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# Two-Dimensional $^{1}H-^{13}C$ Nuclear Magnetic Resonance (NMR)-Based Comprehensive Analysis of Roasted Coffee Bean Extract

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Supporting Information

**ABSTRACT:** Coffee was characterized by proton and carbon nuclear magnetic resonance (NMR) spectroscopy. To identify the coffee components, a detailed and approximately 90% signal assignment was carried out using various two-dimensional NMR spectra and a spiking method, in which authentic compounds were added to the roasted coffee bean extract (RCBE) sample. A total of 24 coffee components, including 5 polysaccharide units, 3 stereoisomers of chlorogenic acids, and 2 stereoisomers of quinic acids, were identified with the NMR spectra of RCBE. On the basis of the signal assignment, state analyses were further launched for the metal ion−citrate complexes and caffeine−chlorogenate complexes. On the basis of the signal integration, the coffee components were successfully quantified. This NMR methodology yielded detailed information on RCBE using only a single observation and provides a systemic approach for the analysis of other complex mixtures.

**KEYWORDS:** NMR, assignment, coffee, complex mixture analysis, edited HSQC−TOCSY, CT-HMBC, relayed COSY, selective *J*-resolved HMBC

## INTRODUCTION

Coffee is one of the most consumed beverages in the world. The quality of the coffee beans used to make coffee is directly related to their chemical composition. The beans comprise carbohydrates, protein fragments, low-molecular-weight acids, caffeine, trigonelline, lipids, many unknown molecules, usually called melanoidins, and more than 800 volatile compounds mainly formed during the roasting process.[1]

Modern nuclear magnetic resonance (NMR) spectroscopy, with its dramatically improved resolution and sensitivity, has been widely applied in food science to achieve a direct and comprehensive observation of foods in a nondestructive way. However, most of these studies have been performed for the purpose of metabolomic analysis, and signal assignment studies have been few.[2−5] This may be in part because food is such a complex mixture, and thus, the NMR spectra tend to show complicated patterns because of signal overlaps and chemical-shift changes, resulting from the interactions among the various components, which creates a huge obstacle in NMR signal assignment. However, if the signal assignment could be accomplished, the qualitative and quantitative information could be obtained after only a single observation. In the past decade, NMR studies with signal assignments have been performed for several foods, such as milk, wine, vinegar, apple, tomato, and green coffee bean.[2−8] However, considering the enormous variety of foods, it is still necessary to develop a broadly applicable NMR approach for the quantitative and qualitative analysis of foods; such a method would be invaluable for application to food quality control.

The $^{1}H$ NMR signal assignment of roasted coffee bean extract (RCBE) has been reported in previous studies.[9,10] However, only a few simple components were assigned because of the complexity of the RCBE NMR spectra. Therefore, to chemically characterize the RCBE by NMR, we analyzed the spectra in detail by various two-dimensional (2D) observations.[11−16]

Using these techniques, we were able to obtain a detailed signal assignment of the high-resolution NMR spectra of RCBE, to quantitatively analyze the concentrations of coffee components, and to carry out an investigation of the state of the coffee components based on the unequivocal signal assignment. It can be expected that the enlargement of the NMR database of foods will be more and more important, because it will allow for rapid and nondestructive assessment of food quality, security, and nutrition.

## MATERIALS AND METHODS

**Materials and Sample Preparation.** The present experiments were performed using arabica coffee beans from Colombia, which were kindly supplied by Ajinomoto General Foods, Inc. (Tokyo, Japan). A 50 g sample of the green beans was roasted in a home coffee roaster (Hearthware Home Products, Inc., Gurnee, IL) for 7 min to achieve a typical level of roasting. The roasted coffee beans were ground into grains about 1−2 mm in size using a Kalita C-120 coffee mill (Kalita Co., Ltd., Tokyo, Japan), and 1.5 g of the crushed beans was incubated at 95 °C in a closed plastic tube with 3.50 mL of 99.7% $D_2O$ (Shoko Co., Ltd., Tokyo, Japan) for 1 h.[10,17] The extract was cooled on ice for 15 min and was then centrifuged. The pH value was checked, and the supernatant was introduced to a 5 mm NMR tube. A trace amount of 2,2-dimethyl-2-silapentane-5-sulfonic acid, sodium salt (DSS; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as an internal reference, and its chemical shift was set to 0 ppm. For an accurate observation of citric acid

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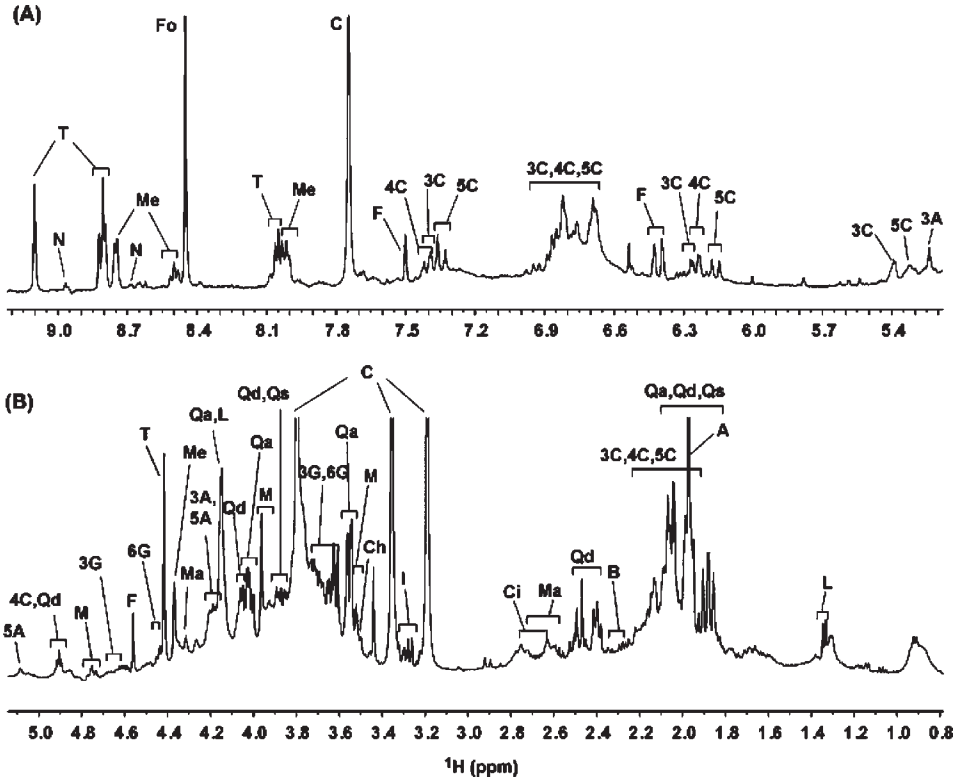


