

Analysis of Eight Capsaicinoids in Peppers and Pepper-Containing Foods by High-Performance Liquid Chromatography and Liquid Chromatography–Mass Spectrometry

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Diverse procedures have been reported for the isolation and analysis of secondary metabolites called capsaicinoids, pungent compounds in the fruit of the *Capsicum* (Solanaceae) plant. To further improve the usefulness of high-performance liquid chromatography (HPLC), studies were carried out on the analysis of extracts containing up to eight of the following capsaicinoids: capsaicin, dihydrocapsaicin, homocapsaicin-I, homocapsaicin-II, homodihydrocapsaicin-I, homodihydrocapsaicin-II, nonivamide, and nordihydrocapsaicin. HPLC was optimized by defining effects on retention times of (a) the composition of the mobile phase (acetonitrile/0.5% formic acid in H₂O), (b) the length of the Inertsil column, and (c) the capacity values (*k*) of the column packing. Identification was based on retention times and mass spectra of individual peaks. Quantification was based on the UV response at 280 nm in HPLC and recoveries from spiked samples. The method (limit of detection of ~15–30 ng) was successfully used to quantify capsaicinoid levels of parts of the pepper fruit (pericarp, placenta, seeds, and in the top, middle, and base parts of whole peppers) in 17 species of peppers and in 23 pepper-containing foods. The results demonstrate the usefulness of the method for the analysis of capsaicinoids ranging from ~0.5 to 3600 μ g of capsaicin equiv/g of product. The water content of 12 fresh peppers ranged from 80.8 to 92.7%. The described freeze-drying, extraction, and analysis methods should be useful for assessing the distribution of capsaicinoids in the foods and in defining the roles of these biologically active compounds in the plant, the diet, and medicine.

KEYWORDS: Capsaicinoids; analysis; HPLC; LC-MS; fresh peppers; freeze-dried peppers; pepper-containing foods

INTRODUCTION

Peppers belong to the Solanaceae plant family, as do potatoes, tomatoes, and jimson weeds. All of these plants produce secondary metabolites, presumably for protection against phytopathogens. Potatoes produce the glycoalkaloids α -chaconine and α -solanine and calystegine alkaloids (1); tomatoes, the glycoalkaloids dehydrotomatine and α -tomatine (2, 3); jimson weed seeds, the alkaloids atropine and scopolamine (4, 5); and peppers (*Capsicum annuum*, *Capsicum chinense*, *Capsicum frutescens*), a series of compounds called capsaicinoids. Peppers also contain high levels of antioxidative vitamin C and carotenoids (6). Interest in capsaicinoids arises from the fact that

some of them exhibit strong pungent sensory properties when consumed as part of the diet. In addition, capsaicinoids are reported to possess a variety of biological properties that may affect human health (7, 8). These include antimicrobial activities against pathogenic bacteria including Bacilli (9, 10), Clostridia (9), Salmonellae (11), and Helicobacter (12); antioxidative activities (13); potential anticancer effects (14); and broad bioactivities against neuronal structures that contain substance P associated with nociception (pain transmission) and neurogenic inflammation (burning and stinging of hands, mouth, and eyes) (7, 15). The latter properties are the basis for the use of capsaicinoids to treat pain and as defensive pepper sprays (16, 17).

Daily per capita consumption of capsaicinoids from peppers in Europe and the United States is estimated at ~1.5 mg and that in India, Mexico, and Thailand at ~25–200 mg (8). The European Scientific Committee on Food concluded that “the

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available data did not allow to establish a safe exposure level of capsaicinoids in food" (8).

A variety of methods have been used for the analysis of capsaicinoids. These include colorimetry/spectrophotometry (18–20), gas chromatography (21), complexation chromatography (22), supercritical fluid chromatography (23), liquid chromatography (24–27), liquid chromatography coupled with gas chromatography–mass spectrometry (28), liquid chromatography–mass spectrometry (16, 17, 29, 30), and enzyme immunoassays (31). The main objectives of this study were to devise and validate improved methods using HPLC and to measure the content of eight capsaicinoids in different parts of the pepper plant, in fresh peppers, in freeze-dried whole peppers, and in dry and liquid pepper-containing foods sold commercially. The approach used complements and extends earlier studies and may have advantages in some applications.

Because the nitrogen atoms of capsaicinoids are not part of a heterocyclic ring, they have been classified as proto- or pseudoalkaloids (18, 32). In this paper, we will use the term "capsaicinoids" to designate the pungent pepper compounds. Other investigators have used such terms as "alkamides", "capsaicins", "capsoids", or "vanilloids".

