Infrared and Nuclear Magnetic Resonance Spectrometry of Caffeine in Roasted Coffee Beans after Separation by Preparative Supercritical Fluid Chromatography

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The isolation of caffeine from coffee powder was performed using supercritical fluid extraction (SFE) and preparative supercritical fluid chromatography (SFC). Two grams of roasted coffee bean powder gave 0.49 g of extract using supercritical carbon dioxide (40°C, 200 kg/cm²) as an extractant. This extract was mixed with 0.6 ml of methanol and after decantation, 0.2 ml of the supernatant was subjected to preparative SFC. Fractionation was carried out with referring to the retention time of the standard caffeine. After evaporation of ethanol added as a modifier from the collected fraction, a 7–8 mg of needle-like crystals was obtained. This crystal was identified to be caffeine by FTIR and by FT-NMR, respectively.

Keywords Caffeine, supercritical fluid extraction, supercritical fluid chromatography, Fourier transform infrared spectrometry, Fourier transform nuclear magnetic resonance spectrometry

Coffee is certainly one of the most frequently consumed beverages not only in North America but all over the world.¹ It is well known that coffee contains caffeine (1,3,7-trimethylxanthine), which is considered to be its most physiologically active component.² In addition, caffeine is present in tea, mate leaves, cocoa, and cola nuts.³

Supercritical fluid extraction (SFE), which uses a supercritical fluid as an extraction medium, has long been used to remove caffeine from green coffee beans in industrial decaffeination processes.⁴

Sugiyama *et al.* demonstrated directly coupled SFE/SFC (supercritical fluid chromatography) analysis of roasted coffee beans, showing that various compounds could be extracted, including caffeine.⁵ They also investigated such optimum conditions as the pressure, temperature, water content and extraction time for caffeine extraction by off-line SFE/HPLC (high performance liquid chromatography).⁵

Since these studies were performed in view of analytical separation, they did not mean to recover sufficient amounts of extracts for further molecular spectroscopic analyses, such as infrared (IR) and nuclear magnetic resonance (NMR) spectrometry for identification.

We investigated how to perform SFE and SFC in larger scale so that it would be possible to recover caffeine fraction for further identification analyses. The amount of obtained caffeine was enough to identify by

Fourier transform infrared spectrometry (FTIR) and by NMR spectrometry.

Experimental

Reagents and a column

Anhydrous caffeine (C₈H₁₀N₄O₂) was obtained from Wako Pure Chemicals (Osaka, Japan). Coffee powder was UCC original blend coffee (medium fine grind) obtained from UESHIMA COFFEE (Kobe, Japan). Carbon dioxide was obtained from Toyoko Kagaku (Kawasaki, Japan). Ethanol and methanol were also from WAKO Pure Chemicals. Water used was purified from tap water using a Millipore Milli-R/Q (Massachusetts, USA) water purification system. SFC was performed using a 5 µm silica gel column (10 mm i.d.×250 mm length) (JASCO, Tokyo, Japan). The KBr crystal used for IR measurements was from JASCO (Tokyo, Japan).

Apparatus

SFE and SFC were carried out using a JASCO Model SUPER-200 SFE/SFC System-2, comprising a carbon dioxide and modifier delivery section; an extraction/chromatography section equipped with a Rheodyne injector Model 7125 (Cotati, CA, USA) with a 200 µl sampling loop; and a JASCO Model 880-81 back-pressure regulator.

A UV/VIS multiwavelength detector (JASCO Model

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MULTI-330) was used for monitoring extraction and chromatographic profiles. DP-L320/98 data processing software was used for data analysis on an NEC PC-9801VM personal computer (NEC, Tokyo, Japan).

Infrared spectral measurements were performed using a microsampling FTIR spectrometer (JASCO Model Micro FTIR-100) with a workstation (JASCO Model WS/IR-8000). NMR spectrometric measurements were performed by using a JEOL EX 270 FT NMR (Akishima, Tokyo, Japan).

Procedures

Coffee pretreatment. Two hundred microliters of water were added to 2 g of coffee powder, according to Sugiyama *et al.*⁵, has been vigorously shaken for 15 min, agitated with a vortex for 15 min, and then left for 3 h before extraction. This process was used to give *ca.* 10% water content to the powder.

