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ORIGINAL ARTICLE

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Composition of pyrolyzate from Japanese green tea

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Abstract Green tea is a beverage that is produced by hot-water extraction of the leaves of tea (*Camellia sinensis* L.). As the amount of green tea consumption has increased, the importance of using the residue has grown. To address this problem, chemical compositions of two pyrolyzates obtained with an electric kiln, L and G, which were obtained from the tea leaf product and its hot-water extraction residue, respectively, were examined. Both pyrolyzates were alkaline, in contrast to the acidic pHs that are typical of pyrolyzates from wood materials. The major constituents were nitrogen-containing compounds, which accounted for up to about 70% (based on gas chromatography-mass spectrometry peak area). Caffeine was the main compound in both samples, although it might not be a pyrolysis product but actually sublimates from the samples and then condenses back into the pyrolyzates. Other nitrogen-containing compounds were formed mainly from proteins and amino acids. Acids and phenols, which are typical components in pyrolyzates obtained from wood materials and contribute to acidic pH, were rare in both samples. Other neutral compounds are known as compounds in pyrolyzates from wood materials. The pyrolyzates obtained in this experiment were novel and may have new uses.

Key words Japanese green tea · Nitrogen-containing compounds · Caffeine · Alkalinity

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Introduction

Green tea is a traditional beverage obtained from the leaves of *Camellia sinensis* (L.) by hot-water extraction and is an important beverage in Japan. Recently, canned and bottled green tea have been produced commercially in industrial plants and the production amount has increased from 610 megaliters in 1998 to 1568 megaliters in 2002.[1] As the amount of green tea consumption has increased, the importance of using the residue has grown. However, no satisfactory usage has been found and hot-water extraction residues are discarded.

Plant pyrolyzates are obtained during the pyrolysis process of biomass like carbonization and are reported to have many biological activities. For example, they act as insecticides[2] and as repellents[3] toward insects. For plants, pyrolyzates promote or inhibit plant growth[4–7] and break seed dormancy.[8–12] In addition, they also have biological effects on microorganisms.[13,14] Pyrolyzates could be obtained from not only the useful parts of plants but also from the useless parts or agricultural waste. Hence, tea leaf residue might be a useful source of pyrolyzates.

In this study, the composition of pyrolyzate samples obtained from two types of tea leaf material, tea leaf product and its hot-water extraction residue, was examined for potential application.

Materials and methods

Plant materials

Two types of tea (*Camellia sinensis*) leaf materials, the tea leaf raw product itself and its hot-water extraction residue, were used in this experiment. The tea leaf raw product was not the raw tea leaves collected in a tea garden but was the product after processing in a tea factory by steaming and drying. The hot-water extraction residue was obtained from tea leaf product after hot-water extraction and drying. Both samples were supplied by Ito-en (Shizuoka, Japan).

Pyrolyzate production

The pyrolyzates were prepared by carbonization of plant materials in an electric kiln. About 200–300 g of plant materials were put into the retort (diameter 10.5 cm, depth 20 cm) and heated from room temperature to 500°C at 5°C/min and held until the end of smoke collection. The smoke released from plant materials was cooled and collected in a condenser. Smoke collection was started at the beginning of carbonization (120°C) and continued until the temperature had fallen to 80°C. After smoke collection, pyrolyzates were left to stand to separate into three layers, namely, a thin layer of oil at the top, a pyrolyzate layer in the middle, and sediment tar at the bottom. The middle layer, which is the main product and is transparent, was collected as the sample.

Pyrolyzates obtained from tea leaf product and its residue are called pyrolyzates L and G, respectively, in this report.

pH, density and concentration of soluble tar

pHs of pyrolyzates were measured with a pH meter (691pH meter, Metrohm, Switzerland). Specific gravity of pyrolyzates was measured with a pycnometer. The content of soluble tar was measured as follows. Three milliliters of pyrolyzate was put into an evaporating basin and held at 120°C until its weight became constant. The percentage of weight of residue against the volume of pyrolyzate was determined as the content of soluble tar.

Gas chromatography-mass spectrometry analysis

Pyrolyzate components were identified by gas chromatography-mass spectrometry (GC-MS) analysis. The GC was equipped with a TC-17 column (30 m × 0.25 mm) which was held at 40°C for 10 min and then heated from 40° to 270°C at 3°C/min and held at 270°C for 30 min, using helium as a carrier gas. Mass spectra were obtained at 70 eV. Each peak area obtained by GC-MS analysis was regarded as being proportional to the amounts of each compound, and the identity of each peak was confirmed by library searches (NIST12, NIST62, and SHIM1607) and comparisons with authentic samples.

Results and discussion

Yield, pH, density and content of soluble tar

The yield of pyrolyzates from tea leaf product and its hot-water extraction residue were 0.10 ml/g (L) and 0.06 ml/g (G) based on dry matter, respectively. Generally, yields of pyrolyzates from wood materials range from about 10% to 20%,[15] so the yields of L and G in this experiment are lower than wood pyrolyzates.

