

Analysis of the Contents of Pungent Compounds in Fresh Korean Red Peppers and in Pepper-Containing Foods

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An HPLC method has been developed for the analysis of extracts of fresh peppers containing capsaicinoids and of both capsaicinoids and piperines in pepper-containing foods produced and sold in Korea. The HPLC method was optimized by defining how composition of the mobile phase affected retention times. Both identification and quantification were based on retention times and the following criteria: linearity of the UV response at 280 nm in HPLC, recoveries from spiked samples, and observed individual molecular ions in the mass spectra of the extracts determined by liquid chromatography–mass spectrometry. This method, with a limit of detection of ~15–30 ng, was used to quantify the distribution of capsaicinoids in 11 Korean whole peppers and in 12 commercial pepper-containing foods. Total capsaicinoid levels of whole peppers ranged from 1.21 $\mu\text{g/g}$ for the PR Gang ja variety to 121.1 $\mu\text{g/g}$ for the Chung yang variety. The levels in food extracts, four of which also included two piperines, ranged from 11.0 $\mu\text{g/g}$ for radish kimuchi to 3752 $\mu\text{g/g}$ for capsaicin sauce. The results demonstrate (a) the usefulness of the HPLC method for the simultaneous analysis of capsaicinoids derived from red peppers and piperines derived from black and white peppers extracted from complex food matrices and (b) the wide-ranging spread of levels of pungent pepper compounds in fresh peppers and in pepper-containing foods consumed in Korea.

KEYWORDS: Capsaicinoids; piperines; HPLC; LC-MS; peppers; pepper-containing foods

INTRODUCTION

Capsaicinoids present in red peppers and piperines present in black and white peppers cause pungent, hot-tasting, sensations when consumed as part of the diet. In addition to sensory properties, capsaicinoids and piperines are reported to possess beneficial biological properties that may affect human health. Reported effects of capsaicinoids include antimicrobial activities against disease-causing bacteria as well as against aquatic microorganisms that coat (foul) submerged surfaces of ships (1–8). They have also exhibited protective effects against mutagens and carcinogens (9, 10), cholesterol (11), obesity (12), and pain (13–15) and are widely used as pain-inducing defensive pepper sprays (16–19). Reported beneficial effects for piperines include antibacterial (20), anticarcinogenic and antigenotoxic (21–24), antidepressant (25), antioxidative (26), and insulin-resistance (27) activities.

Although a variety of methods have been used for the analysis of capsaicinoids (18, 19, 28–36), these considerations suggest the need for improved analyses of pepper compounds, especially in foods. Many of the more recently described procedures were

used to analyze capsaicinoid levels in pepper sprays, but none of these procedures were used to determine the major and minor capsaicinoids in extracts of pepper-containing foods.

Previously, we described HPLC and LC-MS methods for the analysis of capsaicinoids present in various parts of the pepper fruit, in fresh and dry peppers, and in pepper-containing liquid and dry foods sold in the United States (37). Because the capsaicin and nonivamide HPLC peaks overlapped, we had to use two separate HPLC methods to quantitate all of the capsaicinoids. To overcome this problem, the main objectives of this study were (a) to devise and validate an HPLC method for the analysis in a single run of the major and minor capsaicinoid levels in whole fresh peppers and in pepper-containing foods sold commercially in Korea and (b) to confirm the structures of individual capsaicinoids in mixtures by LC-MS. In the course of these studies we also found that some pepper-containing foods contained both capsaicinoids derived from red peppers and piperines derived from black and white peppers.

MATERIALS AND METHODS

Capsaicin ($\geq 97\%$, catalog 21748, lot 1118949) was obtained from Fluka (St. Louis, MO). Dihydrocapsaicin (catalog 37274; lot 427804/1), nonivamide (catalog V9130; lot 054k2610), a mixture of capsai-

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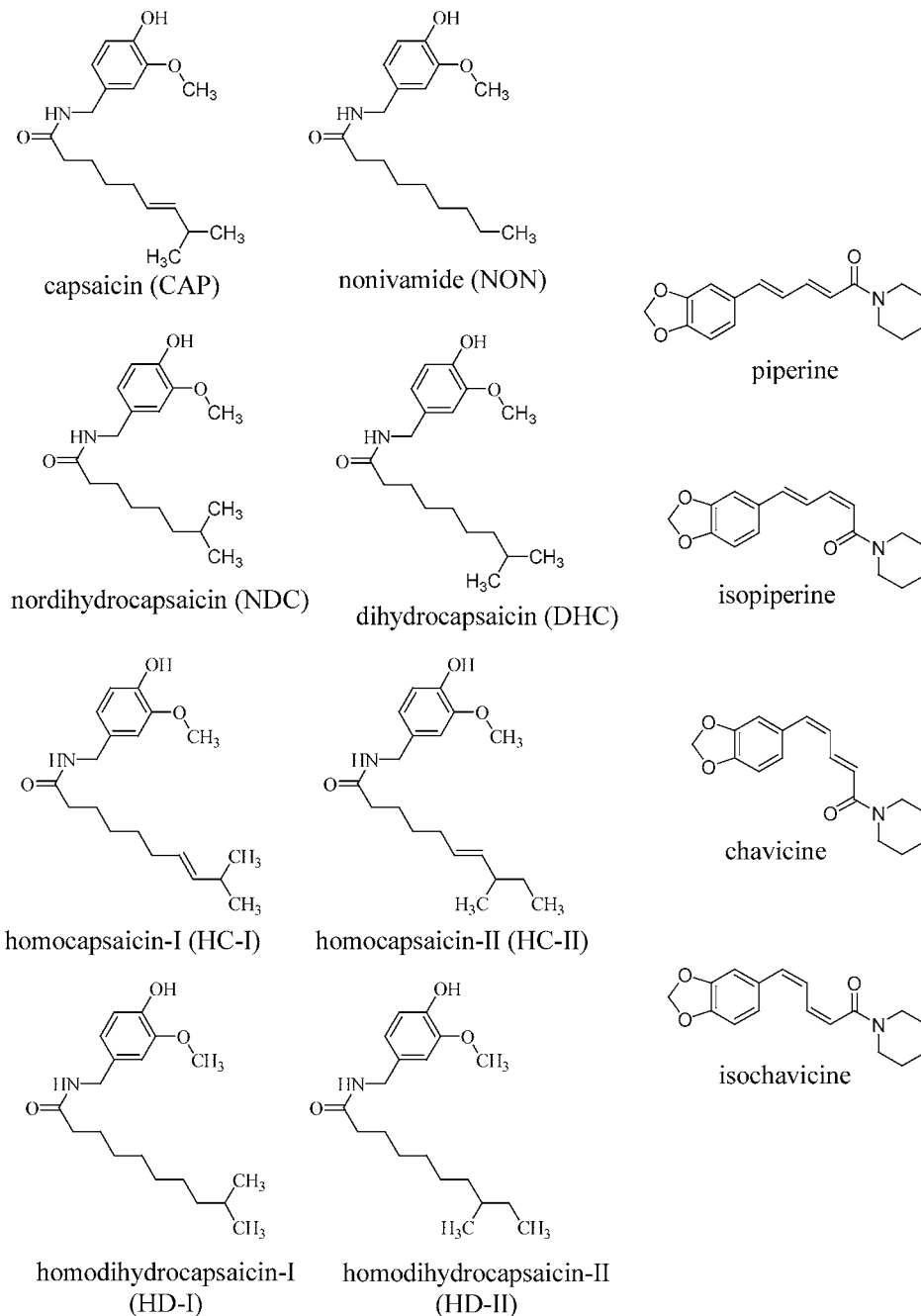


