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Roasting Process of Coffee Beans as Studied by Nuclear Magnetic Resonance: Time Course of Changes in Composition

Feifei Wei, Kazuo Furihata, Masanori Koda, Fangyu Hu, Takuya Miyakawa, and Masaru Tanokura*

Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan

Supporting Information

ABSTRACT: In this paper, we report a ^{1}H and ^{13}C nuclear magnetic resonance (NMR)-based comprehensive analysis of coffee bean extracts of different degrees of roast. The roasting process of coffee bean extracts was chemically characterized using detailed signal assignment information coupled with multivariate data analysis. A total of 30 NMR-visible components of coffee bean extracts were monitored simultaneously as a function of the roasting duration. During roasting, components such as sucrose and chlorogenic acids were degraded and components such as quinic acids, *N*-methylpyridinium, and water-soluble polysaccharides were formed. Caffeine and *myo*-inositol were relatively thermally stable. Multivariate data analysis indicated that some components such as sucrose, chlorogenic acids, quinic acids, and polysaccharides could serve as chemical markers during coffee bean roasting. The present composition-based quality analysis provides an excellent holistic method and suggests useful chemical markers to control and characterize the coffee-roasting process.

KEYWORDS: *NMR, coffee, complex mixture analysis, roasting process, multivariate statistical analysis*

INTRODUCTION

Coffee is a very common beverage throughout the world. Roasting is probably the most important factor in the development of the complex flavors that make coffee enjoyable.[1] During the roasting process, the beans undergo many chemical reactions, leading to important physical changes and to the formation of the substances responsible for the sensory qualities of the beverage.[2] Many coffee components have been targeted for evaluation and characterization in roasted coffee brews.[3−9] The changes of coffee flavor components and chlorogenic acids have been studied by gas chromatography.[10] A detailed analysis of the chlorogenic acids in robusta coffee beans has been performed using liquid chromatography coupled with mass spectrometry.[11−13] Polysaccharides and melanoidins in arabica coffee beans and their detailed changes during roasting have been reported using chromatography and mass spectrometry.[6,14−19] These studies have provided detailed information on coffee components and their changes during roasting. However, all of these studies have been compound-targeted; the changes in one bean component or one class of bean components were monitored during roasting. It is reasonable to consider that the composition of a cup of coffee is quite different from the sum of all of the substances observed after the complicated pretreatments.

Nuclear magnetic resonance (NMR) spectroscopy has been widely applied in food science to achieve quick, direct, and comprehensive information about chemical components present in various foods and drinks.[20−25] Although NMR provides less sensitivity than other techniques, such as chromatography and mass spectrometry, the nondestructive and nontargeted nature of NMR, combined with multivariate data analysis, makes it an attractive tool for the observation of a dynamic process, including changes in the composition of foods as complex mixtures, such as microbial fermentation, the fruit-ripening process, and food processing.[22,26,27] NMR has been used in the observation of coffee in many previous studies. A study by Charlton et al. provided a discrimination method of instant coffee by ^{1}H NMR based on chemometrics.[28] Bosco et al. observed the ^{1}H NMR spectra of espresso coffee, assigned signals to the major components, such as caffeine, quinic acid, and trigonelline, and also observed the roasting process, which provided a reliable overview of the changes during roasting.[29] Changes in the composition of ground coffee beans have also been monitored by high-resolution magic-angle spinning (HR-MAS) NMR spectroscopy by Ciampa et al.[30] However, when coffee was analyzed by NMR in these studies, a detailed signal assignment of spectra from coffee has not been accomplished. Without signal assignment, the evaluated coffee components by NMR are limited, leaving many of the observed signals unknown. Thus, we reported almost all of the NMR signal assignments for green[31] and roasted[32] coffee bean extracts and, in the course of these studies, identified 30 compounds that can be analyzed and monitored during coffee bean roasting.

In the present study, we report for the first time a ^{1}H and ^{13}C NMR-based comprehensive analysis of coffee bean extracts at different degrees of roast and chemically characterize the changes of NMR-visible coffee components during the roasting process using the detailed signal assignment information coupled with multivariate statistical analysis.

