2	3.3 ± 0.3	6.6 ± 0.9	3.0 ± 0.2
Vanillin (p)	16.345	0	4.9 ± 0.5	12.7 ± 2.3	8.1 ± 0.5	0	9.6 ± 1.0
Cinnamic acid (p)	16.544	0	4.5 ± 0	0	4.4 ± 0.8	0	1.9 ± 0.4
Pyrogallol (p)	16.796	0	24.6 ± 3.1	0	5.9 ± 0.1	0	8.7 ± 1.2
p-Hydroxybenzoic acid (p)	18.727	0	1.9 ± 0.3	tr[b]	0.5 ± 0	1.6 ± 0.2	0.7 ± 0.1
Syringaldehyde (p)	20.472	1.5 ± 0	2.1 ± 0.8	5.0 ± 0.9	2.7 ± 0.2	0	1.4 ± 0
Phenylpyruvic acid (p)	20.717	0	1.5 ± 0.4	0	2.3 ± 0.3	0	3.9 ± 0.1
Vanillic acid (p)	21.974	0	0.8 ± 0.1	2.9 ± 0.3	1.8 ± 0.2	0	1.8 ± 0.2
Azelaic acid (d)	22.686	3.3 ± 0.1	6.2 ± 0.1	6.2 ± 0.9	4.3 ± 0.1	8.0 ± 0.7	4.0 ± 0.7
Dihydroconiferyl alcohol (p)	23.208	2.1 ± 0.3	7.0 ± 0.2	5.1 ± 0.2	10.3 ± 1.7	0	3.1 ± 0.6
Protocatechuic acid (p)	23.325	0	0.9 ± 0.1	tr[b]	1.2 ± 0.7	0	0.9 ± 0.1
Syringic acid (p)	24.923	0	0.9 ± 0.1	3.2 ± 0.3	1.9 ± 0.2	1.6 ± 0.5	3.0 ± 0.7
p-Coumaric acid (p)	25.648	0	2.7 ± 0.1	1.2 ± 0.2	2.1 ± 0.3	tr[b]	3.1 ± 1.6
Gallic acid (p)	26.353	0.3 ± 0.4	1.8 ± 0.3	4.5 ± 1.3	1.8 ± 0.1	4.7 ± 0.3	3.6 ± 0.1
Sinapaldehyde (p)	26.954	3.7 ± 0.3	0.4 ± 0	tr[b]	0.7 ± 0.1	0	tr[b]
Palmitic acid (m)	27.695	68.5 ± 2.8	5.7 ± 1.4	22.0 ± 6.3	2.2 ± 0.6	44.4 ± 5.3	8.4 ± 1.6
Ferulic acid (p)	28.693	0.3 ± 0.4	1.1 ± 0.2	tr[b]	tr[b]	5.4 ± 0.2	1.4 ± 0.2
Oleic acid (m)	30.889	0	1.5 ± 0.7	0	0.6 ± 0.1	0	1.2 ± 0.2

Letters in parenthesis after name of compounds show the categories of compounds: (p) phenolic compounds, (m) monocarboxylic acids, and (d) di-carboxylic acids.
[a] Values are expressed as mean value ± SD ($n = 2$).
[b] tr represents trace.

acid, p-hydroxybenzaldehyde, phenylpyruvic acid, gallic acid, p-coumaric acid, salicylic acid, dihydroconiferyl alcohol, syringic acid and p-hydroxyacetophenone were detected over 2%.

4. Discussion

We have conducted rapid extraction of phenolic compounds from tea residue within 2 min. The advantage of MAE is an extreme reduction in extraction time (Ookushi, Sakamoto, & Azuma, 2006). The proportion and variation of phenolic compounds in the chloroform-soluble fraction given after microwave heating of the tea residues increased with increase in heating temperature within temperature range from 110 to 230 °C, showing good agreement with our previous results that polyphenol yield increased with increase in heating temperature and maximised at 230 °C and 2 min, attaining 87.2 mg GAE/g (green tea) to 144.0 mg GAE/g (oolong tea) (Tsubaki et al., 2008).

Vanillin, vanillic acid, dihydroconiferyl alcohol and guaiacol are considered as degraded substances originated from guaiacyl (G) units of lignin since they have a methoxyl group at C-3. In a similar way, syringaldehyde, syringic acid and sinapaldehyde were considered to be originated from syringyl (S) units of lignin (Tsubaki, Ozaki, & Azuma, 2010). p-Hydroxybenzaldehyde, p-hydroxyacetophenone, p-hydroxybenzoic acid, cinnamic acid and p-coumaric acid are probably recovered as decomposed materials of catechins and lignin as well as phenolic acids naturally existing in tea leaves.

Pyrogallol and catechol were found rich in the extracted liquor of tea residues at 230 °C, especially in green tea residue. These compounds, together with gallic acid and protocatechuic acid,

were considered as decomposed materials of catechins. Comparing with the phenolic compounds extracted by physicochemical treatment from other herbal biomass (Cheng, Ye, He, & Liu, 2009) or woody materials (Castro et al., 2008; Conde et al., 2009; Esteves et al., 2008; Takada, Ehara, & Saka, 2004; Tsubaki et al., 2010), abundances in pyrogallol and catechol are characteristic for catechin rich green tea residues. Pyrogallol and catechol posses high antioxidant activity (Thavasi, Bettens, & Leong, 2009), thus, they are usable as bio-based antioxidants in easily-oxidisable chemical products such as biodiesel (Tang, Wang, Salley, & Ng, 2008).

Indeed, oolong tea is a half fermented tea (Graham, 1992). Results in this study revealed that extracted liquor at 230 °C contained G unit derived compounds such as dihydroconiferyl alcohol, vanillin and vanillic acid as the most abundant phenolic compounds. These G unit derivatives were also contained in extracted liquors even at 110 °C, indicating that lignin was slightly decomposed in the course of fermentation process during oolong tea production.

Black tea is a fully oxidised tea, containing catechins as well as theaflavins which are polymerised compounds of catechin (Graham, 1992). The phenolic compounds obtained by heating at 230 °C were a mixture of catechin derivatives and lignin derivatives. Contrary to the oolong tea residues, lignin derivatives were not detected at low temperature heating at 110 °C, showing less progress in lignin degradation occurred in the production process of black tea than oolong tea. The amount of pyrogallol extracted from oolong and black tea residues were much lower than green tea residue, indicating that the pyrogallol moiety in the catechin structure was modified by the oxidation process of tea fermentation.

5-Hydroxymethylfurfural, a typical secondary degraded material from hexoses, was present in high levels in extracted liquors from oolong and black tea residues. Although the extraction efficiencies of polysaccharides were higher in oolong and black tea residues than in green tea residue (Tsubaki et al., 2008), the extracted polysaccharides suffered further degradation to 5-hydroxymethylfurfural by heating above 200 °C. Mono- and di-carboxylic acids were also found in the extracted liquor from tea residues. Fatty acids were also extracted in the methanol-soluble fraction of hot-compressed water treated switchgrass, and they are estimated to be originated from wax and lipids in the leaves (Cheng et al., 2009).

In this study, we have elucidated the constituents of phenolic compounds obtained after MAE under autohydrolytic conditions. Phenolic compounds from plant biomass are attractive renewable resources for chemical feedstock to produce phenolic precursors, polymer substitution, carbon fibre, glue and binders (Zhang, 2008) as well as natural antioxidants.

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