JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

A Novel Convenient Process To Obtain a Raw Decaffeinated Tea Polyphenol Fraction Using a Lignocellulose Column

SENJI SAKANAKA*

School of Bioresources, Hiroshima Prefectural University, 562 Nanatsuka, Shobara-shi, Hiroshima 727-0023, Japan

Lignocellulose prepared from sawdust was investigated for its potential application in obtaining a raw decaffeinated tea polyphenol fraction from tea extract. Tea polyphenols having gallate residues, namely, (−)epigallocatechin gallate (EGCg) and (−)epicatechin gallate (ECg), were adsorbed on the lignocellulose column, while caffeine was passed through it. Adsorbed polyphenols were eluted with 60% ethanol, and the elute was found to consist mainly of EGCg and ECg. The caffeine/EGCg ratio was 0.696 before lignocellulose column treatment, but it became 0.004 after the column treatment. These results suggest that the lignocellulose column provides a useful and convenient process of purification of tea polyphenol fraction accompanied by decaffeination.

KEYWORDS: Lignocellulose; sawdust; tea polyphenols; caffeine; (−)epigallocatechin gallate

INTRODUCTION

Green tea is one of the most popular drinks as well as coffee and cocoa for its attractive flavor and taste. Recently, positive effects of green tea on human health have received valuable attention. These effects are often concerned with green tea polyphenols, which are mainly composed of several polyphenolic compounds, such as EGCg, ECg, GCg, EGC, EC, GC, and C (*1*). It has been reported that green tea polyphenols have various biological, physiological, and pharmaceutical effects, such as the prevention of dental caries (*2*), improvement of renal failure (*3*), improvement of intestinal microfrola (*4*), carcinogenesis inhibitory activity (*5*), antimicrobial activity (*6*), peroxynitrite-scavenging activity (*7*), antioxidative activity (*8*, *9*), antihypertensive action (*10*), and antihypercholesterolemic action (*11*).

Among the polyphenols, EGCg is reported to possess the most important action for health care. Along with the other components, tea extracts also contain a considerable amount of CA, which appears to be undesirable in several cases. CA stimulates the cerebral cortex to induce excitation in the central nervous system, and it also causes irritation of the gastrointestinal tract and sleeplessness for certain people (*12*).

There are several reports about the isolation methods of tea polyphenols (*13*), and crude tea polyphenols have been produced on an industrial scale and used commercially in various fields (*14*). Crude tea polyphenol fractions, however, also contain CA. The decaffeination of tea extract is usually carried out using chloroform or methylene chloride on an experimental scale. However, because of their toxicity, these solvents are in many cases not widely accepted by consumers. Decaffeination using polymerized cyclodextrin columns (*15*) or supercritical carbon dioxide extraction has been reported (*16*, *17*), but those methods need expensive equipment and do not have completely satisfactory results. It has therefore been necessary to study a novel approach to decaffeination by using a natural, nontoxic, and inexpensive ingredient.

On the other hand, there are many vegetable-based ingredients that are discarded. Sawdust is one of them, and it contains lignocellulose. Lignocellulose is a complex substance, and it is composed of a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin (*18*, *19*).

The sawdust (lignocellulose) column has been reported to be utilized for isolation and purification of some enzymes and proteins (*20*−*22*). There are also a few reports of the removal of heavy metals from aqueous solution by sawdust adsorption (*23*, *24*). However, there is no report of isolation and purification of tea polyphenols using the lignocellulose column. In this paper, the application of lignocellulose prepared from sawdust for isolation and purification of tea polyphenol fraction was discussed.

MATERIALS AND METHODS

Materials. Sawdust from North American cedar wood was obtained from a local lumber's workshop. Green tea was obtained from a commercial market. Crude tea polyphenols, EGCg, and ECg were prepared as described previously (*25*) (**Figure 1**). CA and other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). The cellulose used was powdered cellulose (KC Flock, Nippon Paper Industries, Tokyo, Japan).