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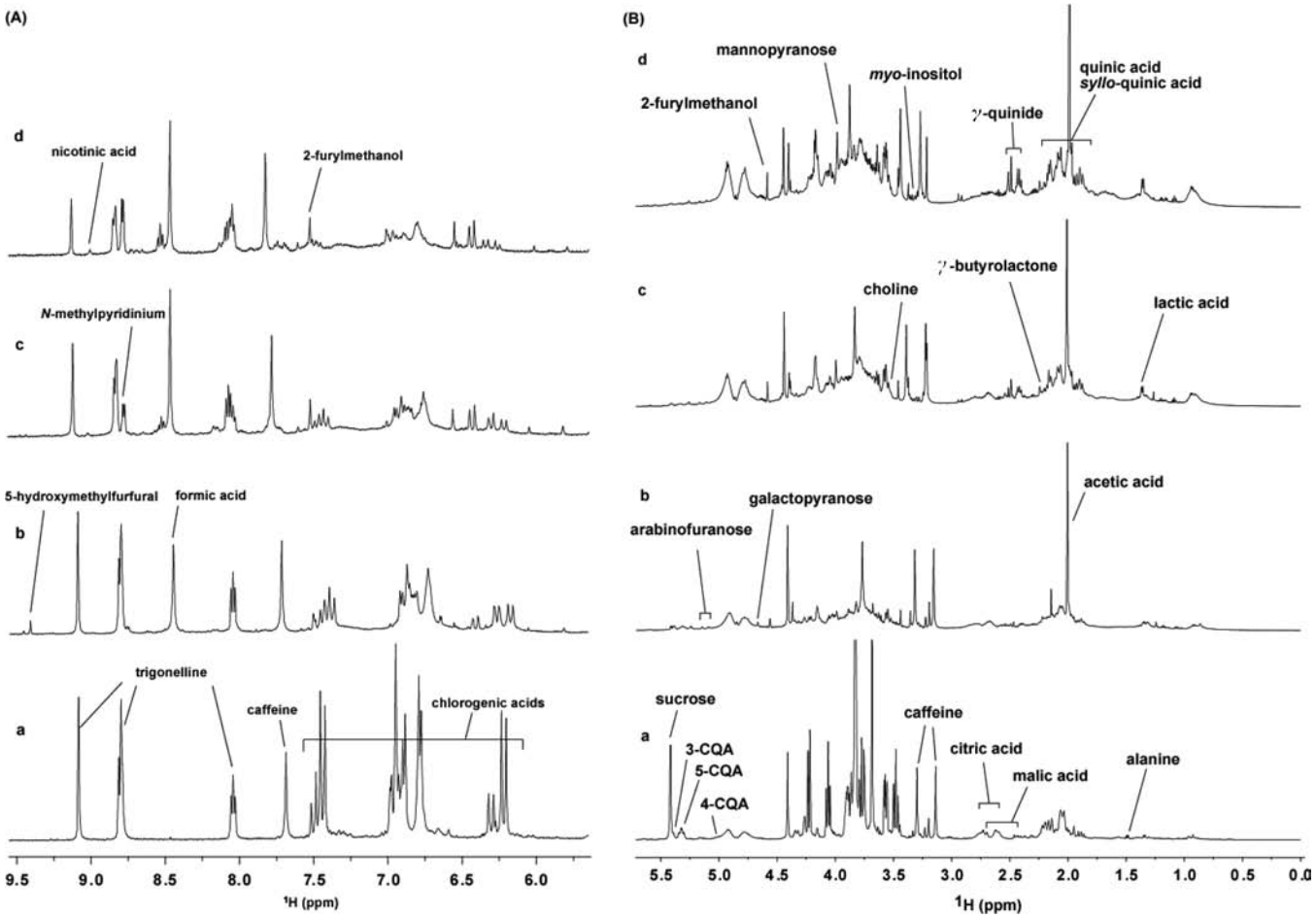

Figure 1. (A) Low-field and (B) high-field ^{1}H NMR spectra of (a) green coffee bean extract and coffee bean extracts at (b) light, (c) medium, and (d) dark degrees of roast.

MATERIALS AND METHODS

Coffee Beans. Arabica coffee beans from Colombia were used and were kindly supplied by Ajinomoto General Foods, Inc. (Tokyo, Japan).

Roasting Process. Green beans (50 g) were roasted in a home coffee roaster (Hearthware Home Products, Inc., Gurnee, IL) at 220 °C for 5 min (L = 26), 7 min (L = 23), and 9 min (L = 17) to reach light, medium, and dark roasting degrees, respectively. To monitor the changes in composition during the roasting process, coffee beans were also roasted for 2, 3, 3.5, 4.5, 5, 5.5, 6, and 6.5 min. All samples were prepared in triplicate (n = 3) for statistical analysis. The green and 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).

NMR Samples. Crushed beans (1.5 g) of different degrees of roast were incubated at 95 °C in a closed plastic tube with D_2O (3.50 mL, 99.7%; Shoko Co., Ltd., Tokyo, Japan) for 1 h, to focus only on water-soluble compounds, such as might occur in the brewed coffee itself. The extracts were cooled on ice for 15 min and then centrifuged at 5000g at 4 °C for 5 min. The supernatants (500 μL), whose pH values ranged from 5.5 for green beans to 4.8 for dark roasted beans, were removed to new tubes and mixed with phosphate buffer (100 μL of 0.2 M sodium phosphate at pH 5.0). 4,4-Dimethyl-4-silapentane-1-sulfonic acid sodium salt (DSS; Wako Pure Chemical Industries, Ltd., Osaka, Japan) was used as the internal reference, and its chemical shift was set to 0 ppm. The coffee bean extracts were then transferred into 5 mm NMR tubes.