**Figure 1.** Assignment of 1D NMR spectra of RCBE. (A) Expansion of the $^{1}$H NMR spectrum from 5.2 to 9.2 ppm. (B) Expansion of the $^{1}$H NMR spectrum from 0.8 to 5.1 ppm.

and malic acid, whose signals were broad and difficult to detect by 2D NMR spectra, ethylenediaminetetraacetic acid (EDTA; Dojindo, Kumamoto, Japan) was added to the supernatant to capture the paramagnetic ions that cause the signal broadening to a final concentration of 1.5 mM prior to the measurement of 2D NMR spectra. The pH values were checked before and after the addition of EDTA and were both 5.3. For quantitative measurements, copper sulfate (Wako Pure Chemical Industries, Ltd.) was used as the relaxation reagent at a final concentration of 5 mM. Hexamethyldisiloxane (HMDS; Wako Pure Chemical Industries, Ltd.) sealed in a capillary was used as a concentration standard, and the concentration was determined according to the previously described method.[5] The extraction of coffee was performed in triplicate, and each extract of the triplicate was quantified. Statistical analysis was performed to confirm the reproducibility. Authentic compounds of formic acid, acetic acid, and nicotinic acid (Wako Pure Chemical Industries, Ltd.) were used to detect these components in coffee extract.

**NMR Spectroscopy.** NMR experiments were performed at 20 °C on a Varian Unity INOVA-500 spectrometer for the $^{1}$H, $^{13}$C, and $^{1}$H−$^{1}$H double-quantum-filtered correlation spectroscopy (DQF-COSY), $^{1}$H−$^{1}$H multiple-step-relayed COSY, $^{1}$H−$^{1}$H rotating frame Overhauser effect spectroscopy (ROESY), $^{1}$H−$^{13}$C edited heteronuclear single-quantum coherence (HSQC), $^{1}$H−$^{13}$C edited heteronuclear single-quantum coherence−total correlation spectroscopy (HSQC−TOCSY), $^{1}$H−$^{13}$C nondecoupling HSQC, and $^{1}$H−$^{13}$C selective *J*-resolved heteronuclear multiple-bond correlation (HMBC)[18,19] spectra and a Varian Unity INOVA-600 spectrometer equipped with a cryogenic probe for the $^{1}$H−$^{13}$C constant-time heteronuclear multiple-bond correlation (CT-HMBC) spectrum. The parameters of each NMR experiment are available in section 1 of the Supporting Information.

**Assignment of NMR Signals.** The free induction decay (FID) NMR data were processed by the program MestRe Nova (version 5.3.0; MestReC, Santiago de Compostela, Spain).

The signals in 1D $^{1}$H and $^{13}$C NMR spectra were tentatively assigned and then verified using different types of 2D NMR spectra as follows. The spin systems were confirmed by the $^{1}$H−$^{1}$H DQF-COSY spectrum. The $^{1}$H−$^{13}$C CT-HMBC spectrum was used to confirm the connections of quaternary carbons to protons through two- or three-bond couplings. The $^{1}$H−$^{13}$C edited HSQC spectrum was used to find the correlations between protons and their neighboring carbons.

When signals were extremely overlapped even in 2D $^{1}$H−$^{1}$H DQF-COSY, $^{1}$H−$^{13}$C edited HSQC, and $^{1}$H−$^{13}$C CT-HMBC spectra, a well-separated $^{1}$H signal was chosen as the target signal and the correlations were pursued from the target signals by observing $^{1}$H−$^{1}$H relayed COSY, $^{1}$H−$^{1}$H double-relayed COSY, $^{1}$H−$^{1}$H triple-relayed COSY, $^{1}$H−$^{13}$C edited HSQC−TOCSY, $^{1}$H−$^{13}$C nondecoupling HSQC, and $^{1}$H−$^{1}$H ROESY spectra.

For assignment of the signals of trace components, whose 2D signals were generally weak or undetectable, authentic compounds were added to the RCBE sample to confirm the existence of the trace components.

Finally, the assignments were compared to the published data.[9,10,20−28]

Detailed information of the signal assignments is available in section 2 of the Supporting Information.

**Quantification of RCBE Components.** The quantitative analysis of RCBE was performed with the 1D $^{13}$C NMR spectrum according to the previously described method.[5] The inverse-gated decoupling method without nuclear Overhauser effects (NOEs) was used, and copper sulfate was used as the relaxation reagent. Detailed information is available in section 3 of the Supporting Information.

## RESULTS AND DISCUSSION

We observed the 1D and 2D NMR spectra of RCBE as a complex mixture and carried out the detailed signal assignment to detect the coffee components.