Preparation of Tea Extracts. Tea extracts were prepared from dried, pulverized Japanese green tea (*Camellia sinensis*), by infusion with water (10% W/V) at 80 °C for 30 min. After the infusion was filtered with filter paper, extracts were subjected to purification and decaffeination of tea polyphenol fraction by the lignocellulose column.

Preparation of Lignocellulose. The sawdust was sifted to screen pieces of sizes between 18 and 32 mesh and dried under natural conditions, and then, they were suspended in 10 volumes of 0.1 N NaOH and kept for 3 days at room temperature. After they were washed,

* To whom correspondence should be addressed. Tel: +81-8247-4-1779. Fax: +81-8247-4-1779. E-mail: sakanaka@bio.hiroshima-pu.ac.jp.

10.1021/jf0208738 CCC: $25.00
Published on Web 03/07/2003

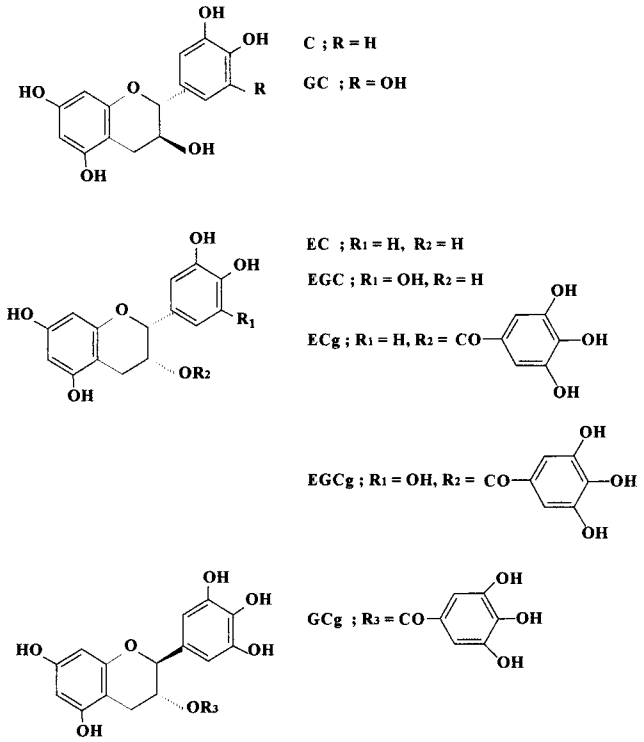

Figure 1. Chemical structures of tea polyphenols.

the procedure was repeated until no further brown color was released. The sawdust was washed thoroughly with water, then suspended in 10 volumes of 0.1 N HCl, and kept overnight at room temperature. The acid-treated sawdust was washed thoroughly with water, then suspended in 60% ethanol solution overnight at room temperature, and washed again with water. The washed sawdust was used as lignocellulose.

Chromatography and Analysis of Tea Extract. Lignocellulose suspended in water was subjected to pack the column. One gram of lignocellulose on a dry basis gave a packed volume of about 7 mL. This column was used as the lignocellulose column.

Tea polyphenols, EGCg, ECg, and CA were analyzed by HPLC using a Hitachi liquid chromatograph 655-A11 (Tokyo, Japan) equipped with and ODS-HG-5 column (150 mm × 4.5 mm, Nomura Chemical Co., Aichi, Japan) at a wavelength of 280 nm. The column was operated at room temperature. The mobile phase was acetic acid/acetonitrile/*N*,*N*-dimethylformamide/water (3:1:15:81, v/v/v/v) at a flow rate of 0.5 mL/min (*26*). Chromatographic peaks in the samples were identified by comparing their retention times with those of the reference standards. Working standard solutions were injected into the HPLC, and peak area responses were obtained. A standard graph of each component was prepared by plotting concentration vs area. Quantification was carried out from the integrated peak area of the sample against the corresponding standard graph. Each sample was analyzed in triplicate. The existence of the polyphenols and CA was also quantitatively detected using a Hitachi-2000 spectrophotometer at 280 nm.