SFE of coffee. SFE was performed using a 10 ml extraction vessel (JASCO Model EV-2). The coffee extract was collected in a 10 ml glass tube connected to the back-pressure regulator by a spring force.⁶

SFC of coffee extract. The extract obtained in SFE was mixed with 600 μ l of methanol and vigorously shaken for 5 min, and then agitated with a vortex for 5 min. After decantation, the supernatant was subjected to preparative SFC separation. A standard solution was prepared by dissolving 4 mg of anhydrous caffeine into 1 ml of methanol. This solution was used for identification of the caffeine peak in the chromatogram of the extract by comparing its retention time with a peak possibly of caffeine in the obtained chromatogram. Caffeine fractionation was performed by collecting a portion of the column effluent with reference to the standard chromatogram. Evaporation of modifier solvent from the collected fractions was carried out using a rotary vacuum evaporator in combination with a thermostated water bath.

Results and Discussion

SFE of coffee

Figure 1 shows the extraction profile monitored at 275 nm, which is about the maximum absorption wavelength of caffeine. As can be seen, it takes 2 h to extract 90% of the compounds which exhibit UV absorption at 275 nm. However, 80% of the compounds could be extracted within 1 h.

The mass of the coffee powder placed in the extraction vessel was 2.00 g, excluding 10% added water. This gave 0.49 g of the extract (oil and xanthine derivatives) and 1.39 g for the residue, corresponding to a recovery of 80.3%. It is considered that most of the added water was extracted together with other compounds. About 20% of the extract was gone with carbon dioxide gas to the exhaust line.

Methanol-soluble compounds in the extract were transferred to methanol by the procedure described in

Fig. 1 Extraction profile of roasted coffee beans. Conditions: mobile phase, CO₂ (5 ml/min); temperature, 40°C; pressure, 200 kg/cm²; detection, 275 nm.

Fig. 2 Chromatogram of coffee extract by SFC. Conditions: mobile phase, CO₂ (5 ml/min)+ethanol (0.5 ml/min); temperature, 40°C; pressure, 200 kg/cm²; sample volume, 200 μ l.

SFC of coffee extract paragraph in Experimental Section.

SFC separation of caffeine from oil in the extract

Figure 2 shows a chromatogram obtained by injecting 200 μ l of the supernatant of the extract. The time frames in which the fractions were collected are shown under the time axis of the chromatogram: fraction no. 1 (0–6 min); fraction no. 2 (6–8 min); fraction no. 3 (8–27 min); fraction no. 4 (27–33 min) and fraction no. 5 (33–43 min).

Figure 3 shows the chromatogram of the caffeine standard solution obtained under the same conditions. It is shown that the retention time of caffeine is 35.46 min.

Comparison of these chromatograms suggested that fraction no. 5 contained caffeine. Components eluted before 20 min were less polar compounds than caffeine.

Fraction no. 5 also contained ethanol, which was added as a modifier. After evaporation of ethanol, we obtained pieces of needle-like crystals on the wall of the glass collection vessel. A slightly yellow ring (0.5 mm width) attached to the tube suggested the presence of minor impurities.

Fig. 3 Chromatogram of standard caffeine. Conditions are same as given in Fig. 2 caption, except that the sample volume was 10 μ l.

A sufficient amount (3.5–4 mg) of caffeine was obtained in a single SFC run to perform further identifications by FTIR and FT-NMR analyses.

Identification of caffeine by infrared spectrometry

Prior to the IR spectrum measurement of the obtained crystal, a measurement of the standard caffeine was performed using the Micro FTIR System by placing the caffeine powder on a KBr disk (diameter=3 mm). The obtained IR spectrum is shown in Fig. 4A.

We first measured the IR spectrum of the crystal having 45 μ m diameter by focusing the IR beam on it while being viewed under a microscope. It was found that the crystal scattered too much of the IR beam to obtain the correct IR spectrum. We therefore pressed

Fig. 4 Micro-FTIR infrared spectra of standard caffeine (A) and isolated caffeine after pressing (B): aperture, 36 $\times$ 45 μ m.

the crystal with a spatula to crash it into a powder. We could thus obtain a high-quality IR spectrum as shown in Fig. 4B.

The identification of caffeine was performed by comparing the standard and measured IR spectra as shown in Figs. 4A and 4B. The result shows that there is excellent agreement in the wavenumbers of the specific bands of both spectra. The similarity of the shapes of the spectra can be justified by visually comparing the spectra shown in Figs. 4A and 4B. The crystal was thus identified to be caffeine.