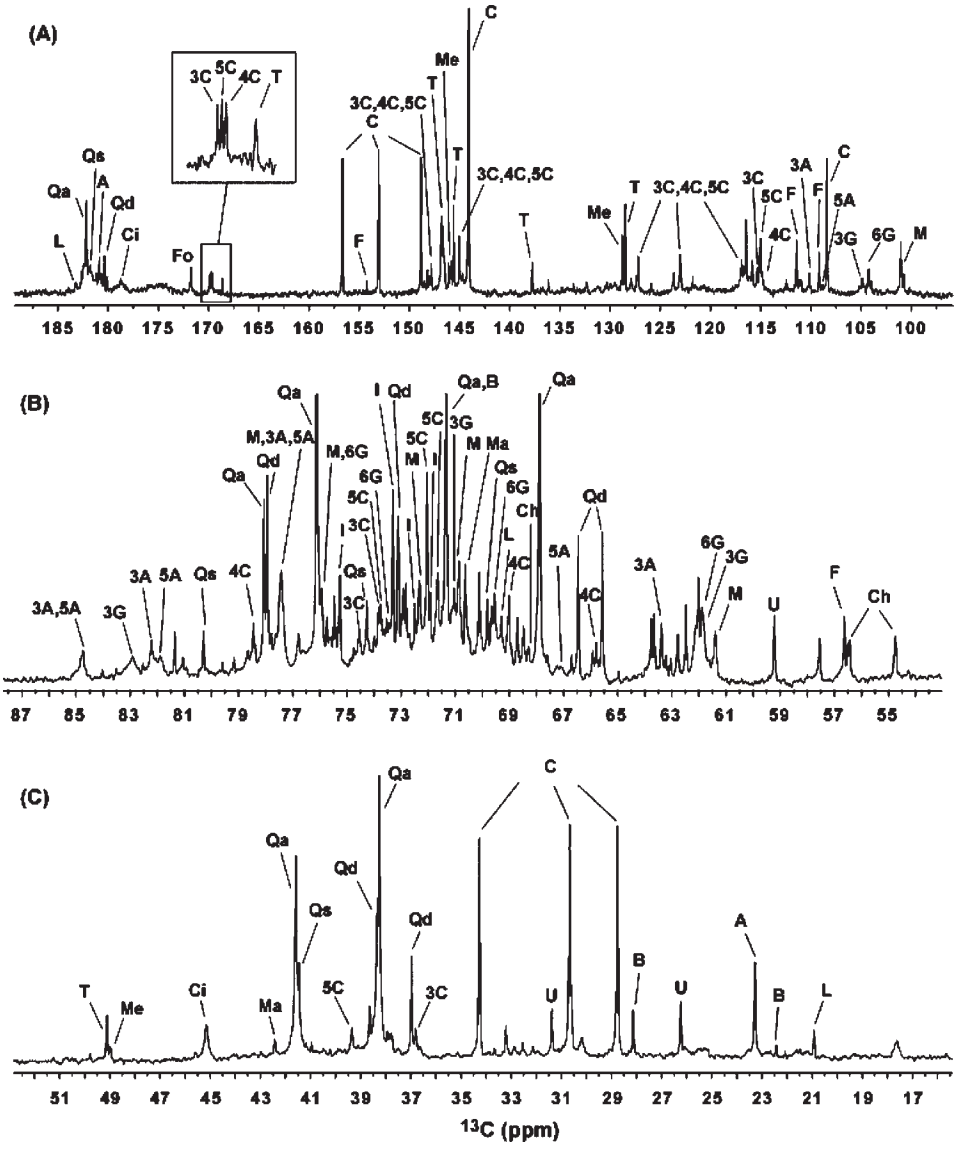


**Figure 2.** (A) Expansion the $^{13}$C NMR spectrum of RCBE from 100 to 185 ppm. (B) Expansion of the $^{13}$C NMR spectrum from 54 to 87 ppm. (C) Expansion of the $^{13}$C NMR spectrum from 17 to 51 ppm.

**Detection of Compounds in RCBE.** The identification of compounds in coffee extract was carried out by assigning 2D NMR correlations or using the spiking method. A total of 21 components, including 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA), 5-caffeoylquinic acid (5-CQA), quinic acid, $\gamma$-quinide, *syllo*-quinic acid, α-(1−3)-L-arabinofuranose, α-(1−5)-L-arabinofuranose, $\beta$-(1−3)-D-galactopyranose, $\beta$-(1−6)-D-galactopyranose, $\beta$-(1−4)-D-mannopyranose, acetic acid, $\gamma$-butyrolactone, caffeine, choline, citric acid, 2-furylmethanol, formic acid, *myo*-inositol, lactic acid, malic acid, *N*-methylpyridinium, nicotinic acid, and trigonelline, were detected. Detailed information on the signal assignments is available in section 2 of the Supporting Information.

**Signal Assignment: $^{1}$H and $^{13}$C NMR Spectrum of RCBE.** The assignment of $^{1}$H and $^{13}$C NMR signals arising from the coffee components is shown in Figures 1 and 2; the NMR data are summarized in parts A−C of Table 1.

As shown in Figure 1A, the low-field region between 5.2 and 9.2 ppm was assigned to signals arising from the aromatic ring parts of caffeine, trigonelline, *N*-methylpyridinium, 2-furylmethanol, nicotinic acid, and CQAs, signals arising from olefinic protons of CQAs, and signals arising from formic acid. This region is symptomatic of aromatic ring compounds, and many other less intense resonances can be clearly seen within it.

The high-field region between 0.8 and 5.1 ppm is shown in Figure 1B. The signals between 1.2 and 3.0 ppm were assigned to the protons arising from aliphatic groups of organic acids or their esters. The signals between 3.0 and 5.1 ppm were assigned to the $^{1}$H signals arising from aliphatic groups with a covalent binding to the oxygen atom of saccharides, choline, *myo*-inositol, 2-furylmethanol, lactic acid, malate, quinic acid, *syllo*-quinic acid, $\gamma$-quinide, CQAs, and $\gamma$-butyrolactone or arising from methyl groups with a covalent binding to the nitrogen atom of choline, caffeine, trigonelline, and *N*-methylpyridinium. In this region, the signals are very broad and heavily overlapped and there are still a few additional anomeric signals originating from minor polysaccharides or oligosaccharides, which are difficult to assign because of the strong signal overlap in both the 1D and 2D

**Table 1. Assignment of the $^{1}H$ and $^{13}C$ Signals of Compounds in Coffee: (A) Chlorogenic Acids, Quinic Acids, and Quinide, (B) Carbohydrates, and (C) Other Compounds**

| (A) | compound[a] | | | | | |
|---|---|---|---|---|---|---|
| number[b] | 3-CQA (3C) | 4-CQA (4C) | 5-CQA (5C) | quinic acid (Qa) | γ-quinide (Qd) | *syllo*-quinic acid (Qs) |
| 1 | 76.67 | 77.44 | 77.81 | 78.08 | 73.12 | 74.28 |
| 2 | 36.83[c] [2.22a/2.07e] | 38.12 [2.18a/2.04e] | 38.30 [2.15a/2.05e] | 38.26[b,c] [2.07a/1.98e] | 38.35[c] [1.95a/2.14e] | 40.46[c] [1.88a/2.09e] |
| 3 | 73.85 [5.40] | 69.01 [4.36] | 71.66 [4.25] | 71.35 [4.15] | 66.46 [3.87] | 69.82 [3.84] |
| 4 | 74.55 [3.77] | 78.47 [4.92] | 73.77 [3.88] | 76.12 [3.57] | 65.57 [4.18] | 80.30 [3.32] |
| 5 | 67.99 [4.19] | 65.80 [4.30] | 72.04 [5.32] | 67.89 [4.03] | 77.95 [4.91] | 69.82 [3.84] |
| 6 | 41.46 [1.92a/2.07e] | 41.46[c] [2.04a/2.17e] | 39.36[c] [2.04a/2.21e] | 41.59 [1.88a/2.07e] | 36.98 [2.49a/2.41e] | 41.46 [1.88a/2.09e] |
| 7 | 181.83 | 181.83 | 182.09 | 182.22 | 180.39 | 181.81 |
| 1′ | 169.89 | 169.61 | 169.76 | | | |
| 2′ | 115.38 [6.26] | 114.87 [6.25] | 115.03 [6.17] | | | |
| 3′ | 146.89 [7.39] | 146.68 [7.42] | 146.58 [7.36] | | | |
| 4′ | 127.22 | 127.22 | 127.22 | | | |
| 5′ | 123.04 [6.90] | 123.04 [6.90] | 123.04 [6.90] | | | |
| 6′ | 116.91 [6.78] | 116.91 [6.78] | 116.91 [6.78] | | | |
| 7′ | 147.84 | 147.84 | 147.84 | | | |
| 8′ | 145.04 | 145.04 | 145.04 | | | |
| 9′ | 115.03 [6.86] | 115.03 [6.86] | 115.03 [6.86] | | | |