Statistical Analysis. Values represent means of triplicate analysis and are given with standard deviations. Differences among experimental data were analyzed by Tukey's studentized range test (*27*), and those at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Separation and Decaffeination of Tea Polyphenols by Linear Gradient Elution from Lignocellulose Column. Tea extract (300 mL) was applied to the 100 mL lignocellulose column equipped with deionized water, and the column was washed with 1 L of deionized water. Tea polyphenols adsorbed on the lignocellulose were eluted with a linear gradient solution of water−ethanol and collected in 10 mL fractions (**Figure 2**). Data obtained from quantitative analyses by spectrophotometer

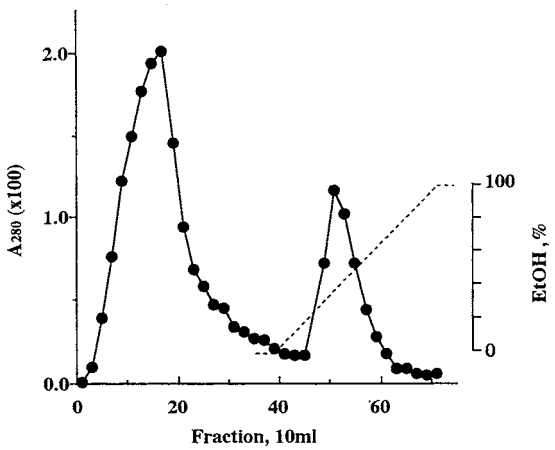

Figure 2. Chromatogram of tea polyphenols on lignocellulose column with aqueous ethanol by linear gradient elution. Column, 2.5 cm i.d. × 22 cm; flow rate, 6 mL/min. The adsorbed tea polyphenols were eluted with a linear gradient (- - -) of ethanol from 0 to 100%. ●, absorbance at 280 nm.

show that the elution of tea polyphenols started after the ethanol concentration exceeded 30%. **Figure 3** shows the comparison of the HPLC chromatograms of the initial tea extract and eluted fractions. Results indicate that CA, along with several other components of tea extracts, is mainly passing through the column, while those of the tea polyphenols with gallate residues, namely, EGCg, ECg, and GCg, are adsorbed on the lignocellulose column. Those compounds were eluted with an increasing concentration of ethanol.

Stepwise Elution of Tea Polyphenols from the Lignocellulose Column. The same lignocellulose column was reactivated by washing with deionized water, charged with 330 mL of tea extract, and washed with 1 L of water, and tea polyphenols adsorbed on the column were eluted with four kinds of solutions: 100 mL portions of ethanol with concentrations of 20, 40, 60, and 80%, respectively. The results shown in **Table 1** indicate that while a 60% concentration of ethanol is sufficient to extract almost all of the adsorbed EGCg, about 75% of the initially existing amount in the tea extract appears to be in the effluent. At the same time, the amount of CA in the effluent was only 0.4% of the initial. The ratio of CA/EGCg of the charged tea extract was 0.696, and that of the total effluent came to be 0.004. CA was removed well, and the tea polyphenols of the effluent consisted mainly of EGCg and ECg.

Efficiency of Cellulose Powder on the Separation of CA from the Tea Extract. The efficiency of cellulose powder was compared with that of the lignocellulose. Cellulose powder was packed in the column, and then, tea extract was charged with the column similarly to the lignocellulose column. Both CA and tea polyphenols were not adsorbed on the cellulose column and most of them passed through the column. The ratios of CA/EGCg were not significantly changed before and after column treatments (**Table 2**). Lignocellulose is a complex substance, and it is composed of a mixture of carbohydrate polymers (cellulose and hemicellulose) and lignin. Lignin is a complex, variable, hydrophobic, cross-linked, three-dimensional aromatic polymer of *p*-hydroxyphenylpropanoid units connected by C−C and C−O−C links (*19*). This suggests that the affinity of tea polyphenols with lignocellulose might be dependent on the lignin moisture in the lignocellulose prepared from sawdust.