Figure 1. Structures of capsaicinoids and piperines.

Table 1. Programming of Mobile Phases A and B for the Separation of Capsaicinoids and Piperines in Peppers and Pepper-Containing Foods on the Inertsil ODS-3v (4.0 × 250 mm) HPLC Column

time (min)	A, acetonitrile (%)	B, 0.5% formic acid (%)	elution mode
0.0	31	69	isocratic
100.0	31	69	isocratic
170.0	53	47	gradient
170.1	80	20	isocratic
180.0	80	20	isocratic
180.1	31	69	isocratic
200.0	31	69	isocratic
end			

cinoids from *Capsicum* fruits (~60%; catalog M3403, lot N101k1166), and piperine (≥97%, lot 01902TD) were obtained from Sigma-Aldrich (St. Louis, MO). Solvents used were HPLC grade acetonitrile, methanol, and analytical grade formic acid, obtained from commercial sources.

The solvents were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA) and degassed in an ultrasonic bath before use.

Eleven red pepper varieties (hot to mild) were obtained from the Youngyang Pepper Experimental Station, Gyeongsangbuk, Korea, and from a farm in Deagu City, Korea. Dry pepper powder, sliced pepper, and commercial pepper-containing foods (**Table 1**) were purchased from local stores and restaurants in Daegu City, Korea.

Extraction of Capsaicinoids from Korean Fresh Whole Peppers (*Capsicum annuum* L.). Three uniform-sized peppers (average weight, 12.8 g; length, 101.1 mm; width, 17.8 mm) were used for analyses of capsaicinoids. Each whole pepper was cut into 1 × 1 mm slices by a clasp knife and mixed well. Each sample was thoroughly macerated in a mortar with 25 mL of methanol and filtered on Advantec no. 2 filter paper. The residue was then rinsed four more times with 5 mL aliquots of methanol. The filtrate was collected and brought to a volume of 50 mL with methanol. The filtrate was centrifuged at 18000g for 10 min at 1 °C. All capsaicinoid extracts were filtered through a 0.45 μm

Millipore nylon filter before HPLC. The colors of the extracts corresponded to the surface colors of the peppers.

Extraction of Capsaicinoids and of Piperines from Pepper Sauces and Pastes. Pepper sauces (extremely hot to mildly hot) and pastes (very hot) from two restaurants and a department store (Daegu, Korea) were used to determine the capsaicinoid contents of the extracts. Each sample (~0.5–1.0 g) was placed into a 20 mL volumetric flask to which was added 20 mL of methanol. The flask was then placed into an ultrasonic bath for 60 min. After filtration, the filtrate was centrifuged at 18000g for 10 min at 1 °C. The extracts were then passed through a 0.45 μ m Millipore nylon filter before HPLC analysis.

Extraction of Capsaicinoids from Dry Sliced Peppers and Pepper Powders. The commercial sliced pepper was finely ground into powders with a homogenizer. Each powder (~0.5–1.0 g) was placed into a 10 mL volumetric flask to which was added 10 mL of methanol. The flask was then placed into an ultrasonic bath for 60 min. The filtrate was centrifuged at 18000g for 10 min at 1 °C. The capsaicin extracts were then filtered through a 0.45 μ m Millipore nylon filter before HPLC analysis.

Extraction of Capsaicinoids from Kimchi Pickles. Kimchi pickles were removed from the plastic bags, cut into 5 × 5 mm slices with a clasp knife, and then thoroughly mixed. The sample (~5–7 g) was macerated in a mortar with 10 mL of methanol and filtered on Advantec no. 2 filter paper. The residue was then rinsed three more times with 4 mL of methanol. The supernatant was collected and brought to a volume of 25 mL with methanol. The filtrate was centrifuged at 18000g for 10 min at 1 °C. The capsaicinoid-containing extracts were filtered through a 0.45 μ m Millipore nylon filter before HPLC.