MATERIALS AND METHODS

Capsaicin ($\geq 97\%$, catalog no. 21748, lot 1118949) and dihydrocapsaicin ($\geq 90\%$, catalog no. 37274, lot 427804/1) were obtained from Fluka (St. Louis, MO). Capsaicin ($\geq 95\%$, catalog no. M2028, lot 023K70321), a mixture of capsaicinoids from *Capsicum* fruits ($\sim 60\%$ capsaicin and $\sim 30\%$ dihydrocapsaicin, catalog no. M3403, lot 101K1166), and nonivamide (*N*-vanillylnonanamide, $\geq 97\%$, catalog no. V9130, lot 054K2610) were obtained from Sigma (St. Louis, MO). HPLC grade acetonitrile, methanol, and analytical grade formic acid were obtained from commercial sources. The solvents were filtered through a $0.45\text{-}\mu\text{m}$ membrane filter (Millipore, Bedford, MA) and degassed in an ultrasonic bath before use.

Ten red pepper varieties (very hot to mild) grown in Korea were obtained from the Youngyang Pepper Experimental Station, Gyeongsangbuk, Korea. Whole fresh and dry peppers, canned peppers, and commercial pepper-containing foods were purchased from local stores in California. The sources of the test substances are listed in the footnotes to the tables.

Freeze-Drying of Fresh and Canned Peppers. Fresh peppers from a local market (Safeway, Albany, CA) were rinsed in distilled water and patted dry. The caps were snapped off and discarded. The peppers were then sliced, immediately frozen in a freezer, and freeze-dried the next day in a lyophilizer. The moisture was determined by weighing the sample before and after lyophilization. Dried samples were then ground in a Waring blender to all pass through a 1-mm sieve, with $\sim 95\%$ passing through 0.5 mm . Canned peppers were prepared by draining the excess liquid, weighing, slitting the pepper to allow the liquid in the cavity to drain, and then rinsing them with distilled water. The canned peppers were then treated the same way as the fresh peppers.

Extraction of Capsaicinoids from Fresh Korean Peppers (*Capsicum annuum* L.). Fresh red pepper parts from three uniform-sized peppers (average weight, 11.9 g; length, 104.3 mm; width, 18.2 mm) were used for analysis. Peppers were cut into halves from the stem end to the opposite end, and then the placenta and seeds were removed. After they were divided into three parts of equal length, the middle parts of the pericarp were cut into $1 \times 1\text{ mm}$ slices by a clasp knife and mixed well. Each weighed sample ($\sim 2.0\text{ g}$) was macerated in a mortar with 5 mL of methanol and filtered on Advantec (Dublin, CA) no. 2 filter paper. The residue was then rinsed three more times with 5 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 $1\text{ }^\circ\text{C}$. All capsaicinoid extracts were filtered through a $0.45\text{-}\mu\text{m}$ Millipore nylon filter (Bedford, MA) before HPLC. The colors of the extracts corresponded to the surface colors of the peppers.

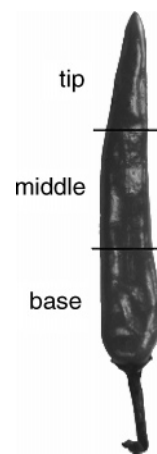


Figure 1. Photograph of the surface of a Korean Hansui hot pepper showing horizontal cuts into three segments used for analysis of capsaicinoids.

Extraction of Capsaicinoids from the Tip (Top), Middle, and Base Parts of Korean Hansui Hot Peppers. The pepper parts from three uniform-sized peppers (average weight, 17.6 g; length, 117.1 mm; width, 17.6 mm) were used for the analysis of capsaicinoids. The peppers were cut horizontally with a clasp knife from the stem end to the opposite end and divided equally into three parts as shown in **Figure 1**. The capsaicinoids were extracted from each part by the above-described procedure.

Extraction of Capsaicinoids from Pericarp, Placenta, and Seeds of Seongrok Korean Hot Peppers. Capsaicinoids were extracted from the pericarp, placenta, and seeds of three uniform-sized fresh red peppers (weight, 11.8 g; length, 109.2 mm; width, 18.8 mm). The peppers were cut in half from the stem end to the opposite end. They were then divided into the pericarp (middle part), placenta, and seeds. The extraction from each individual part was carried out as described above.

Isolation of Capsaicinoids from Freeze-Dried and Whole Dry Peppers. Each freeze-dried pepper powder ($\sim 0.3\text{--}0.8\text{ g}$) was placed in a 10 mL vial to which was added 5 mL of methanol. The suspension was then sonicated for 20 min in the ultrasonic bath and centrifuged at 18000g for 10 min at $1\text{ }^\circ\text{C}$. The extracts were then filtered through a $0.45\text{-}\mu\text{m}$ Millipore nylon filter (Bedford, MA) before HPLC analysis. Whole dry peppers were ground to powders in a Waring blender to pass through a 1-mm sieve. The powder ($\sim 0.25\text{ g}$) was then treated in the same way as described above for freeze-dried peppers.