NMR Spectroscopic Analysis. The one-dimensional (1D) ^{1}H and ^{13}C NMR spectra were measured at 500 and 125.65 MHz, respectively, on a Varian Unity INOVA-500 spectrometer. For the ^{1}H NMR spectra, the H_2O signal was suppressed by the presaturation method,[33] and the parameters for observation were as follows: number of data points, 64 000; spectral width, 8000 Hz; acquisition time, 4.00 s; delay time, 2.0 s; and number of scans, 128. The parameters for the ^{13}C NMR spectra were as follows: number of data points, 64 000; spectral width, 31 422 Hz; acquisition time, 1.04 s; delay time, 2.0 s; and number of scans, 30 000. The delay time was determined after checking the spin−lattice relaxation time (T_1), which is available in the Supporting Information.

NMR Data Processing. The free-induction decay (FID) NMR data were processed by the program MestRe Nova (version 5.3.0; MestReC, Santiago de Compostela, Spain). Signal assignments were made essentially according to the previously described method based on two-dimensional (2D) NMR correlations.[31,32] To investigate the changes in the chemical composition over time, the integral values of the signals were calculated automatically, relative to that of caffeine (3.20 ppm for ^{1}H and 28.77 ppm for ^{13}C), which is thermostable during roasting,[34,35] and were set to a constant of 100. The integrated signals were consistent with the previous studies.[31] In each NMR spectrum of coffee bean extracts in the roasting process, relative integral values of signals of the same carbon atoms were calculated. Changes over time in the concentrations of coffee components were determined by changes in the relative integral values at different degrees of roast.

Multivariate Statistical Analysis. The ^{13}C NMR spectral data were reduced into 0.8 ppm spectral buckets, and all spectra were aligned using the correlation optimized warping (COW) method and then normalized.[22] The resulting data sets were then imported into SIMCA-P, version 11.5 (Umetrics, Umeå, Sweden), for multivariate statistical analysis. The mean center was applied for all multivariate analyses by SIMCA-P, version 11.5. Principle component analysis