Nuclear magnetic resonance measurements

Figures 5A and 5B show ^1H -NMR spectra of the standard caffeine and isolated caffeine in CDCl_3 at 270 MHz (45 degrees pulse with intervals of 2 s). The NMR spectrum of the isolated caffeine shown in Fig. 5A

has almost the same profile as that of the standard, except for a shift of 1/10 of the ppm. The relative intensities of the peaks are almost the same except for the reference peak and the impurity peak at 5.614 ppm (in the isolated caffeine) of which intensity is three-times higher than that in the standard caffeine spectrum (5.518 ppm). Impurities were found in both spectra. These results suggest that the impurities in the isolated caffeine spectrum originated from the solvent used for NMR measurements, not only from the isolated caffeine. The relationships between the NMR peaks (standard caffeine and a crystal of caffeine) and the functional groups are listed in Table 1.

Figures 6A and 6B show the ^{13}C -NMR spectra of the standard caffeine and the isolated caffeine in CDCl_3 at 67.5 MHz (45 degrees pulse with intervals of 5 s).

The peak shifts in the isolated caffeine show a variation

Fig. 5 ^1H -NMR spectra of standard caffeine (A) and isolated caffeine (B). Conditions: frequency, 270 MHz, 45 deg pulse with intervals of 2 s; Fourier transform data points, 32768; sample tube, 5 mm i.d.

Fig. 6 ^{13}C -NMR spectra of standard caffeine (A) and isolated caffeine (B). Conditions: frequency, 67.5 MHz, 45 deg pulse with intervals of 5 s; Fourier transform data points, 65536. Other conditions were same as given in Fig. 5 caption.

Table 1 Identification of methyl groups contained in caffeine (standard and extracted) according to chemical shifts in its ^1H -NMR spectra

Chemical shifts, ppm		Functional groups in caffeine structure
Standard	Isolated	
0.000	0.000	reference TMS
1.649	1.759	methyl group $\text{CH}_3^{(11)}$
1.826	1.925	methyl group $\text{CH}_3^{(10)}$
2.237	2.341	methyl group $\text{CH}_3^{(12)}$
5.518	5.614	(*)
5.751	5.853	

(*) impurities. Numbers in parentheses give the place where the functional groups are located in the caffeine structure in Fig. 4A.

of 2/10 of ppm higher than that in the standard caffeine, except for around 141.3 ppm and those due to CHCl_3 impurities between 77.475 and 76.532 ppm.

Although the peak shift of the C(6) of the ketone group should have appeared at 167.5 ppm⁷, no such peak shift appeared for this ketone group, because of nuclear Overhauser effect.

The peak intensities were lower in the isolated caffeine than those in the standard caffeine because of the lower sample concentration. An additional peak appeared in the isolated caffeine spectrum at 77.202 ppm with a low intensity compared with the three peaks at 76.532, 77.000 and 77.475 ppm, respectively. This peak may have come from the isolated caffeine.

Table 2 summarizes the NMR signal assignments of the functional groups related to the peaks in the caffeine structure.

In this way, the NMR measurements confirmed that the crystal was caffeine.

SFE and SFC allowed the isolation of caffeine from roasted coffee beans as a crystal in less than three hours. Two runs offered about 8 mg of caffeine. This amount was sufficient to allow IR and NMR measurements. In this work, we focused on the isolation and identification of caffeine, however, we also obtained oil fractions which gave the coffee aroma. An analysis of flavor compounds in the SFE extract of coffee is currently under way; the results will be published elsewhere.

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Table 2 Identification of carbon atoms contained in caffeine (standard and extracted) according to chemical shifts in its ^{13}C -NMR spectra

Chemical shifts, ppm		Functional groups in caffeine structure
Standard	Isolated	
27.736	27.916	methyl group $\text{CH}_3^{(12)}$
29.565	29.930	methyl group $\text{CH}_3^{(11)}$
33.415	33.567	methyl group $\text{CH}_3^{(10)}$
76.532	76.532	(*)
77.000	77.000	
—	77.202	
77.475	77.475	(**)
107.395	107.611	
141.303	141.368	
148.517	148.718	C ⁽⁴⁾
151.526	151.734	C ⁽⁸⁾
155.212	155.442	C ⁽²⁾
		C ⁽⁵⁾

(*) contamination of solvent CDCl_3 ; (**) impurity. Numbers in parentheses give the place where the functional groups or atoms are located in the caffeine structure in Fig. 4A.

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