High-Performance Liquid Chromatography (HPLC). HPLC was carried out on a Hitachi liquid chromatograph model 665-II equipped with an autosampler (model 655A-40). The capsaicinoids were separated using a stainless steel column (250 mm × 4.0 mm i.d.) packed with Inertsil ODS-3v (5 μ m particle diameter) (GL Sciences). The binary gradient elution system used consisted of acetonitrile (A) and distilled water containing 0.5% formic acid (B). Separation was achieved using the following gradient program: 0–100 min, 31% A; 100–170 min, 31% A–53% B; 170.1–180 min, 80% A; finally, reconditioning the column with 31% A isocratic for 20 min. The column temperature was maintained with a Shimadzu column oven CTO-10vp (Shimadzu). The flow rate was 1 mL/min at 30 °C. UV detection was set at 280 nm. The capsaicinoid extract (~20–50 μ L) was injected directly into the column. Two separate analyses, each in triplicate, were carried out with separate extracts.

Liquid Chromatography–Mass Spectrometry (LC-MS). The identification of the individual capsaicinoids was achieved by LC-MS using a Finnigan LCQ Advantage MAX (Finnigan, San Jose, CA). The capsaicinoids and piperines were separated using a Zorbax Eclipse XDB-C18 column (150 × 4.6 mm, 3.5 μ m) (Agilent Technologies, Palo Alto, CA) with the gradient system described above for HPLC analysis. Acetonitrile was used as mobile phase A and distilled water containing 0.5% formic acid as mobile phase B at a flow rate of 750 μ L/min and at a column temperature of 30 °C. The mass spectrometer was equipped with an electrospray ionization source and was operated in the selected-ion monitoring (SIM) mode. Nitrogen gas was used as the sheath gas at 1.2 L/min and the auxiliary gas at 0.225 L/min with a capillary temperature of 350 °C and the spray voltage set to 5.5 kV. The mass spectrometer was operated in the positive ion mode in m/z range of 100–500. Helium was used as the collision gas for the tandem mass/mass (MS/MS = MS²) spectrometric procedures, followed by the isolation of ions over a selected mass window of 1 Da.

The following protonated molecules were monitored: nordihydrocapsaicin (m/z 294), capsaicin (m/z 306), dihydrocapsaicin (m/z 308), homocapsaicin (m/z 320) (two isomers), homodihydrocapsaicin (m/z 322) (two isomers), nonivamide (m/z 293.9), piperine (m/z 286.2), and piperine isomer (m/z 286.2).

The isomeric capsaicinoids homocapsaicin I (HC-I) and homocapsaicin II (HC-II) (m/z 308) and homodihydrocapsaicin I (HDC-I) and homodihydrocapsaicin II (HDC-II) (m/z 320) each differ in the position of the terminal CH₃ groups but not in molecular weight (Figure 2). LC-MS analysis of the mass spectra does not tell us which chromatographic peak is associated with which isomer. The relative intensity of

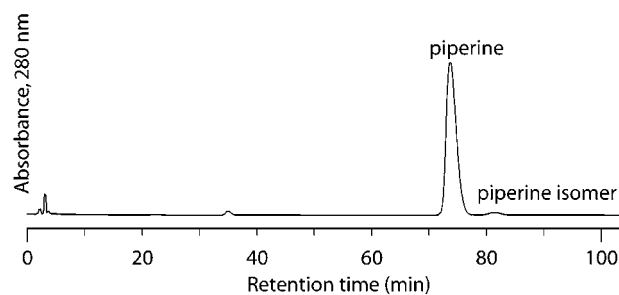


Figure 2. HPLC chromatogram of a commercial standard containing piperine and a piperine isomer. Column, Inertsil ODS-3v (5 μ m, 4.0 × 250 mm); column temperature, 30 °C; mobile phase, gradient mode of acetonitrile/0.5% formic acid; detector, UV at 280 nm; flow rate, 1 mL/min.

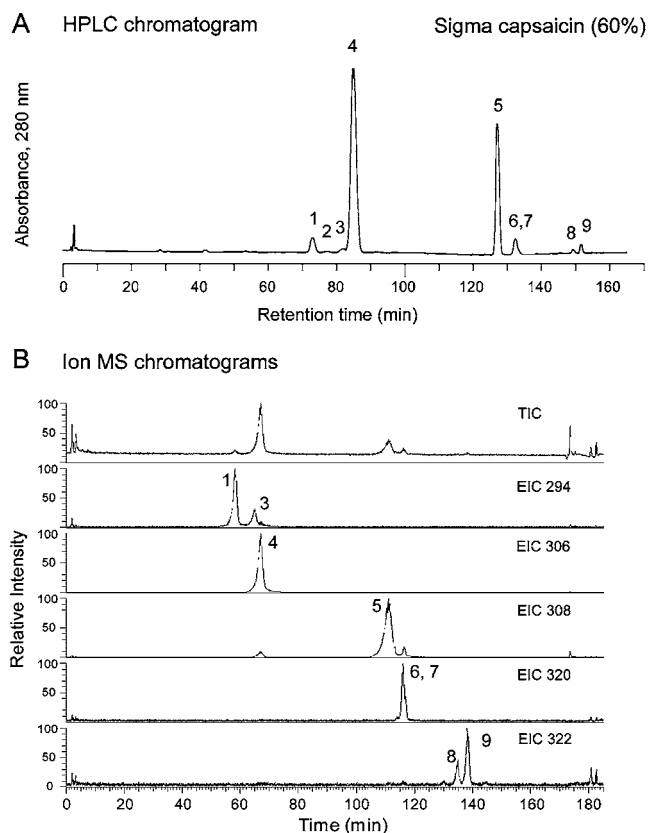


Figure 3. Sigma capsaicin (60%) used as a standard: (A) HPLC chromatogram; (B) LC-MS chromatograms. Peaks: 1, nordihydrocapsaicin (NDC); 2, unknown peak (UKP); 3, nonivamide (NON); 4, capsaicin (CAP); 5, dihydrocapsaicin (DHC); 6, 7, homocapsaicins I and II (HC-I and II); 8, homodihydrocapsaicin I (HDC-I); 9, homodihydrocapsaicin II (HDC-II).

m/z 320 of HC-II to that of m/z 320 of HC-I equals 0.33, whereas the corresponding ratio of the relative intensity of m/z 322 of the HDC-II peak to that of m/z 322 of the HDC-I peak equals 1.71.