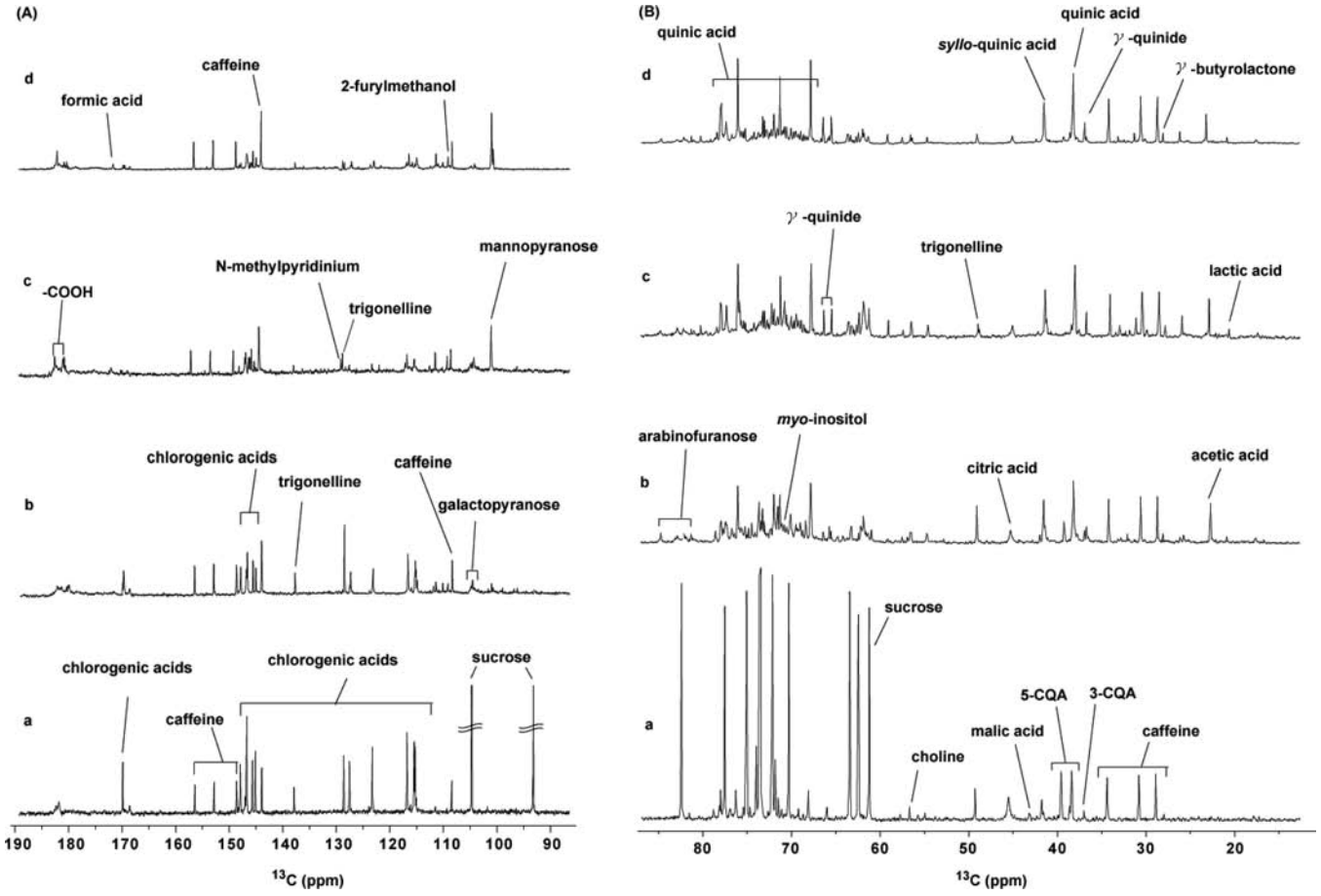

Figure 2. (A) Low-field and (B) high-field ^{13}C NMR spectra of (a) green coffee bean extract and coffee bean extracts at (b) light, (c) medium, and (d) dark degrees of roast.

(PCA), an unsupervised pattern-recognition method, was performed to examine the intrinsic variations in the data set. Hotelling's T2 region, shown as an ellipse in the score plots, defined the 99% confidence interval of the modeled variation. The quality of the model was described by Rx^2 and Q^2 values. Rx^2 was defined as the proportion of variance in the data explained by the model and indicates goodness of fit. Q^2 was defined as the proportion of variance in the data predictable by the model and indicates predictability.

RESULTS

^1H and ^{13}C NMR Spectra of Coffee Bean Extracts at Different Degrees of Roast. Figures 1 and 2 show representative 1D ^1H and ^{13}C NMR spectra of green coffee bean extract and coffee bean extracts at light, medium, and dark roasting degrees. A total of 30 components were identified through analyses of 2D NMR and spiking experiments in previous studies.[31] As shown in Figures 1 and 2, the spectra of coffee bean extracts in the roasting process were dominated by a number of components, including those present in green coffee bean extract: chlorogenic acids, including 3-, 4-, and 5-caffeoylquinic acid (3-CQA, 4-CQA, and 5-CQA, respectively), acetic acid, caffeine, choline, citric acid, γ-aminobutyrate (GABA), L-alanine, L-asparagine, L-glutamic acid, quinic acid, malic acid, *myo*-inositol, sucrose, and trigonelline, and those produced during the roasting process: 5-hydroxymethylfurfural, γ-quinide, *syllo*-quinic acid, α-(1−3)-L-arabinofuranose, α-(1−5)-L-arabinofuranose, β-(1−4)-D-mannopyranose, β-(1−3)-D-galactopyranose, β-(1−6)-D-galactopyranose, γ-butyrolactone, 2-furylmethanol, formic acid, lactic acid, nicotinic acid, and *N*-methylpyridinium. The procedures and results of the detailed signal assignments of green and roasted coffee bean extracts are described in our previous studies.[31,32]

Evolution of Coffee Components in the Roasting Process. To investigate the changes in coffee composition during the roasting process, the relative concentrations were plotted as a function of the roasting duration for all of the components identified (Figure 3). The evolution of coffee components, except nicotinic acid, 5-hydroxymethylfurfural, and formic acid, were obtained from ^{13}C signal integral values, because the signal separation in ^{13}C spectra was better than that in the complicated ^1H spectra, where the signals were heavily overlapped and could not be integrated accurately. However, for nicotinic acid, 5-hydroxymethylfurfural, and formic acid, the integral values of ^1H signals were traced during roasting, because sensitivity was better in the ^1H spectra than in the ^{13}C spectra for these compounds.

As shown in Figure 3A, chlorogenic acids were severely degraded during the roasting process. After a slight roasting for 2 min, a sharp decrease in 5-CQA and stable decreases in 4-CQA and 3-CQA were observed. Almost all three of the chlorogenic acid isomers disappeared after dark roasting for 9 min.