| (B) | compound | | | | |
|---|---|---|---|---|---|
| number | α-(1−3)-L-arabino-furanose unit (3A) | α-(1−5)-L-arabino-furanose unit (5A) | β-(1−4)-D-manno-pyranose unit (M) | β-(1−3)-D-galacto-pyranose unit (3G) | β-(1−6)-D-galacto-pyranose unit (6G) |
| 1 | 110.17[c] [5.25] | 108.88[c] [5.09] | 101.06[c] [4.74] | 104.91[c] [4.67] | 104.25[c] [4.43] |
| 2 | 82.22 [4.22] | 81.90 [4.12] | 70.87 [4.12] | 71.06 [3.78] | 71.74 [3.55] |
| 3 | 77.44 [3.95] | 77.44 [3.94] | 72.33 [3.81] | 82.93 [3.84] | 73.55 [3.65] |
| 4 | 84.76 [4.13] | 84.76 [4.07] | 77.44 [3.93] | 69.30 [4.21] | 69.55 [3.92] |
| 5 | 63.39 [3.65−3.73] | 67.14 [3.77/3.90] | 75.96 [3.55] | 76.12 [3.71] | 75.96 [3.69] |
| 6 | | | 61.41 [3.91/3.75] | 61.86 [3.70/3.65] | 61.90 [3.78/3.73] |

| (C) | compound | | | | | | |
|---|---|---|---|---|---|---|---|
| number | acetic acid (A) | γ-butyro-lactone (B) | caffeine (C) | choline (Ch) | citric acid[d] (Ci) | 2-furyl-methanol (F) | formic acid (Fo) |
| 1 | 23.28[c] [1.97] | 178.00 | 28.77[c] [3.20] | 68.30 [3.50] | 181.69 | 56.64[c] [4.56] | 171.76 [8.46] |
| 2 | 180.90 | 28.14 [2.59] | 30.67 [3.37] | 56.44[c] [4.05] | 178.79 | 154.28 | |
| 3 | | 22.47[c] | 34.27 | 54.77 | 76.12 | 109.19 | |


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**Table 1. Continued**

| (C) number | compound | | | | | | |
|---|---|---|---|---|---|---|---|
| | acetic acid (A) | γ-butyro-lactone (B) | caffeine (C) | choline (Ch) | citric acid[d] (Ci) | 2-furyl-methanol (F) | formic acid (Fo) |
| | [2.29] | [3.81] | [3.19] | | [6.39] | | |
| 4 | | 71.35 | 153.08 | 54.77 | 45.16[c] | 111.43 | |
| | | [4.44] | | [3.19] | [2.61/2.74] | [6.43] | |
| 5 | | | 148.84 | 54.77 | 45.16[c] | 144.11 | |
| | | | | [3.19] | [2.61/2.74] | [7.50] | |
| 6 | | | 108.43 | | 181.69 | | |
| 7 | | | 156.69 | | | | |
| 8 | | | 144.11 | | | | |
| | | | [7.76] | | | | |

| (C) number | compound | | | | | |
|---|---|---|---|---|---|---|
| | *myo*-inositol (I) | lactic acid (L) | malic acid[d] (Ma) | *N*-methyl-pyridinium (Me) | nicotinic acid (N) | trigonelline (T) |
| 1 | 75.27[c] | 183.67 | 181.51 | 146.13 | | 49.12[c] |
| | [3.28] | | | [8.51] | [8.68][e] | [4.42] |
| 2 | 71.93 | 69.30 | 179.82 | 145.89 | | 146.83 |
| | [3.52] | [4.13] | | [8.75] | [7.74][e] | [8.80] |
| 3 | 73.31 | 20.95[c] | 70.65 | 128.81 | | 128.52 |
| | [3.62] | [1.34] | [4.33] | [8.02] | [8.55][e] | [8.05] |
| 4 | 72.50 | | 42.46[c] | 49.00[c] | | 145.62 |
| | [4.06] | | [2.45/2.72] | [4.37] | [8.95][e] | [8.81] |
| 5 | 73.31 | | | 128.81 | | 137.78 |
| | [3.62] | | | [8.02] | | |
| 6 | 71.93 | | | 145.89 | | 146.69 |
| | [3.52] | | | [8.75] | | [9.11] |
| 7 | | | | | | 168.64 |

[a] For each compound, the abbreviation used in the present study is given in parentheses. Chemical shifts (in ppm) of $^{1}H$ are given in brackets under their directly bound $^{13}C$. [b] Corresponding to the atom numbers in Figure S-2 in the Supporting Information. [c] The carbon signals chosen for quantitative analysis. [d] Assignment accomplished in the presence of EDTA. [e] The proton signals assigned by the $^{1}H-^{1}H$ DQF-COSY spectrum and the addition of authentic compounds into the coffee.

NMR spectra. The reason for this may be that the molecular weights of polysaccharides or oligosaccharides are so large that their signals would be very broad and low and, thus, difficult to detect in the 2D NMR spectra.

The expansion of the $^{13}C$ NMR spectrum of RCBE and the assignment details are shown in Figure 2. The signals were narrow and less overlapped than those in the $^{1}H$ NMR spectrum.