Effect of pH on the Separation of CA and EGCg by the Lignocellulose Column. Tea polyphenols have been known to be stable at acidic conditions better than at neutral or alkali conditions (*28*, *29*). Experiments were carried out to study the

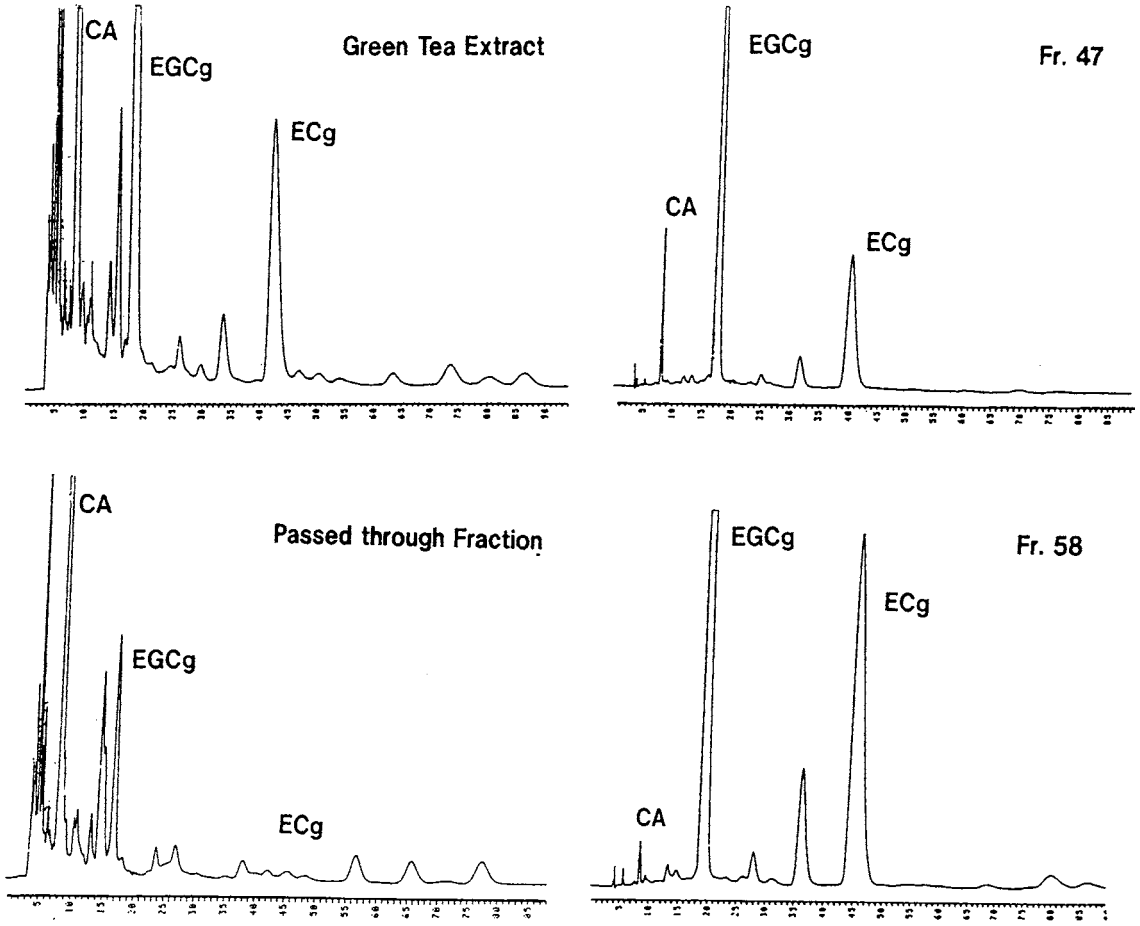

Figure 3. HPLC profiles of effluents from lignocellulose column. Green tea extract, passed through fraction, and effluents (fractions 47 and 58) from lignocellulose column shown in **Figure 2** were analyzed by the method described in the text.