The evolutions of quinic acid, γ-quinide, and *syllo*-quinic acid are shown in Figure 3B. The free quinic acid present in green coffee beans was found to be increased along with the roasting time from the start to 4.5 min of roasting, decreased from 4.5 to 5.5 min, and increased again until dark roasting for 9 min. In

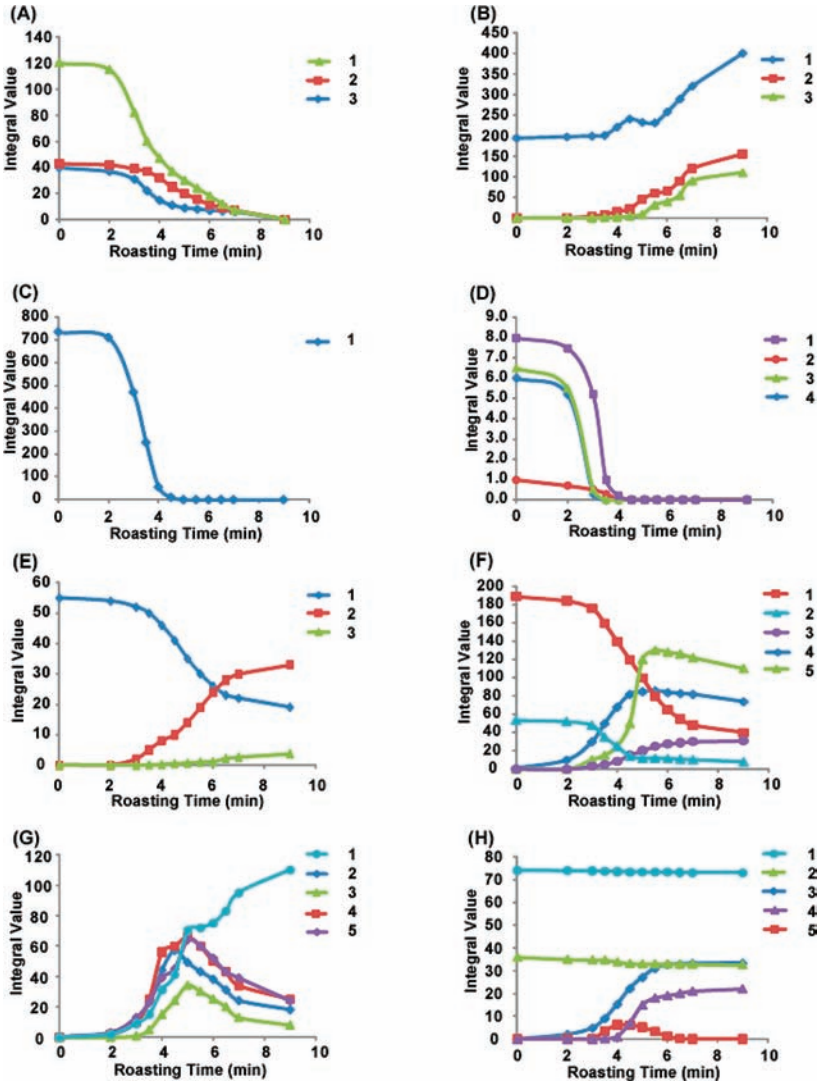

Figure 3. Evolutions of coffee components during the roasting process: (A) 1, 5-CQA; 2, 4-CQA; and 3, 3-CQA; (B) 1, quinic acid; 2, γ-quinide; and 3, *syllo*-quinic acid; (C) sucrose; (D) 1, L-glutamic acid; 2, L-alanine; 3, L-asparagine; and 4, GABA; (E) 1, trigonelline; 2, *N*-methylpyridinium; and 3, nicotinic acid; (F) 1, citric acid; 2, malic acid; 3, lactic acid; 4, acetic acid; and 5, formic acid; (G) 1, β-(1−4)-D-mannopyranose; 2, α-(1−3)-L-arabinofuranose; 3, α-(1−5)-L-arabinofuranose; 4, β-(1−3)-D-galactopyranose; and 5, β-(1−6)-D-galactopyranose; and (H) 1, *myo*-inositol; 2, choline; 3, 2-furylmethanol; 4, γ-butyrolactone; and 5, 5-hydroxymethylfurfural. The integral value of the signal of caffeine was set to a constant of 100.

addition, γ-quinide and *syllo*-quinic acid were found to be produced during the roasting process, and continuous increases in the amounts of both were observed.

The evolution of sucrose, the most abundant simple carbohydrate in green coffee bean extract, is shown in Figure 3C. Sucrose is found to be destroyed quickly by roasting for 2−4 min. After roasting for 5 min, no ^{13}C NMR signal of sucrose was observed.

As shown in Figure 3D, free amino acids decreased during roasting. Although four kinds of amino acids, i.e., L-glutamic acid, L-alanine, L-asparagine, and GABA, were identified from the NMR spectra of green coffee bean extract,[31] they disappeared after roasting for 4 min.

The evolutions of trigonelline, *N*-methylpyridinium, and nicotinic acid are shown in Figure 3E. Trigonelline, present at high levels in green coffee beans, decreased continuously during the roasting process. *N*-Methylpyridinium[8] and nicotinic acid,[36] two thermal decomposition products of trigonelline, increased continuously during the roasting.