The low-field region between 180 and 100 ppm is shown in Figure 2A. In the part between 100 and 115 ppm, signals were assigned to anomeric carbons in fragments of polysaccharides. There are a few undetermined signals between 103 and 107 ppm in this region, which can be considered to have arisen from other fragments of polysaccharides or glycoproteins that were destroyed during the coffee-roasting process.[29] It was difficult to assign all of the signals in this region, because the NMR signals arising from the fragments of high-molecular-weight components, which have similar chemical structures, are broad and overlapped in both the 1D and 2D NMR spectra. The region higher than 110 ppm was assigned to symptomatic unsaturated carbons in aromatic heterocyclic rings, aliphatic double-bond parts, and the carboxyl groups of coffee components.

The region between 87 and 54 ppm is shown in Figure 2B. The signals between 60 and 65 ppm were assigned to the carbons from methylene groups in the fragments of polysaccharides. In the region from 65 to 86 ppm, the $^{13}C$ signals show an extremely complex pattern and the base part of the signals is very broad. The signals in this region were mainly assigned to secondary or tertiary carbons in aliphatic rings that were joined by a single C−O bond. The quaternary carbons in aliphatic rings were also assigned in this region. One possible reason for the broad signals at the base part could be that they were due to high-molecular-weight components, such as polysaccharides, proteins, and Maillard reaction products.[24,25,30,31]

In Figure 2C, the signals in the region below 51 ppm are assigned to the primary carbons arising from $N-CH_3$ groups of caffeine, trigonelline, *N*-methylpyridinium, and choline, the primary carbons of acetic acid and lactic acid, and the secondary carbons of citric acid, malic acid, and aliphatic ring parts in CQA isomers, quinic acid, *syllo*-quinic acid, γ-quinide, and γ-butyrolactone.

**State Analysis of Coffee Components.** One of the advantages of NMR is that the observation of a complex mixture can be carried out without the separation of the components, and the interactions among some components in aqueous media can also be detected. As for coffee, information concerning the states of certain components in aqueous media was obtained by proton NMR spectra.

As shown in the top trace of Figure 3A, the signals because of citric acid and malic acid from the $^{1}H$ NMR spectrum appear

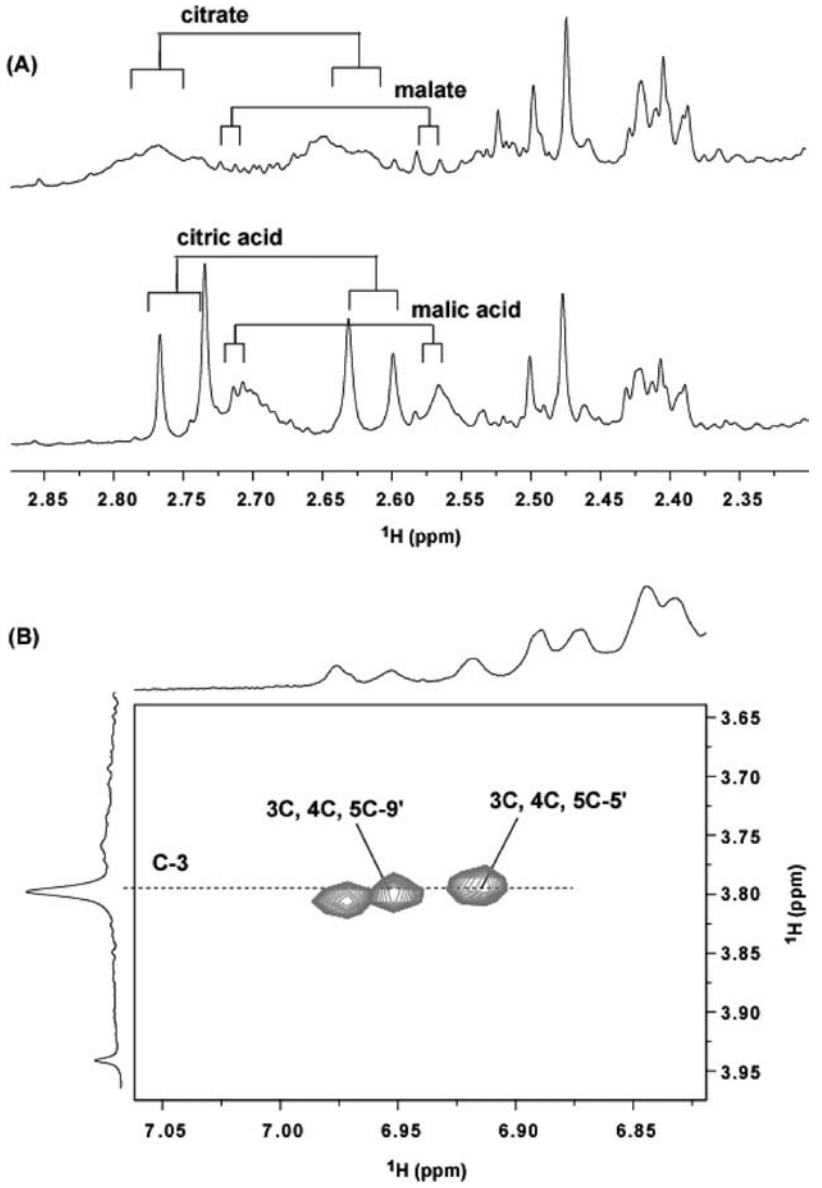


**Figure 3.** (A) Expansion of the 1D $^{1}$H NMR spectrum of coffee. (Top trace) $^{1}$H NMR spectrum of RCBE without EDTA. (Bottom trace) $^{1}$H NMR spectrum in the presence of EDTA. (B) Expansion of the $^{1}$H−$^{1}$H ROESY spectrum of RCBE.

much broader than the other signals; their line width is about 27−29 Hz, more than 7 times broader than the other signals. The explanation for this line broadening is considered to be paramagnetic relaxation of the metal−citrate/malate complexes. It is a weak complexation of citrate or malate carboxylic groups with paramagnetic metal ions ($Fe^{2+}$ and $Mn^{2+}$) present in coffee. The addition to the coffee mixture of EDTA, which is a strong cation-chelating reagent for metal ions, selectively reduces the citrate and malate line width, as shown in the bottom trace of Figure 3A. This indicates that some paramagnetic metal−citrate complexes are present in coffee.