The following abbreviations relate to the described mass spectra: EIC represents extracted ion current of the analyte of interest during the chromatographic analysis; TIC represents the total ion current equal to the summation of all intensity fragment ions and parent ion intensity. MS/MS = MS² represents tandem mass spectrometry, whereby mass selection of the analyte by m/z (mass divided by charge) is followed by fragmentation and analysis of the fragments.

Quantification of Capsaicinoids and Piperines. Because only three standard capsaicinoids (capsaicin, dihydrocapsaicin, and nonivamide) were available to us, the concentrations of capsaicinoids and piperines present as mixtures in all extracts of peppers as well as in pepper-containing foods were determined as capsaicin (CAP) and piperine equivalents. This was done by calculating the relative contribution of

Korean Sliced Red Pepper

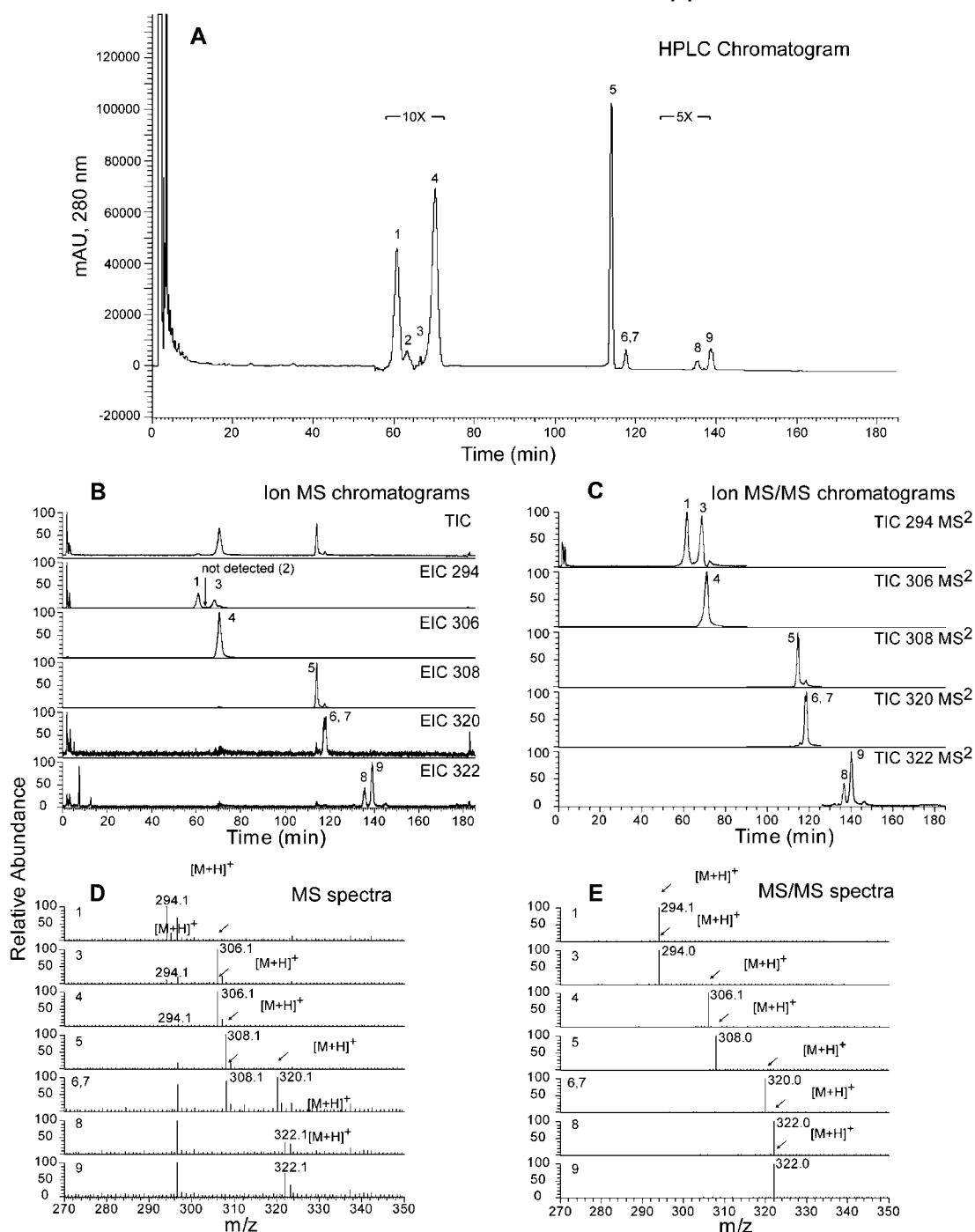


Figure 4. HPLC chromatograms of capsaicinoids in an extract from Korean sliced red pepper: (A) HPLC chromatogram; (B) LC-MS ion mass chromatograms; (C) LC-MS ion mass/mass chromatograms; (D) mass spectra; (E) mass/mass spectra. Peaks: 1, nordihydrocapsaicin (NDC); 2, unknown peak; 3, nonivamide (NON); 4, capsaicin (CAP); 5, dihydrocapsaicin (DHC); 6, 7, homocapsaicins I and II (HC-I and II); 8, homodihydrocapsaicin I (HDC-I); 9, homodihydrocapsaicin II (HDC-II).

each UV response peak to the sum of all the peaks contained in the Sigma capsaicin mixture (~60%) and Sigma piperine standard using the Hitachi Chromato-integrator model D-2500. The calibration curve of each compound was obtained by plotting the content obtained against the amount injected. Each peak was identified by comparing retention times obtained by HPLC and by LC-MS. The observed retention times with the Inertsil HPLC column, but not the order of elutions, differed somewhat from the corresponding retention times observed with Zorbax LC column.