The evolutions of aliphatic acids are shown in Figure 3F. Decreases in citric acid and malic acid and a steady increase in lactic acid were observed. For acetic and formic acids, increases followed by steady decreases after roasting for 5.5 min were observed.

The evolutions of polysaccharide residues are shown in Figure 3G. An increase of at least two steps was found in the extractable β-(1−4)-D-mannopyranose content during the roasting progress, while sharp increases in α-(1−3)-L-arabinofuranose, α-(1−5)-L-arabinofuranose, β-(1−3)-D-galactopyranose, and β-(1−6)-D-galactopyranose were seen after 2 min of roasting. Simultaneous decreases after roasting for 5 min were observed for α-(1−5)-L-arabinofuranose, β-(1−3)-D-galactopyranose, and β-(1−6)-D-galactopyranose, while a decrease in α-(1−3)-L-arabinofuranose was detected after roasting for 4.5 min.

The evolutions of other coffee components, including *myo*-inositol, choline, 2-furylmethanol, γ-butyrolactone, and 5-hydroxymethylfurfural, are shown in Figure 3H. Slight

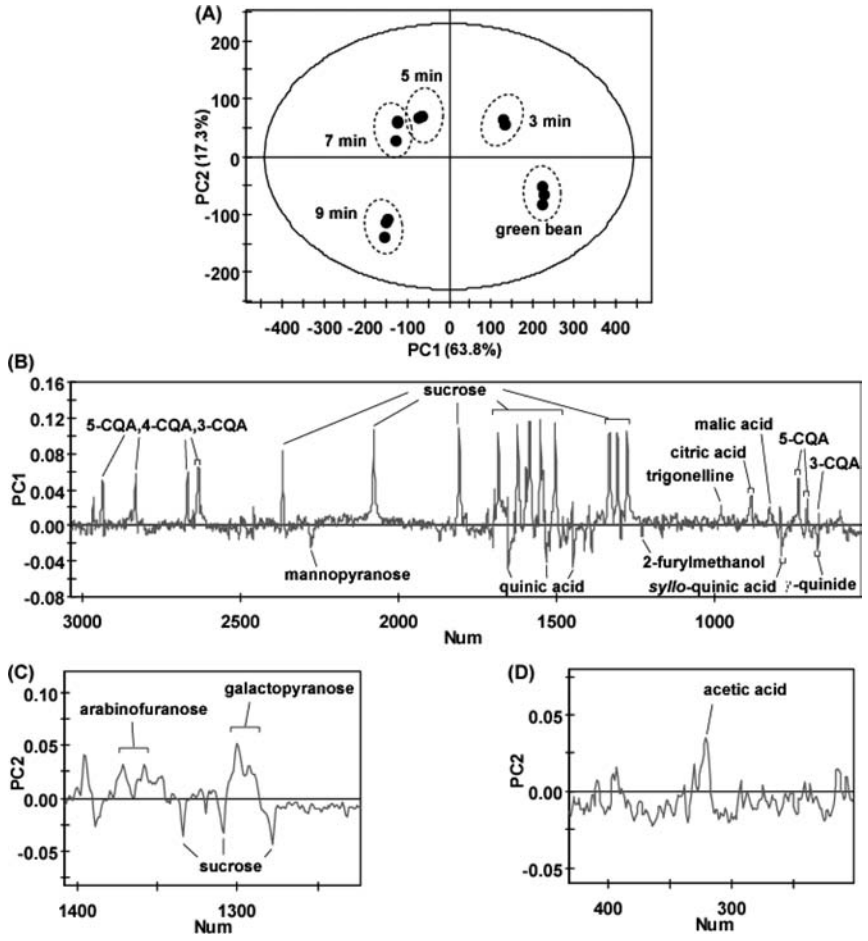

Figure 4. (A) PCA score, (B) loading plots for PC1, and (C) loading plots for PC2 derived from the ^{13}C NMR spectra of coffee bean extracts at different degrees of roast. Green bean, 3, 5, 7, and 9 min represent the green coffee bean extract and coffee bean extracts roasted for 3, 5, 7, and 9 min, respectively. The fitness and predictability of the models are indicated by Rx^2 values of 63.8 and 17.3% for PC1 and PC2, respectively, and Q^2 of 0.99.

decreases in *myo*-inositol and choline were found during the roasting process. 2-Furylmethanol and γ-butyrolactone were generated during the roasting process with steadily continuous increases. 5-Hydroxymethylfurfural increased during roasting for up to 4 min, after which its level quickly degraded, becoming undetectable after 6.5 min.