Table 1. Stepwise Elution of the Absorbed CA and EGCg from the Lignocellulose Column by Different Concentrations of Ethanol[a]

	CA (mg)[b]	EGCg (mg)[b]	CA/EGCg
tea extract (330 mL)	291.4 ± 4.9	418.8 ± 7.4	0.696 ± 0.012
ethanol concentration			
20%	0.78 ± 0.03	54.2 ± 1.4	0.014 ± 0.001
40%	0.28 ± 0.02	190.0 ± 5.7	0.002 ± 0.001
60%	0.10 ± 0.07	58.4 ± 2.2	0.002 ± 0.001
80%	0.02 ± 0.02	9.6 ± 2.5	0.002 ± 0.002
total effluent	1.18 ± 0.11	312.2 ± 6.9	0.004 ± 0.001

[a] Lignocellulose column, 2.5 cm i.d. × 22 cm. Each value is expressed as mean ± standard deviation ($n = 3$). [b] CA and EGCg were analyzed by HPLC as described in the text.

Table 2. Elution of CA and EGCg from the Cellulose Column by 60% Ethanol[a]

	CA (mg)	EGCg (mg)	CA/EGCg
tea extract (300 mL)	264.9 ± 5.4	380.7 ± 10.0	0.696 ± 0.015
passed through and after washed liquid	246.4 ± 5.0	338.8 ± 7.1	0.727 ± 0.019
effluent with 60% ethanol	9.5 ± 0.5	30.5 ± 5.9	0.313 ± 0.043

[a] Each value is expressed as mean ± standard deviation ($n = 3$).

efficiency of the lignocellulose columns, in regard to the pH of the equilibrated column, tea extracts, and the washing water. The differences among the recoveries of EGCg could not be regarded as significant when the pH values were between 2.5 and 5.9. The recoveries of EGCg were 63.6−71.4% (**Table 3**). On the other hand, the recoveries of CA were less than 1% in all of the effluents. These results show that EGCg and CA can be clearly separated by the lignocellulose column in acidic conditions (pH 5.9−2.0).

Table 3. Effect of pH on the Separation of EGCg and CA by Lignocellulose Column[a]

	% from initial in effluent[b]	
pH	CA	EGCg
5.9	0.38 ± 0.12ac	63.6 ± 5.6a
5.5	0.40 ± 0.09ac	69.8 ± 6.2a
4.5	0.33 ± 0.14c	68.2 ± 4.9a
3.5	0.47 ± 0.07ac	69.1 ± 5.9a
3.0	0.94 ± 0.17b	71.4 ± 7.9a
2.5	0.70 ± 0.19ab	68.0 ± 6.5a

[a] Each effluent was eluted with 300 mL of 60% ethanol. Each value is expressed as mean ± standard deviation ($n = 3$). [b] Different alphabetical letters within a column are significantly different ($p < 0.05$).

Lignocellulose, which is the main constituent in wood, contains lignin, a complex hydrophobic compound. In this experiment, tea polyphenols were purified by using a lignocellulose column prepared from sawdust. Tea polyphenols were previously prepared by using various column treatments, such as reversed phase column chromatography or Sephadex gel chromatography (*30, 31*). Lignocellulose has never been used to prepare tea polyphenols, but the results in this experiment showed it could be used as a simple method of separating EGCg and CA. It is also important to mention that lignocellulose

columns can be easily reactivated and used more than several times. During our experiments, each of the columns was used six times, and the activity of the column did not decrease. Lignocellulose used in this experiment was a porous and pressure resistant grain, and it had little swelling and contraction, which took place by environmental change. The lignocellulose column did not have the fall of the flow rate during the experiment. These advantages of lignocellulose and the low price of sawdust make this technique promising for large-scale procedures.

In conclusion, the presented results suggest that the lignocellulose column is a useful process for the purification of tea polyphenols and it is also possible to obtain a raw decaffeinated tea polyphenol fraction. The lignocellulose column may be able to be used for separation and purification of various vegetable constituents.