Recovery of Capsaicinoids and Piperine after Spiking. Peppers were analyzed before and after the addition of known amounts of the

mixture of capsaicinoids and piperine: $\text{recovery (\%)} = \frac{\text{concentration in spiked sample}}{\text{concentration of endogenous compound} + \text{spike}} \times 100$.

RESULTS AND DISCUSSION

Analytical Aspects. Figure 1 displays the structures of the eight known capsaicinoids and of four piperines. Figure 2 shows an HPLC chromatogram of a commercial sample labeled "piperine" that also contained a small amount of a piperine isomer. Figure 3A illustrates HPLC chromatograms of the

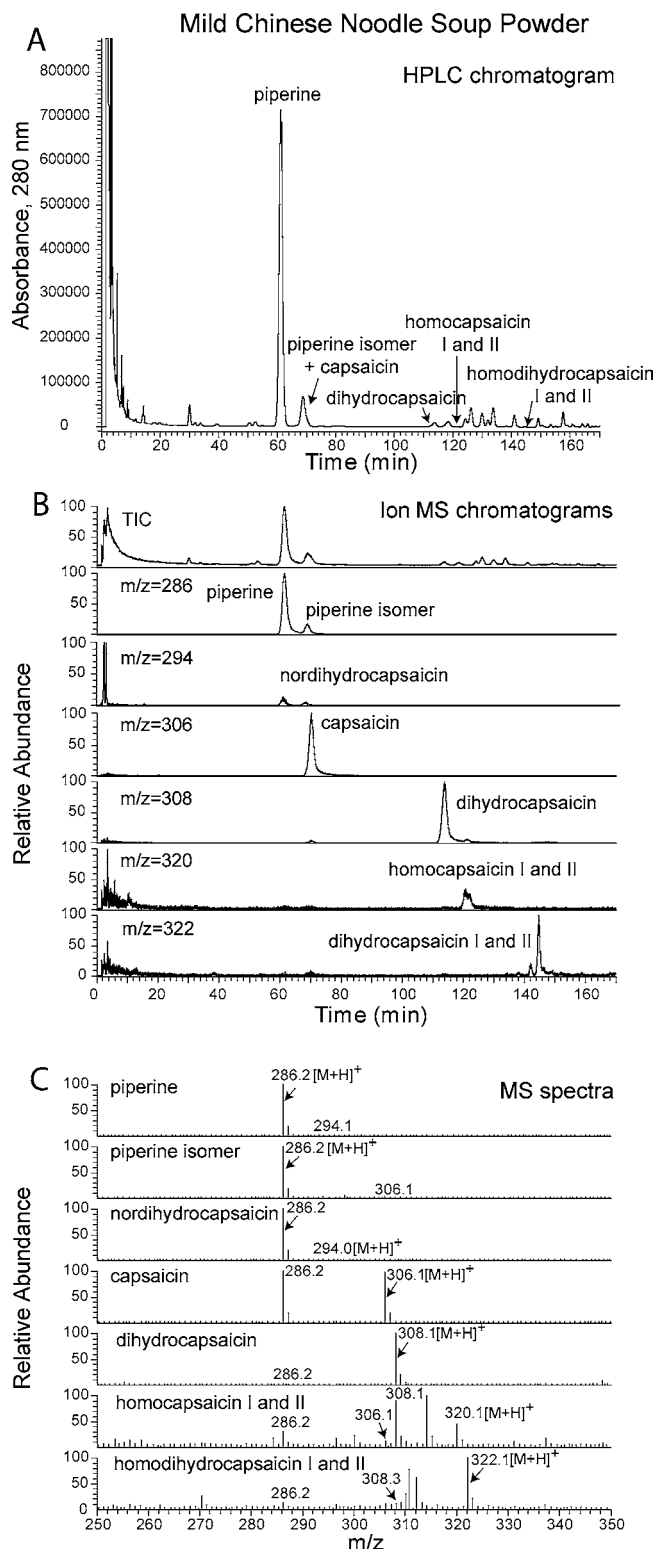


Figure 5. (A) HPLC chromatogram of an extract of mild Chinese noodle powder; (B) LC-MS ion MS chromatograms; (C) MS spectra.

capsaicinoids present in a commercial pepper extract used as a standard for establishing elution positions of chromatographic peaks associated with specific compounds, and **Figure 3B** shows the corresponding mass spectra. **Figure 4A** shows an HPLC chromatogram of Korean sliced red peppers, **Figure 4B** shows the ion mass chromatograms, **Figure 4C** shows the corresponding mass/mass chromatograms, and parts **D** and **E** of **Figure 4** show the corresponding mass spectra. **Figure 5A** shows an HPLC chromatogram of an extract of Chinese noodle soup

powder, and parts **B** and **C** of **Figure 5** show the corresponding mass spectra.

Identification and quantitation of each compound was determined by LC-MS of elution profiles of each peak associated with each of the protonated molecular ion peaks $[M + H]^+$. The measured values in terms of CAP equivalents for each constituent of the commercial Sigma pepper extract were similar to those previously determined with the dual HPLC assay (37).