Variations in Coffee Components during the Roasting Process. To investigate the overall composition changes during the roasting process, PCA was performed on the data for all coffee bean extracts at different degrees of roast. In this study, instead of ^1H NMR spectra, which are commonly used in the multivariate statistical analysis, ^{13}C NMR spectra were used to investigate the variations in coffee components during the roasting process. This is because signals of caffeine and chlorogenic acids in the ^1H NMR spectra of coffee bean extracts were shifted remarkably by the formation of the caffeine−chlorogenate complex when the relative concentration changed during the roasting process,[37] while no such effect was found in the ^{13}C NMR spectra. The reason for this difference may be that the noncovalent correlations between caffeine and chlorogenic acids are not strong, and thus, they affect the chemical environments in ^1H nuclei but not those in ^{13}C nuclei.

Coffee bean extract at each roasting degree was clearly distinguished from the others by the PCA score plots, which had high statistical values of Rx^2 (63.8 and 17.3% for PC1 and PC2, respectively) and Q^2 (0.99), as shown in Figure 4A. The PCA loading plots revealed the components responsible for variables contributing to the classification in the complementary PCA score plot. As shown in Figure 4B, the positive side of the PC1 loading plot indicates higher levels of components in green beans and beans roasted up to 3 min than in those roasted for longer times, whereas the negative side indicates lower levels of components. As the results show, the differentiation between the green and roasted coffee bean extracts was attributable to higher levels of sucrose, 5-CQA, 4-CQA, 3-CQA, trigonelline, citric acid, and malic acid and to lower levels of β-(1−4)-D-mannopyranose, quinic acid, γ-quinide, and *syllo*-quinic acid in the green coffee bean extracts. The PC2 explained 17.3% of all of the variables, and the loading plot of PC2 is shown in panels C and D of Figure 4. The PC2 loading plot reveals that the significant changes in α-(1−3)-L-arabinofuranose, α-(1−5)-L-arabinofuranose, β-(1−3)-D-galactopyranose, β-(1−6)-D-galactopyranose, and acetic acid accounted for the variations, with higher levels of these components found in coffee beans roasted for 3−7 min, while lower levels were found in both green coffee beans and dark roasted beans.

DISCUSSION

Chlorogenic Acids, Quinic Acids, and Quinide. It has been reported that there are at least 30 chlorogenic acids in

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coffee beans,[11−13] of which the three most abundant kinds (3-CQA, 4-CQA, and 5-CQA) have been detected and assigned by NMR of green coffee bean extracts[31] and roasted coffee bean extracts.[32] Other chlorogenic acid isomers were not detected by NMR in our studies, even though they may have been present in the coffee bean extracts. The reason can be considered as their trace concentrations. In this study, we found that the levels of chlorogenic acids, including 5-CQA, 4-CQA, and 3-CQA, decreased considerably, whereas the levels of quinic acid, γ-quinide, and *syllo*-quinic acid increased during the roasting process. Chlorogenic acids are the major polyphenols in green coffee beans, and their degradation leads to the release of quinic acid, which is known as the dominant acid in roasted coffee, and cinnamic acid.[1,11] During the roasting process, γ-quinide was formed as an internal ester of the quinic acid and *syllo*-quinic acid, which is another isomeric product of quinic acid, was also produced. The decrease in the amount of quinic acid from beans roasted for 4.5−5.5 min might be attributable to the occurrence of the internal esterification and isomeric reaction under the prevalent conditions of roasting, which include a thermal atmosphere, low water content, and moderate acidity.[38] The loading plot of PC1 also indicated that the degradation of chlorogenic acids and the formation of quinic acids and quinide were remarkable changes during the roasting of coffee beans, and these components may be regarded as the most prominent chemical markers during the coffee-bean-roasting process. The other thermal decomposition products of chlorogenic acids are cinnamic acids. According to the previous study by Vitzthum,[10] cinnamic acids may participate in the next chemical reactions to form other flavor components, which are at trace concentrations and absent from NMR spectra in the present study. The other reason may be that cinnamic acids are hot-water-soluble but cool-water-insoluble according to their physical properties. After extraction, the samples were cooled and the spectra were observed at 20 °C, at which temperature the soluble cinnamic acids were not visible and, therefore, present in only trace amounts, if at all.