As shown in Figure 3B, which is the expansion of the $^{1}$H−$^{1}$H ROESY spectrum, the $^{1}$H signal at 3.81 ppm (H1 of caffeine) showed the cross-peaks with 6.95 ppm (H9′ of CQAs) and 6.90 ppm (H5′ of CQAs), which indicated the intramolecular NOEs between caffeine and CQAs in the RCBE sample used in the present study. It has been reported that, in freshly prepared espresso coffee brews, caffeine and chlorogenic acids were emitted as a caffeine−chlorogenic acid complex.[32] In that study, the intramolecular NOEs were detected between caffeine-H1/5-CQA-H9′, caffeine-H2/5-CQA-H6′, caffeine-H3/5-CQA-H2′, and caffeine-H3/5-CQA-H9′, which was different from the findings in the present study. The reason for the discrepancy was likely that both caffeine and chlorogenic acids were at higher concentrations in espresso coffee than in regular arabica coffee brew,[33] which resulted in the different interaction modes and intensity.

**Concentrations of RCBE Components.** In the quantitative analysis of RCBE, the $^{1}$H NMR spectrum was not used because of its extreme overlapping of signals. As shown in Figure 4, the concentration of coffee components was analyzed by the 1D $^{13}$C NMR spectrum obtained by the addition of copper sulfate and the inverse-gated decoupling method. The concentrations of RCBE components are summarized in Table 2 and Figure 4,

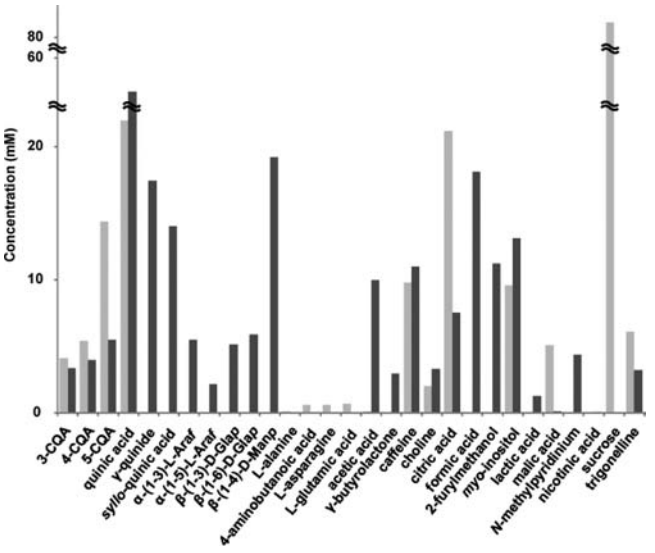


**Figure 4.** Concentrations of RCBE components as compared to those in GCBE. Gray bars indicate the concentrations in GCBE, and black bars indicate those in RCBE. α-(1−3)-L-Ara*f*, α-(1−5)-L-Ara*f*, *β*-(1−3)-D-Gal*p*, *β*-(1−6)-D-Gal*p*, and *β*-(1−4)-D-Man*p* indicate α-(1−3)-L-arabinofuranose, α-(1−5)-L-arabinofuranose, *β*-(1−3)-D-galactopyranose, *β*-(1−6)-D-galactopyranose, and *β*-(1−4)-D-mannopyranose, respectively.

together with the quantitative results of GCBE in the previous study.[5] One advantage of an assigned NMR spectrum is that it provides quantitative information of each assigned component in quite a obvious way when similar data are compared. In comparison to the results of the comprehensive analysis of green coffee bean extract (GCBE) by NMR,[5] both the 1D and 2D NMR spectra of RCBE became much more complicated after the process of coffee bean roasting.

As shown in both Table 2 and Figure 4, quinic acid is the dominant acid in RCBE and was also detected in GCBE. The increase of quinic acid in roasted beans is due to the decomposition of chlorogenic acids. Quinic acid is one of the most bitter-tasting components in coffee, along with its isomer, *syllo*-quinic acid, and its lactone, *γ*-quinide.[34,35] It has been reported that isomers and lactones of quinic acid can be obtained by heating a mixture of quinic acid, acetic acid, and $H_2SO_4$,[26] which means that the isomerization of quinic acid can happen during the heating process. The generation of *syllo*-quinic acid and *γ*-quinide must be related to the isomerization of quinic acid during the coffee-bean-roasting process. As for chlorogenic acids, each of 5-CQA, 4-CQA, and 3-CQA was degraded after roasting, and 5-CQA was still the most abundant chlorogenic acid in coffee extract, just as it is in GCBE.

Polysaccharides are present in RCBE. The water-soluble polysaccharides in coffee are said to be responsible for the creamy mouthfeel of coffee known as "body".[36] There were no NMR signals of polysaccharides appearing in the NMR spectra of GCBE. The reason for this finding could be that the polysaccharides in green coffee beans are mainly in the form of cellulose and glycoproteins, which in turn are in the form of insoluble polysaccharides.[24] During the roasting, the heating process destroys some of the cellulose or glycoproteins, and thereafter, the polysaccharides become water-soluble and observable by NMR spectroscopy.

Formic acid, acetic acid, and lactic acid form during roasting, while the amounts of citric acid and malic acid decrease after

**Table 2. Concentrations of Compounds in RCBE Quantified by NMR as Compared to Those in GCBE[a]**

| | concentration (mM)[b] | |
|---|---|---|
| compound | RCBE | GCBE[a] |
| 3-CQA | 3.37 ± 0.03 | 4.1 ± 0.1 |
| 4-CQA | 4.00 ± 0.03 | 5.4 ± 0.1 |
| 5-CQA | 5.50 ± 0.03 | 14.4 ± 0.1 |
| quinic acid | 57.32 ± 0.03 | 22.0 ± 0.1 |
| *γ*-quinide | 17.48 ± 0.10 | – |
| *syllo*-quinic acid | 14.03 ± 0.03 | – |
| α-(1−3)-L-arabinofuranose | 5.50 ± 0.05 | – |
| α-(1−5)-L-arabinofuranose | 2.16 ± 0.05 | – |
| *β*-(1−3)-D-galactopyranose | 5.12 ± 0.10 | – |
| *β*-(1−6)-D-galactopyranose | 5.87 ± 0.02 | – |
| *β*-(1−4)-D-mannopyranose | 19.23 ± 0.10 | – |
| L-alanine | – | 0.1 ± 0.0 |
| 4-aminobutanoic acid | – | 0.6 ± 0.0 |
| L-asparagine | – | 0.6 ± 0.0 |
| L-glutamic acid | – | 0.7 ± 0.0 |
| acetic acid | 9.99 ± 0.04 | 0.1 ± 0.0 |
| *γ*-butyrolactone | 2.96 ± 0.05 | – |
| caffeine | 11.05 ± 0.07 | 9.8 ± 0.1 |
| choline | 3.33 ± 0.01 | 2.0 ± 0.1 |
| citric acid | 7.53 ± 0.04 | 21.2 ± 0.1 |
| formic acid | 18.15 ± 0.03 | – |
| 2-furylmethanol | 11.25 ± 0.04 | – |
| *myo*-inositol | 13.13 ± 0.05 | 9.6 ± 0.3 |
| lactic acid | 1.26 ± 0.03 | – |
| malic acid | 0.10 ± 0.01 | 5.1 ± 0.1 |
| *N*-methylpyridinium | 4.38 ± 0.03 | – |
| nicotinic acid | 0.08 ± 0.04 | – |
| sucrose | – | 81.2 ± 0.1 |
| trigonelline | 3.21 ± 0.04 | 6.1 ± 0.0 |