The most significant result is that both L and G showed alkalinity and the pH values of L and G were 9.26 and 9.13, respectively. Generally, the pH values of pyrolyzates from wood are acidic. Therefore, the pyrolyzates obtained in this experiment having showed alkaline pH are novel and may have new uses. For example, wood pyrolyzates are used for agricultural purposes, but cannot be applied with alkaline agricultural chemicals, because acidic wood pyrolyzates would neutralize the alkaline agricultural chemicals and reduce the efficacy of both chemicals. Pyrolyzates obtained in this experiment would show no neutralization effects with alkaline reagents and therefore may be one new application for these novel materials.

The densities of L and G were 1.067 and 1.062 g/ml, respectively, which are higher than those of typical pyrolyzates from wood, which range from about 1.000 to 1.040 g/ml.[15] One major cause of this result might be the difference in the content of soluble tar. Both samples obtained in this experiment were rich in soluble tar and the content of soluble tar was 8.2% (w/v) (L) and 14.9% (w/v) (G), which are higher than the content of soluble tar in pyrolyzate from woods (trace amounts to about 2.6%).[15]

Composition analysis with GC-MS analysis

Table 1 shows the results of pyrolyzate composition analysis with GC-MS. Compounds that covered about 85% of the total peak area were identified. Note that both L and G contained large amounts of nitrogen-containing compounds of over 70%. Acids and phenols, which are typical constituents of pyrolyzates obtained from wood and contribute to acidic pH values were rare in both samples. This composition is responsible for the high pH of pyrolyzates.

Nitrogen-containing compounds such as nitriles, amines, pyrroles, pyridines, indole, and caffeine were detected in pyrolyzates, although pyrazines were not identified. Both L and G samples contained propannitrile, pyrrole, and caffeine in high concentrations, and 2-methyl-1H-pyrrole, pyridine, and 2-pyrrolidinone were also formed in G. The most abundant compound was caffeine and its content was up to 61.8% (L) and 36.3% (G) (based on peak area %). Caffeine is one of the major compounds in tea leaf[16] and its content ranges from about 4% to 6% (on dry matter).[17] Caffeine is released from tea leaf by hot-water extraction and about 65% of caffeine is released in the first extraction.[18] Therefore, about 35% of caffeine remains in the hot-water extraction residue and the concentration of caffeine in the residue is lower than that of tea leaves before hot-water extraction. Pyrolyzates, L, and G also kept the same relationship. Caffeine tends to sublimate[19] and its content decreases during roasting.[17] Hence, caffeine detected in both pyrolyzates might not have been formed during the pyrolytic treatment of leaf tissue but may have sublimated from leaf tissue during pyrolysis and then condensed in the pyrolyzates when the smoke was cooled in the condenser. There are no reports on the action of caffeine molecules during the pyrolytic process, and further study is required to clarify this matter.

N-Methylene-ethenamine and three nitriles, propane nitrile, 1-methyl-pentanedinitrile and 2-methylene-butanenitrile, were detected in the pyrolysis experiments. Nitriles were formed in two stages of thermal degradation

Table 1. Composition of pyrolyzates obtained from two tea leaf materials

	L	G
Nitrogen-containing compounds		
Propanenitrile	2.74	6.2
1-Methyl-pentanedinitrile	0.06	0.35
N-Methylene-ethenamine	0.1	0.55
Pyrrole	3.58	14.68
Pyridine	0.3	1.29
2-Methylene-butanenitrile	0.14	0.91
2-Methyl-1H-pyrrole	0.93	1.88
2-Methyl-pyridine	–	0.51
2-Pyridinamine	–	0.02
2,3-Dimethyl-1H-pyrrole	0.31	0.78
2-Pyrrolidinone	0.82	1.41
Indole	0.44	0.61
Caffeine	61.83	36.27
Total nitrogen compounds	71.25	65.46
Acids		
Acetic acid	0.67	0.85
Propanoic acid	0.34	0.1
Total acids	1.01	0.95
Phenols		
Phenol	6.58	5.06
o-Cresol (2-methyl-phenol)	0.74	0.54
m-Cresol (3-methyl-phenol)	1.57	2.88
Mequinol (4-methoxy-phenol)	0.45	0.92
2,6-Dimethylphenol	0.11	0.13
Hydroquinone (1,4-benzenediol)	1.68	–
Total phenols	11.13	9.53
Neutral compounds		
2-Propen-1-ol	0.14	1.47
2-Methyl-furan	0.02	0.04
Benzene	0.1	0.14
Toluene	0.19	1.75
Ethylbenzene	–	0.39
Cyclopentanone	0.1	–
2-Furanmethanol	0.96	2.66
2-Methyl-2-cyclopenten-1-one	0.27	1.06
1-(2-Furanyl)-ethanone	0.38	1.14
Total neutral compounds	2.16	8.65
Unidentified		
Total unidentified nitrogen compounds[a]	6.97	8.33
Total others	7.48	7.08
Total unidentified	14.45	15.41

Compositions expressed as percentages based on gas chromatography-mass spectrometry peak areas

L, pyrolyzate obtained from tea leaf product; G, pyrolyzate obtained from hot-water extraction residue

[a] Compounds with an odd number for the molecular ion peak

from protein via amines.[20] Amines present as part of the structures of proteins were released and nitriles were subsequently formed from them.