Table 1 shows the composition of the mobile phase consisting of acetonitrile (A) and distilled water containing 0.5% formic acid (B). **Table 2** shows that retention times ranged from 73.2 min for nordihydrocapsaicin (NDC) to 152.3 min for homodihydrocapsaicin (HDC-II); the plots of concentration versus peak areas (calibration plots) were linear from 0 to 2500 ng. The limit of detection estimated from the calibration plots ranged from 15.4 to 30.4 ng, and recoveries of spiked samples ranged from 89.6 ± 7.1 to $112.3 \pm 5.9\%$ ($n = 3$). The absence of baseline noise in the chromatograms, linear concentration responses of integrated peak areas, and good recoveries of all compounds from spiked peppers all support the validity of the HPLC method. The excellent reproducibility of the analyses and the ability of the method to accurately measure $\sim 0.5\%$ of a capsaicinoid demonstrate its high sensitivity at nanogram levels.

The retention times on the Inertsil HPLC column, but not the order of elutions, differed somewhat from the corresponding retention times on the Zorbax LC-MS column. In addition, because the peaks associated with minor capsaicinoids HC-I and HC-II were not separated on either column, we list the sum of concentrations for these two compounds.

The piperine data deserve additional comment. It is known that piperine can exist as four distinct geometric isomers: *E,E*-(*trans-trans*)-piperine (piperine), *Z,E*-(*cis-trans*)-piperine (isopiperine), *E,Z*-(*trans-cis*)-piperine (isochavicine), and *Z,Z*-(*cis-cis*)-piperine (chavicine) (**Figure 1**) (38). *E,E*-(*trans-trans*)-Piperine appears to largely contribute the pungency of peppers of black and white peppers. The other three isomers with low or no pungency appear to be formed by UV-light-induced isomerization of the double bonds of the parent piperine molecule. Note the presence of a small peak in the chromatogram of the commercial piperine sample shown in **Figure 2**. Detailed NMR studies are needed to establish the structure of this isomer. Much larger amounts of the same isomer were present in some of the pepper-containing foods (see below).

Capsaicinoid Content of Korean Whole Red Fresh Peppers. To demonstrate the applicability of the analytical method to fresh peppers, we analyzed the capsaicinoid levels of the fruits of 11 Korean whole red peppers ranging from very hot to very mild (**Table 3**). Levels of total capsaicinoids ranged from 1.2 μg of CAPS equiv/g of fresh weight for the PR Gang ja pepper variety to 121 $\mu\text{g}/\text{g}$ for the very hot Chung yang variety, a 100-fold difference from highest to lowest value. The relative content of the individual capsaicinoids also varies widely. Only the first two peppers listed (Chung yang and Buchon) contained measurable amounts of all nine compounds. Chung yang extracts contained the following levels of capsaicinoids (in percent relative abundance; total of 121 $\mu\text{g}/\text{g} = 100\%$): nordihydrocapsaicin (NDC), 4.4; unknown peak (UKP), 0.2; nonivamide (NOV), 0.2; capsaicin (CAP), 29.4; dihydrocapsaicin (DHC), 54.1; sum of homocapsaicin I (HC-I) and homocapsaicin II (HC-II), 6.3; homodihydrocapsaicin I (HDC-I) 1.7; and homodihydrocapsaicin II (HDC-II), 3.6. The corresponding values for the Buchon pepper variety (total of 80.3 $\mu\text{g}/\text{g} = 100\%$ total) are

Table 2. HPLC Analysis of Standards: Retention Times, Minimum Detection Limits, Tests for Linearity, and Recoveries after Spiking of Peppers

compound	retention time (min)	detection limit (ng)	linearity range ^a (ng)	recovery after spiking ^b (%)
nordihydrocapsaicin (NDC)	73.2 ± 0.2	19.7	0–2000	94.4 ± 3.8
unknown peak (UKP)	77.5 ± 0.2	15.4	0–2000	92.3 ± 4.6
nonivamide (NOV)	82.3 ± 0.2	18.6	0–2000	93.8 ± 4.2
capsaicin (CAP)	85.4 ± 0.2	17.3	0–2500	95.2 ± 4.5
dihydrocapsaicin (DHC)	127.7 ± 0.1	19.7	0–1800	98.2 ± 2.1
homocapsaicin I (HC-I)	127.7 ± 0.1	17.7	0–500	106.7 ± 4.3
homocapsaicin II (HC-II)	127.7 ± 0.1	15.3	0–1100	112.3 ± 5.9
homodihydrocapsaicin I (HDC-I)	150.1 ± 0.1	30.4	0–500	98.5 ± 7.5
homodihydrocapsaicin II (HDC-II)	152.3 ± 0.1	23.9	0–500	89.6 ± 7.1
piperine	73.3 ± 0.8	10.1	0–2000	92.6 ± 3.9
piperine isomer	81.4 ± 0.2			

^a Linear plots of concentration versus peak area in μV ; $n = 3$; $r^2 = 0.99$ for all compounds. ^b Korean Chung yang red peppers were spiked before extraction with a 60% commercial mixture of capsaicinoids (Sigma).