[a] Values are quoted from ref 5. [b] Values are expressed by the average of three separate samples ± standard deviation ($n$ = 3), and "–" indicates the concentrations below the limit of detection.

roasting. A portion of trigonelline degrades into *N*-methylpyridinium and nicotinic acid.[21] In the present study, sucrose, which is a main component of green coffee beans, was reduced to only a trace amount that could not even be detected by the $^1$H NMR spectrum after roasting. It can be considered that the degradation of sucrose and Maillard reactions between sucrose and proteins in green coffee beans happens during the roasting process, the products of which were melanoidin complexes with high molecular weights that are difficult to detect by NMR.[30,31] The free amino acids in the extracts of green coffee bean disappear in the extracts of roasted coffee bean, perhaps because the free amino acids participate in the Maillard reactions with saccharides during roasting.[37] The abundance of caffeine was likely attributable to the water loss after roasting, which would have resulted in an abundance of each coffee component, even though a bean sample of equal mass was used for each extraction. In this study, components of RCBE at concentrations higher than 0.1 mM (nicotinic acid and malic acid) were quantified.

In conclusion, in this work, we accomplished the comprehensive identification, quantification, and state analysis of coffee

components by NMR spectroscopy without any separation as a complex mixture analysis. Unlike in the previous NMR studies of coffee,[9,10] the signals because of α-(1−3)-L-arabinofuranose, α-(1−5)-L-arabinofuranose, β-(1−3)-D-galactopyranose, β-(1−6)-D-galactopyranose, β-(1−4)-D-mannopyranose, 3-CQA, 4-CQA, γ-quinide, *syllo*-quinic acid, γ-butyrolactone, choline, 2-furylmethanol, *myo*-inositol, malic acid, and nicotinic acid were directly assigned from the NMR spectra of RCBE as a complex mixture. The methodology used here to analyze the NMR spectra of RCBE might also be considered applicable to the nondestructive analysis of foods as complex mixtures by NMR.

## ■ ASSOCIATED CONTENT

**Supporting Information.** Detailed descriptions of the NMR experiments, signal assignments, and quantification of RCBE. This material is available free of charge via the Internet at http://pubs.acs.org.

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## ■ REFERENCES

(1) Charles-Bernard, M.; Kraehenbuehl, K.; Rytz, A.; Roberts, D. D. Interactions between volatile and nonvolatile coffee components. 1. Screening of nonvolatile components. *J. Agric. Food Chem.* **2005**, *53*, 4417–4425.

(2) Caligiani, A.; Acquotti, D.; Palla, G.; Bocchi, V. Identification and quantification of the main organic components of vinegars by high resolution $^1$H NMR spectroscopy. *Anal. Chim. Acta* **2007**, *585*, 110–119.

(3) Sobolev, A. P.; Segre, A.; Lamanna, R. Proton high-field NMR study of tomato juice. *Magn. Reson. Chem.* **2003**, *41*, 237–245.

(4) Hu, F.; Furihata, K.; Ito-Ishida, M.; Kaminogawa, S.; Tanokura, M. Nondestructive observation of bovine milk by NMR spectroscopy: Analysis of existing states of compounds and detection of new compounds. *J. Agric. Food Chem.* **2004**, *52*, 4969–4674.

(5) Wei, F. F.; Furihata, K.; Hu, F. Y.; Miyakawa, T.; Tanokura, M. Complex mixture analysis of organic compounds in green coffee bean extract by two-dimensional NMR spectroscopy. *Magn. Reson. Chem.* **2010**, *48*, 857–865.

(6) Hu, F.; Furihata, K.; Kato, Y.; Tanokura, M. Nondestructive quantification of organic compounds in whole milk without pretreatment by two-dimensional NMR spectroscopy. *J. Agric. Food Chem.* **2007**, *55*, 4307–4311.

(7) Nilsson, M.; Duarte, I. F.; Almeida, C.; Delgadillo, I.; Goodfellow, B. J.; Gil, A. M.; Morris, G. A. High-resolution NMR and diffusion-ordered spectroscopy of port wine. *J. Agric. Food Chem.* **2004**, *52*, 3736–3743.

(8) Belton, P. S.; Delgadillo, I.; Gil, A. M.; Roma, P.; Casuscelli, F.; Colquhoun, I. J.; Dennis, M. J.; Spraul, M. High-field proton NMR studies of apple slices. *Magn. Reson. Chem.* **1997**, *35*, S52–S60.

(9) Bosco, M.; Toffanin, R.; de Palo, D.; Zatti, L.; Segre, A. High-resolution H-1 NMR investigation of coffee. *J. Sci. Food Agric.* **1999**, *79*, 869–878.

(10) Tavares, L. A.; Ferreira, A. G. Quali- and quantitative analysis of commercial coffee by NMR. *Quim. Nova* **2006**, *29*, 911–915.

(11) Parella, T.; Sanchez-Ferrando, F.; Virgili, A. Improved sensitivity in gradient-based 1D and 2D multiplicity-edited HSQC experiments. *J. Magn. Reson.* **1997**, *126*, 274–277.

(12) Furihata, K.; Seto, H. Constant time HMBC (CT-HMBC), a new HMBC technique useful for improving separation of cross peaks. *Tetrahedron Lett.* **1998**, *39*, 7337–7340.