Pyrrole is a substructure of the porphyrin ring of chlorophyll *a*, and pyrrole derivatives were obtained when chlorophyll *a* was degraded with pyrolysis–gas chromatography.[21] Pyrroles are also formed during the pyrolysis of proline, hydroxy proline, glutamine and asparagine,[20] and hydrolyzable amino acids.[22] Pyrrolidinones are reported to be formed from γ-aminobutyric acid,[23] which is formed from amino acids under anaerobic conditions.[24] The content of pyrrole and its derivatives in G was about 17% and was higher than that in L. The high content of pyrroles in G might be caused by the increase of relative content of compounds that release pyrroles after hot-water extraction and the decrease of other water-soluble compounds in tea leaves. For example, chlorophyll *a* is hydrophobic, and hence hot-water extraction might increase the relative concentration of chlorophyll *a* in the tea residue. On the contrary, the content of water-soluble compounds, like tannins, in leaves is decreased by water extraction. However, the increase of the content of pyrroles could not only be due to the reason mentioned above, because pyrroles formed from only chlorophyll would require the content over 80% of the component from leaf. Other compounds from which pyrroles are derived probably include those categorized as unidentified nitrogen-containing compounds.

Guillen and Manzanos[25] obtained pyridine derivatives in pyrolyzate of the aerial part of sage (*Salvia lavandulifolia*) after pyrolysis at 338°C. Viani and Horman[26] suggested these compounds were formed from the alkaloid trigoneline. Pyridines were also formed during the decomposition of alanine[27] and polypeptides.[28] Therefore pyridine and its derivatives might be formed from alkaloids and proteins in tea leaf tissue.

Indole formation during pyrolysis was reported to occur from the amino acid tryptophan.[20] Generally, it is clear that nitrogen compounds are mainly formed from proteins and amino acids.

Unidentified compounds in Table 1 with an odd number of molecular ion peaks were categorized as unidentified nitrogen-containing compounds, because compounds that contained an odd number of nitrogen atoms showed odd numbers of M^+ and those that contained an even number of nitrogen atoms showed even numbers of M^+.

Acids, which are one of the main compounds in wood pyrolyzates, are only present in small amounts in L and G. Pyrolyzates obtained from wood materials contain several types of acid and the total acid content of the total pyrolyzate present ranges from 10% to about 60%.[15] However, in this experiment, two acids, acetic acid and propanoic acid, were detected in only small amounts and their combined concentration was only about 1.0%. Generally, acids are formed from cellulose, hemicellulose, and lignin. Acetic acid is derived from acetyl groups during pyrolysis.[29–31] However, leaf materials contain smaller amounts of these groups than wood materials and contain proteins and alkaloids that release basic compounds such as amines and pyridine during pyrolysis. Hence, the relatively small amount of acetic acid formation and its neutralization with basic compounds that are formed simultaneously might cause the low total acid content in pyrolyzates and make pyrolyzates alkaline.

Six phenols, phenol, *o*-cresol, *m*-cresol, mequinol, 2,6-dimethyl phenol, and hydroquinone, were detected in pyrolyzates. All of these phenols and their content in L and G are similar to those in pyrolyzates obtained from wood materials. Phenols obtained during pyrolysis are formed from the pyrolysis of lignin. In addition to lignin, tea leaves contain catechins, which are another possible source of phenol. Catechins are reported to account for 11.4% of tea leaf.[32] Catechins contain ortho-substituted dihydroxylbenzene groups (catechols) in their molecule; however, after pyrolysis, catechol could not be detected in L and G in this experiment, because of changes to the catechol structure during pyrolysis.

Other compounds typical of pyrolyzates, such as alcohols, aromatics, and carbonyl compounds, are known to exist in pyrolyzates from wood materials. The content of neutral compounds was larger in G than in L and the content of neutral compounds in G was similar to that in wood materials.

When aromatic herbs were pyrolyzed, some compounds that contributed to the flavors of aromatic herbs, such as terpenes, were detected in smoke;[25,33] however, no flavoring compounds of tea were detected in the pyrolyzates in this study.

Conclusions

Pyrolyzates obtained from two types of tea leaf materials (tea leaf and hot-water extract residue) showed lower yields and higher soluble tar contents than wood pyrolyzate. Pyrolyzates showed alkaline pH in contrast to the acidic pH typical of wood pyrolyzates. The major constituents were nitrogen-containing compounds, which were present in concentrations of up to 70% and largely responsible for the high pHs of pyrolyzates. The main compound in both pyrolyzates was caffeine, although it might not be formed during pyrolysis but from sublimation and is condensed with the pyrolyzates. Other nitrogen-containing compounds originated mainly from proteins and amino acids in leaf tissue. Acids and phenols, which are known as typical components of wood pyrolyzates, were present in low concentration and this contributed to the high pHs of the samples. Other neutral compounds were not found to be specific to tea leaf materials. The pyrolyzates obtained in this experiment were novel and may lead to new uses for wood pyrolyzate.

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