Table 3. Capsaicinoid Content of Korean Whole Red Peppers^a

pepper variety	NDC ^b	UKP	NOV	CAP	DHC	HC-I; HC-II	HD-I	HD-II	total
Chung yang	5.4 ± 0.19	0.3 ± 0.03	0.2 ± 0.04	35.7 ± 1.9	65.5 ± 2.7	7.6 ± 0.66 ^c	2.1 ± 0.16	4.4 ± 0.38	121
Buchon	6.5 ± 0.34	0.3 ± 0.02	0.2 ± 0.02	41.5 ± 1.8	24.0 ± 1.3	4.1 ± 0.32	1.0 ± 0.10	2.8 ± 0.22	80.3
Chun ha tong il	0.9 ± 0.06	ND	ND	5.2 ± 0.33	3.0 ± 0.20	0.1 ± 0.01	0.3 ± 0.03	0.8 ± 0.09	10.4
Hanbando	1.4 ± 0.09	ND	ND	2.0 ± 0.13	5.2 ± 0.48	0.3 ± 0.03	0.1 ± 0.02	0.6 ± 0.04	9.7
PR Da dda	1.7 ± 0.09	ND	ND	3.5 ± 0.27	1.1 ± 0.08	ND	0.1 ± 0.01	0.6 ± 0.05	7.1
PR Bulsajo	0.7 ± 0.03	ND	ND	0.9 ± 0.11	1.0 ± 0.03	ND	ND	ND	2.6
Keum hyang	0.9 ± 0.04	ND	ND	0.7 ± 0.06	0.9 ± 0.01	ND	ND	ND	2.5
Wang dae bark	0.7 ± 0.01	ND	ND	1.4 ± 0.13	0.3 ± 0.01	ND	ND	ND	2.4
Yeuck gang hong jang gun	1.0 ± 0.08	ND	ND	0.4 ± 0.01	0.6 ± 0.06	ND	ND	ND	2.0
Dock ya chung chung	0.5 ± 0.05	ND	ND	0.7 ± 0.06	0.6 ± 0.05	ND	ND	ND	1.8
PR Gang ja	0.6 ± 0.04	ND	ND	0.1 ± 0.01	0.5 ± 0.03	ND	ND	ND	1.2

^a Values in $\mu\text{g/g} \pm \text{SD}$ of fresh weight; $n = 3$; ND = not detected. ^b See **Table 3** for names of compounds shown as abbreviations. ^c Sum of HC-I and HC-II.

Table 4. Sources of Korean Pepper-Containing Commercial Foods Evaluated in This Study

pepper food	company name and location
pepper sauces	
boiled fish paste seasoning sauce (o-den sauce)	Pal Gan Ji Bung Co., Daegu, Korea
rice cake seasoning sauce (topkogi)	Hong Cho Bul Dak Co., Daegu, Korea
spicy rice cake sauce (topkogi)	Sin Dduck Co., Daegu, Korea
capsaicin sauce	Green Food, Co., Daegu, Korea
pepper soup powders	
hot Chinese noodles powder	Nong Shim. Co., Seoul, Korea
mild Chinese noodles powder	Nong Shim. Co., Seoul, Korea
pepper powders	
chili powder	Hyangwon Spice Co., Seoul, Korea
red pepper powder	Agricultural Cooperative of Koesangun, Koesan, Korea
pepper pastes	
gochujang hot pepper paste (A)	Gobul Restaurant Co., Daegu, Korea
sunchang gochujang hot pepper paste (B)	Desang Food Co., Seoul, Korea
pickles	
radish kimuchi	Restaunt Co., Gyeongjiu, Korea
pepper slices	
red pepper, sliced	Hanguk Sang San Co., Seoul, Korea

($\mu\text{g/g}$): NDC, 8.1; UKP, 0.4; NOV, 0.2; CAP, 51.7; DHC, 29.9; sum of HC-I and HC-II, 5.1; HDC-I, 1.2; and HDC-II, 3.4.

The cited results show that dihydrocapsaicin contributes the highest amount (54.1%) to the total capsaicinoid level of the Chung yang pepper variety and that capsaicin contributes the second highest amount (29.4%). In contrast, capsaicin contributes the highest amount (51.7%) to the total of the Buchon variety, and dihydrocapsaicin, the second highest amount of 29.90%. **Table 4** also shows that two varieties (Chun ha tong il, Hanbando) contained six, one variety (PR Da dda) five, and the other six varieties only three measurable levels of capsaicinoids. Both absolute and relative amounts of capsaicinoids differ widely among the evaluated red peppers grown in two nearby locations in Korea.

Relevant previous studies include the following observations. Using LC-MS, Reilly et al. (39) determined levels of six capsaicinoids including nonivamide in six peppers grown in the United States. Total values ranged from 0.0018 $\mu\text{g/g}$ (yellow bell pepper) to 510 $\mu\text{g/g}$ (habanero peppers). These authors note that standard HPLC methods did not chromatographically separate capsaicin from nonivamide and that GC-MS methods were ineffective in differentiating nordihydrocapsaicin from nonivamide. Karnka et al. (33) used a sequential simplex HPLC method to measure levels of two capsaicinoids (capsaicin and dihydrocapsaicin) in mature chili pods grown in Thailand. Pyorazolgu et al. (40) analyzed by HPLC three capsaicinoids (capsaicin, dihydrocapsaicin, and nordihydrocapsaicin) in four Turkish chili peppers and three pepper seeds. Thompson et al.

Table 5. HPLC Analysis of Individual and Total Piperine and Capsaicinoid Content of Korean Peppers and Pepper-Containing Commercial Foods^a