ABBREVIATIONS USED

C, (+)catechin; EC, (−)epicatechin; GC, (+)gallocatechin; EGC, (−)epigallocatechin; ECg, (−)epicatechin gallate; EGCg, (−)epigallocatechin gallate; GCg, (−)gallocatechin gallate; CA, caffeine; EtOH, ethyl alcohol; HPLC, high-performance liquid chromatography.

ACKNOWLEDGMENT

I am grateful to Mr. T. Okubo (Taiyo Kagaku Co., Ltd.) for his helpful suggestions and Dr. T. Yamamoto for his valuable discussions on this study.

LITERATURE CITED

(1) Bokuchava, M. A.; Skobeleva, N. I. The biochemistry and technology of tea manufacture. *Crit. Rev. Food Sci. Nutr*. **1980**, 303−370.

(2) Sakanaka, S.; Shimura, N.; Aizawa, M.; Kim, M.; Yamamoto, T. Preventive effect of green tea polyphenols against dental caries in conventional rats. *Biosci., Biotechnol., Biochem*. **1992**, *56*, 592−594.

(3) Yokozawa, T.; Oura, H.; Shibata, T.; Ishida, K.; Kaneko, M.; Hasegawa, M.; Sakanaka, S.; Kim, M. Effects of green tea tannin in dialysis patients. *J. Tradit. Med.* **1996**, *13*, 124−131.

(4) Okubo, T.; Ishihara, N.; Oura, A.; Serit, M.; Kim, M.; Yamamoto, T.; Mitsuoka, T. In vitro effects of tea polyphenols intake on human intestinal microflora and metabolism. *Biosci., Biotechnol., Biochem.* **1992**, *56*, 588−591.

(5) Wang, Z. N.; Hong, j. Y.; Huang, M. T.; Reuhl, K. R.; Conney, A. H.; Yang, C. S. Inhibition of *N*-nitrosodiethylamine- and 4-(methyl-nitrosoamino)-1-(3-pyridyl)-1-butanone-induced tumorigensis in A/J mice by green tea. *Cancer Res.* **1992**, *52*, 1943−1947.

(6) Diker, K. S.; Hascelik, G. The bactericidal activity of tea against *Helicobacter pylori*. *Lett. Appl. Microbiol.* **1994**, *19*, 299−300.

(7) Chung, H. Y.; Yokozawa, T.; Soung, D. Y.; Kye, I. S.; No, J. K.; Baek, B. S. Peroxynitrite-scavenging activity of green tea tannin. *J. Agric. Food Chem*. **1998**, *46*, 4484−4486.

(8) Amarowicz, R.; Shahidi, F. Antioxidant activity of green tea catechins in β-carotene-linoleate model system. *J. Food Lipids* **1995**, *2*, 47−56.

(9) Unten, L.; Koketsu, M.; Kim, M. Antidiscoloring activity of green tea polyphenols on β-carotene. *J. Agric. Food Chem*. **1997**, *45*, 2009−2012.

(10) Yokozawa, T.; Oura, H.; Sakanaka, S.; Ishigaki, S.; Kim, M. Depressor effect of tannin in green tea on rats with renal hypertension. *Biosci., Biotechnol., Biochem*. **1994**, *58*, 855−858.

(11) Chisaka, T.; Matsuda, H.; Kubomura, Y.; Mochizuki, M.; Yamahara, J.; Fujimura, H. The effects of crude tea drugs on experimental hypercholesteremia: Mode of action of (−)-epigallocatechin gallate in tea leaves. *Chem. Pharm. Bull*. **1988**, *36*, 227−233.

(12) Nehlig, A.; Daval, J.-L.; Debry, G. Caffeine and the central nervous system: mechanism of action, biochemical, metabolic and psychostimulant effects. *Brain Res. Rev.* **1992**, 139−170.

(13) Finger, A.; Kuhr, S.; Engelhardt, U. H. Chromatography of tea constituents. *J. Chromatogr*. **1992**, *624*, 293−315.