(13) Homans, S. W.; Dwek, R. A.; Fernandes, D. L.; Rademacher, T. W. Multiple-step relayed correlation spectroscopy: Sequential resonance assignments in oligosaccharides. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 6286–6289.

(14) Bax, A.; Drobny, G. Optimization of 2-dimensional homonuclear relayed coherence transfer NMR-spectroscopy. *J. Magn. Reson.* **1985**, *61*, 306–320.

(15) Ikura, M.; Hikichi, K. Two-dimensional H-1-NMR studies of cello-oligosaccharides—The utility of multiple-relay chemical-shift-correlated spectroscopy. *Carbohydr. Res.* **1987**, *163*, 1–8.

(16) Crouch, R. C.; Spitzer, T. D.; Martin, G. E. Strategies for the phase editing of relayed responses in 2D HMQC−TOCSY spectra. *Magn. Reson. Chem.* **1992**, S71–S73.

(17) Muller, C.; Hofmann, T. Screening of raw coffee for thiol binding site precursors using "in bean" model roasting experiments. *J. Agric. Food Chem.* **2005**, *53*, 2623–2629.

(18) Furihata, K.; Seto, H. *J*-Resolved HMBC, a new NMR technique for measuring heteronuclear long-range coupling constants. *Tetrahedron Lett.* **1999**, *40*, 6271–6275.

(19) Furihata, K.; Tashiro, M.; Seto, H. Selective *J*-resolved HMBC, an efficient method for measuring heteronuclear long-range coupling constants. *Magn. Reson. Chem.* **2009**, *47*, 814–818.

(20) Somoza, V.; Lindenmeier, M.; Wenzel, E.; Frank, O.; Erbersdobler, H. F.; Hofmann, T. Activity-guided identification of a chemopreventive compound in coffee beverage using in vitro and in vivo techniques. *J. Agric. Food Chem.* **2003**, *51*, 6861–6869.

(21) Stadler, R. H.; Varga, N.; Hau, J.; Vera, F. A.; Welti, D. H. Alkylpyridiniums. 1. Formation in model systems via thermal degradation of trigonelline. *J. Agric. Food Chem.* **2002**, *50*, 1192–1199.

(22) Naidu, M. M.; Sulochanamma, G.; Sampathu, S. R.; Srinivas, P. Studies on extraction and antioxidant potential of green coffee. *Food Chem.* **2008**, *107*, 377–384.

(23) Iwai, K.; Kishimoto, N.; Kakino, Y.; Mochida, K.; Fujita, T. In vitro antioxidative effects and tyrosinase inhibitory activities of seven hydroxycinnamoyl derivatives in green coffee beans. *J. Agric. Food Chem.* **2004**, *52*, 4893–4898.

(24) Capek, P.; Matulova, M.; Navarini, L.; Suggi-Liverani, F. Structural features of an arabinogalactan-protein isolated from instant coffee powder of *Coffea arabica* beans. *Carbohydr. Polym.* **2010**, *80*, 180–185.

(25) Nunes, F. M.; Reis, A.; Silva, A. M.; Domingues, M. R.; Coimbra, M. A. Rhamnoarabinosyl and rhamnoarabinoarabinosyl side chains as structural features of coffee arabinogalactans. *Phytochemistry* **2008**, *69*, 1573–1585.

(26) Scholz-Böttcher, B. M.; Ernst, L.; Maier, H. G. New stereoisomers of quinic acid and their lactones. *Liebigs Ann. Chem.* **1991**, 1029–1036.

(27) Yamaji, T.; Saito, T.; Hayamizu, K.; Yanagisawa, M.; Yamamoto, O. *Integrated Spectral Data Base System for Organic Compounds*; Integrated Spectral Data Base System: Tokyo, Japan, 2001; http://riodb01.ibase.aist.go.jp/sdbs/ (accessed on July 25, 2010).

(28) Ulrich, E. L.; Akutsu, H.; Doreleijers, J. F.; Harano, Y.; Ioannidis, Y. E.; Lin, J.; Livny, M.; Mading, S.; Maziuk, D.; Miller, Z.; Nakatani, E.; Schulte, C. F.; Tolmie, D. E.; Wenger, R. K.; Yao, H.; Markley, J. L. BioMagResBank. *Nucleic Acids Res.* **2007**, *36*, D402–D408.

(29) Navarini, L.; Gilli, R.; Gombac, V.; Abatangelo, A.; Bosco, M.; Toffanin, R. Polysaccharides from hot water extracts of roasted *Coffea arabica* beans: Isolation and characterization. *Carbohydr. Polym.* **1999**, *40*, 71–81.

(30) Gniechwitz, D.; Reichardt, N.; Ralph, J.; Blaut, M.; Steinhart, H.; Bunzel, M. Isolation and characterisation of a coffee melanoidin fraction. *J. Sci. Food Agric.* **2008**, *88*, 2153–2160.

(31) Reichardt, N.; Gniechwitz, D.; Steinhart, H.; Bunzel, M.; Blaut, M. Characterization of high molecular weight coffee fractions and their fermentation by human intestinal microbiota. *Mol. Nutr. Food Res.* **2009**, *53*, 287–299.

(32) D'Amelio, N.; Fontanive, L.; Uggeri, F.; Suggi-Liverani, F.; Navarini, L. NMR reinvestigation of the caffeine−chlorogenate complex in aqueous solution and in coffee brews. *Food Biophys.* **2009**, *4*, 321–330.

(33) Alves, R. C.; Costa, A. S. G.; Jerez, M.; Casal, S.; Sineiro, J.; Nunez, M. J.; Oliveira, B. Antiradical activity, phenolics profile, and hydroxymethylfurfural in espresso coffee: Influence of technological factors. *J. Agric. Food Chem.* **2010**, *58*, 12221–12229.

(34) Rubico, S. M.; Mcdaniel, M. R. Sensory evaluation of acids by free-choice profiling. *Chem. Senses* **1992**, *17*, 273–289.

(35) Frank, O.; Zehentbauer, G.; Hofmann, T. Screening and identification of bitter compounds in roasted coffee brew by taste dilution analysis. *Dev. Food Sci.* **2006**, *43*, 165–168.

(36) Illy, A.; Viani, R. Roasting. In *Espresso Coffee: The Chemistry of Quality*, 1st ed.; Academic Press: New York, 1995; pp 105−106.

(37) Murkovic, M.; Derler, K. Analysis of amino acids and carbohydrates in green coffee. *J. Biochem. Biophys. Methods* **2006**, *69*, 25–32.