sample	piperine ^b	piperine isomer ^b	NDC	UKP	NON	CAP	DHC	HC-1; HC-II ^c	HDC-I	HDC-II	total
capsaicin sauce	ND	ND	220 ± 5.6	146 ± 15.0	75.7 ± 1.4	1719 ± 79	1325 ± 18.4	122 ± 2.7 ^c	45.4 ± 11.0	96.9 ± 7.5	3752 ^d
chili powder	ND	ND	288 ± 5.3	0.2 ± 0.01	86.0 ± 1.4	1461 ± 0.76	1415 ± 6.8	126 ± 3.2	31.7 ± 6.9	122 ± 10.2	3530 ^d
hot Chinese noodle	658 ± 41.5	198 ± 6.1	ND	ND	ND	67.8 ± 9.97	41.4 ± 3.02	ND	ND	3.0 ± 0.06	928 (857; 71) ^e
mild Chinese noodle	475 ± 24.9	24.5 ± 3.4	ND	ND	ND	44.4 ± 1.8	33.9 ± 3.4	4.5 ± 0.27	ND	2.0 ± 0.18	584 (499; 85) ^e
red pepper powder	ND	ND	33.9 ± 1.4	ND	7.2 ± 1.7	236 ± 2.7	118 ± 12.8	16.5 ± 0.78	4.8 ± 0.35	8.8 ± 1.2	425 ^d
rice cake seasoning	ND	ND	109 ± 0.53	ND	5.6 ± 1.0	90.0 ± 8.5	79.8 ± 3.03	12.7 ± 0.35	1.6 ± 0.52	6.7 ± 0.04	305 ^d
sauce (Tokpogi)											
hot pepper paste (A) (gochujang)	128 ± 1.7	14.0 ± 2.5	ND	ND	ND	58.5 ± 6.6	57.6 ± 0.14	7.1 ± 0.28	ND	3.2 ± 0.27	269 (142; 127) ^e
hot spicy rice cake sauce (tokpogi)	91.5 ± 3.4	26.0 ± 1.9	ND	ND	ND	67.3 ± 5.6	46.7 ± 1.9	6.6 ± 0.02	2.0 ± 0.35	3.5 ± 0.33	244 (117; 127) ^e
red pepper, pepper	ND	ND	7.3 ± 0.48	ND	4.6 ± 0.69	63.4 ± 3.3	32.5 ± 3.0	12.9 ± 1.9	4.0 ± 1.1	6.4 ± 1.3	131 ^d
boiled fish paste	ND	ND	8.1 ± 0.07	12.3 ± 0.03	ND	7.1 ± 0.31	12.9 ± 0.16	4.1 ± 0.29	ND	1.46 ± 0.22	46.0 ^d
seasoning sauce (o-den sauce)											
hot pepper paste (B) (sun-chang gochujang)	ND	ND	3.6 ± 0.16	ND	ND	22.8 ± 1.0	14.8 ± 0.85	1.3 ± 0.02	ND	1.7 ± 0.24	44.2 ^d
radish kimuchi	ND	ND	1.0 ± 0.22	ND	ND	5.1 ± 0.67	3.1 ± 0.63	0.60 ± 0.15	0.52 ± 0.31	0.74 ± 0.37	11.0 ^d

^a Values in $\mu\text{g/g} \pm \text{SD}$; $n = 3$; ND = not detected. ^b Piperine equivalents. ^c Sum of HC-I and HC-II. ^d Capsaicin equivalents. ^e Piperine; capsaicin equivalents.

(18, 36) used liquid chromatography–electrospray ionization–mass spectrometry (LC-ESI-MS) to determine nine known and one unknown capsaicinoid in a frozen jalapeno and a fresh habanero pepper.

Capsaicinoids Content of Korean Dry and Liquid Pepper-Containing Foods. Table 4 lists the names and sources of 12 Korean pepper-containing commercial foods, and Table 5 shows the determined levels of capsaicinoids in these dry and liquid foods. The total capsaicinoid levels of eight foods containing only capsaicinoids (in CAP equivalents per gram of original product) ranged from 11.0 $\mu\text{g/g}$ for radish kimuchi to 3752 $\mu\text{g/g}$ in capsaicin sauce. The total amounts in six other products were as follows: chili powder, 3530 $\mu\text{g/g}$; red pepper powder, 425 $\mu\text{g/g}$; rice cake seasoning sauce, 305 $\mu\text{g/g}$; sliced red pepper, 131 $\mu\text{g/g}$; boiled fish paste seasoning sauce, 46.0 $\mu\text{g/g}$; and hot pepper paste (B), 44.2 $\mu\text{g/g}$. There is a 341-fold difference in total capsaicinoids from the highest to lowest amounts in the tested pepper foods.

The chromatograms of one food (capsaicin sauce) contained an unknown compound (146 $\mu\text{g/g} = 3.9\%$ of total). Four foods contained, in addition to capsaicinoids, two piperine isomers with the same parent ion peaks (m/z 286.2). The following levels of piperines and capsaicinoids (in $\mu\text{g/g}$) were measured in these foods: hot Chinese noodles soup powder, piperines, 857, and capsaicinoids, 71; mild Chinese noodles soup powder, piperines, 499, and capsaicinoids, 85; hot pepper paste (A), piperines, 142, and capsaicinoids, 127; and hot spice rice cake sauce, piperines, 117, and capsaicinoids, 127.

The cited data show that piperines are present at high concentrations in four pepper-containing foods but not in the other eight foods evaluated. The following is a possible explanation for why some Korean foods contain both capsaicinoids and piperines. In Korea, dry red pepper fruits and powders containing capsaicinoids are quite expensive. In contrast, black and white pepper fruits and powders containing piperines are not expensive. For economic reasons, some food companies therefore mix the black and white powders with red pepper powders to produce less expensive pepper products and pepper-containing foods.

Conclusions. The data on the content of capsaicinoids in fresh peppers and pepper-containing foods listed in Tables 4 and 5

complement a larger set (17 species of peppers and 23 pepper-containing foods) of analogous data on capsaicinoid levels of peppers and pepper foods sold in the United States (37). With the possible exceptions of analyzed capsaicinoid levels in paprikas by Peusch et al. (41) and of salsa seasonings by Perkins et al. (35), to our knowledge there are no other recently reported studies on the capsaicinoid content of numerous commercial pepper-containing foods consumed worldwide. Taken together, the two sets show that there is wide variation in both individual and total amounts of capsaicinoids among pepper-containing foods sold in the United States and Korea and that some foods may contain both pungent capsaicinoids and piperines. Because a variety of factors including agricultural practices, soil and plant nutrition, geographical origin, and pre- and postharvest exposure to light may influence the nature and amounts of both classes of pungent compounds in fresh and processed peppers and pepper-containing foods (42), labeling for capsaicinoid and piperine content would undoubtedly benefit consumers.

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