(14) Hara, Y. Practical and industrial applications. *Green Tea*; Marcel Dekker: New York, 2001; pp 183−219.

(15) Yu, E. K. C. Novel decaffeination process using cyclodextrins. *Appl. Microbiol. Biotechnol.* **1988**, *28*, 546−552.

(16) Chang, C. J.; Chiu, K. L.; Chen, Y. L.; Yang, P. W. Effect of ethanol content on carbon dioxide extraction of polyphenols from tea. *J. Food Compos. Anal.* **2001**, *14*, 75−82.

(17) Ramalakshmi, K.; Raghavan, B. Caffeine in coffee: its removal. Why and how? *Crit. Rev. Food Sci. Nutr.* **1999**, *39*, 441−456.

(18) Lee, J. Biological conversion of lignocellulosic biomass to ethanol. *J. Biotechnol.* **1997**, *56*, 1−24.

(19) Adler, E. Lignin chemistry−past, present and future. *Wood Sci. Technol.* **1977**, *11*, 169−218.

(20) Fujimoto, K.; Ogawa, M.; Saito, N.; Kosaki, G.; Minamiura, N.; Yamamoto, T. A novel method of isolation and some characteristic properties of human pancreatic elastases. *Biochim. Biophys. Acta* **1980**, *612*, 262−267.

(21) Kobayashi, O.; Matsui, K.; Minamiura, N.; Yamamoto, T. Isolation of human urine urokinase by column chromatography on sawdust and some properties of the enzyme obtained. *J. Chromatogr*. **1981**, *210*, 180−185.

(22) Šafarik, I. Chromatography of trypsin on a sawdust column. *J. Chromatogr*. **1984**, *294*, 504−506.

(23) Yu, B.; Zhang, Y.; Shukla, A.; Shukla, S. S.; Dorris, K. L. The removal of heavy metal from aqueous solutions by sawdust adsorption−removal of copper. *J. Hazard. Mater*. **2000**, *80*, 33−42.

(24) Shukla, A.; Zhang, Y. H.; Dubey, P.; Margave, J. L.; Shukla, S. S. The role of sawdust in the removal of unwanted materials from water. *J. Hazard. Mater*. **2002**, *95*, 137−152.

(25) Sakanaka, S.; Kim, M.; Taniguchi, M.; Yamamoto, T. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. *Agric. Biol. Chem.* **1989**, *53*, 2307−2311.

(26) Hirose, S.; Tamada, S. A Quantitative determination of flavanols in tea by high-pressure liquid chromatography. (In Japanese) *Chagyo Kenkyu Hokoku* **1979**, *50*, 51−55.

(27) Nelson, P. R. In *Handbook of Statistical Methods for Engineers and Scientists: Design and Analysis of Experiments*; Wadsworth, H. M., Ed.; McGraw-Hill Publishing: New York, 1989; Chapter 14.

(28) Zhu, Q. Y.; Zhang, A.; Huang, Y.; Tsang, D.; Chen, Z. Y. Stability of green tea catechins. *J. Agric. Food Chem.* **1997**, *45*, 4624−4628.

(29) Ho, Y.; Lee, Y. L.; Hsu, K. Y. Determination of (+)-catechin in plasma by high-performance liquid chromatography using fluorescence detection. *J. Chromatogr. B* **1995**, *665*, 383−389.

(30) Nonaka, G.; Kawahara, O.; Nishioka, I. Tannins and related compounds. XV. A new class of dimmeric flavan-3-ol gallate, teasinensins A and B, and proanthocyanidin gallates from green tea leaf. *Chem. Pharm. Bull.* **1983**, *31*, 3906−3914.

(31) Amarowicz, R.; Shahidi, F. A rapid chromatographic method for separation of individual catechins from green tea. *Food Res. Int.* **1996**, *29*, 71−76.

Received for review August 9, 2002. Revised manuscript received December 30, 2002. Accepted January 22, 2003.

JF0208738