Chlorogenic acids have an astringent taste, and their reduction in coffee of a darker roast contributes to a smoother cup taste. Meanwhile, the quinic acids and quinide were found to contribute considerably to the bitterness of roasted coffee.[1,39,40] The decomposition of chlorogenic acids could also be used as an index of the roasting degree.[41]

Sucrose, Aliphatic Acids, and 5-Hydroxymethylfurfural. Sucrose is the most abundant simple carbohydrate in green coffee beans.[31] Even when the beans were roasted for only about 5 min, under the conditions of the present study, almost all of the sucrose was already degraded. The PC1 loadings captured this change as a prominent feature of the pattern describing the most important source of variance. In the PC1 loading plot, the positive side was dominated by sucrose, which confirmed the degradation of sucrose during roasting. It has been reported that sucrose acts as an aroma precursor during roasting, generating several classes of compounds, such as furans, aldehydes, and aliphatic acids, that affect the flavor of the beverage.[42−44] The formation of organic acids, such as acetic acid, formic acid, and lactic acid, and aroma compounds of 2-furylmethanol and γ-butyrolactone could be attributed to the degradation of sucrose. The formation of 2-furylmethanol was also captured by PCA. Because sucrose during roasting contributes to the formation of sourness and to the aroma, which are key contributors, along with bitterness, to the total sensory impact of a coffee beverage, the final cup will not be enjoyable if the sucrose is not degraded. 5-Hydroxymethylfurfural is very common in a wide variety of heat-processed foods and is generated from the fructose moiety produced from sucrose during pyrolysis at high temperatures.[45] In the present study, the maximum concentration of 5-hydroxymethylfurfural occurred after a slight roasting; it degraded quickly upon further roasting. This is consistent with the results of a previous study.[46]

Polysaccharides. On a dry-weight basis, almost half of green coffee beans are reported to be made of polysaccharides, which include cellulose, mannan, and arabinogalactan.[47] However, no polysaccharide was found by NMR in green coffee bean extract in either our previous study[30] or the present study. This may be attributable to the fact that, in green coffee beans, polysaccharides are retained in the coffee bean cell wall as part of the insoluble polysaccharide complex.[19,48] The roasting process increases the solubility of both arabinogalactans and mannans from the bean by the loosening of the cell-wall structure as it swells and by the depolymerization of the polysaccharides during the roasting process. The degradation in the present study of α-(1−3)-L-arabinofuranose, α-(1−5)-L-arabinofuranose, β-(1−3)-D-galactopyranose, and β-(1−6)-D-galactopyranose units from arabinogalactans after light roasting could be attributed to the higher thermal lability of arabinogalactans compared to that of β-(1−4)-D-mannopyranose from mannan, which was less susceptible to degradation even after a dark roast.[7] The water-soluble polysaccharides that appear after roasting, which play an important role in the retention of volatile substances, contribute to the viscosity of the coffee brew and, thus, to the creamy sensation known as "body" in the mouth.[1] The PCA showed that β-(1−4)-D-mannopyranose, which contributes to the negative side of PC1, formed during roasting; α-(1−3)-L-arabinofuranose, α-(1−5)-L-arabinofuranose, β-(1−3)-D-galactopyranose, and β-(1−6)-D-galactopyranose contribute to the positive side of PC2, which means a formation followed by degradation. These results suggest that the polysaccharides in coffee beans may be used as chemical markers during the roasting process, especially β-(1−4)-D-mannopyranose.

Trigonelline, *N*-Methylpyridinium, and Nicotinic Acid. The importance of trigonelline, not only as a precursor of flavor and aroma compounds but also as a beneficial nutritional factor, has been well-documented in previous studies.[36,49] Reports on the thermal degradation of trigonelline have revealed nicotinic acid and nicotinamide, as well as their *O*- and *N*-methyl derivatives, as reaction products.[49] However, data obtained in both our previous and present studies confirmed that *N*-methylpyridinium and nicotinic acid are the major nonvolatile products of trigonelline pyrolysis.[8] The decrease in the *N*-methylpyridinium level after roasting for 7 min was probably attributable to further decomposition and/or interaction with other thermolytic products. Nicotinic acid, which is an important vitamin as well as the second major thermal degradation product of trigonelline, was positively correlated with the roasting degree. Trigonelline and its thermolytic products undoubtedly have direct and indirect effects on other physicochemical properties of a cup of coffee, such as flavor and aroma. Our PCA results also indicate that trigonelline and *N*-methylpyridinium can serve as chemical markers during roasting for their remarkable changes in the concentration.

In the present study, the coffee bean extracts were well-distinguished by PCA according to their different degrees of roast.

The coffee-bean-roasting process has been characterized by ^{1}H and ^{13}C NMR spectra based on detailed signal assignment information coupled with multivariate statistical analysis, which enables the overall observation of coffee bean extracts to be studied nondestructively and simultaneously. The 30 NMR-visible components of coffee bean extracts were monitored simultaneously as a function of the roasting duration, and our composition-based quality analysis offered an excellent holistic method and significant chemical markers to control and characterize the coffee-bean-roasting process.

■ ASSOCIATED CONTENT

Supporting Information

Detailed descriptions of NMR experiments upon checking of spin−lattice relaxation time (T_1) of green and roasted coffee bean extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +81-3-5841-5165. Fax: +81-3-5841-8023. E-mail: amtanok@mail.ecc.u-tokyo.ac.jp.

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