Isolation of Capsaicinoids from Commercial Dry Peppers and Pepper Foods. The commercial peppers and dry pepper foods were finely ground into powders with a mortar and pestle or a homogenizer. Each powder ($\sim 0.5\text{--}1.0\text{ g}$) was placed into a 50 mL volumetric flask to which was added 50 mL of methanol. The flask was then placed into an ultrasonic bath for 20 min. The filtrate was centrifuged at 18000g for 10 min at $1\text{ }^\circ\text{C}$. The capsaicin extracts were then passed through a $0.45\text{-}\mu\text{m}$ Millipore nylon filter (Bedford, MA) before HPLC analysis.

Isolation of Capsaicinoids from Commercial Pepper Sauces. After stirring, an aliquot ($\sim 2.0\text{ g}$) of the sauce was placed into a 20-mL volumetric flask. The flask was brought up to volume with methanol, placed into an ultrasonic bath for 20 min, and centrifuged at 18000g for 10 min at $1\text{ }^\circ\text{C}$. The extract was then passed through a $0.45\text{-}\mu\text{m}$ Millipore nylon filter. An aliquot ($40\text{ }\mu\text{L}$) of the filtrate was injected directly into the HPLC column.

High-Performance Liquid Chromatography (HPLC). HPLC was carried out on a Hitachi liquid chromatograph model 665-II equipped with an autosampler (model 655A-40). Two column sizes were used for two separate HPLC methods (see below): stainless steel columns ($250\text{ mm} \times 4.0\text{ mm i.d.}$ and $150\text{ mm} \times 4.0\text{ mm i.d.}$) connected in series and a $250\text{ mm} \times 4.0\text{ mm i.d.}$ column, each packed with Inertsil ODS-3v ($5\text{-}\mu\text{m}$ particle diameter) (GL Sciences, Tokyo, Japan). The column temperature was maintained with a Shimadzu column oven CTO-10vp (Shimadzu, Kyoto, Japan). The flow rate was 1 mL/min at $30\text{ }^\circ\text{C}$. UV detection was set at 280 nm. The capsaicinoid extract ($\sim 20\text{--}40\text{ }\mu\text{L}$) was injected directly into the column. Separate analyses, each

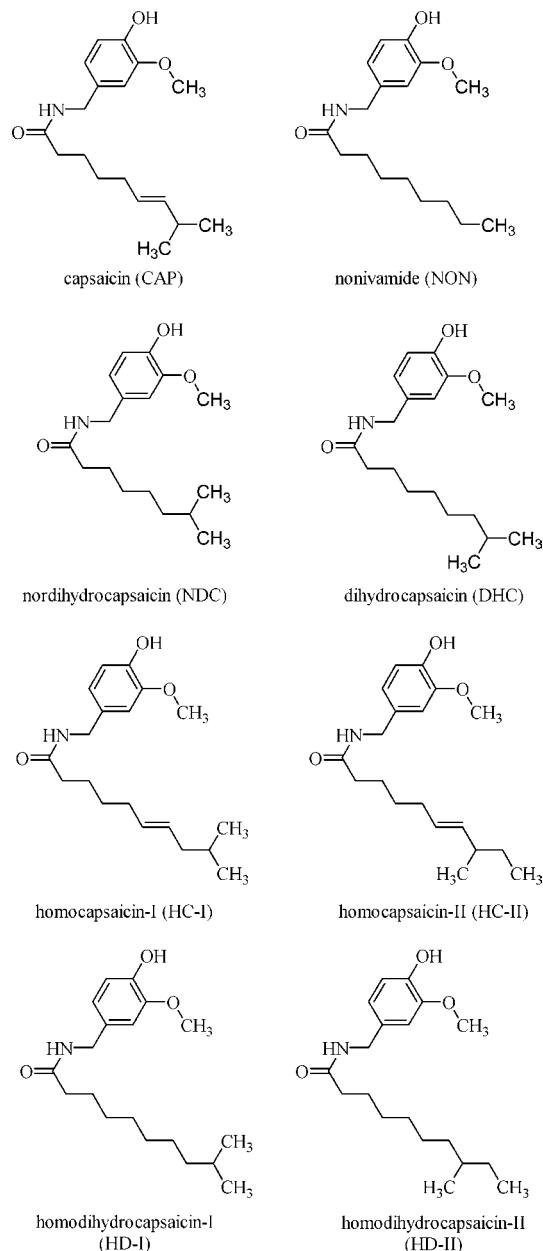


Figure 2. Structures of capsaicinoids evaluated in this study.

in triplicate, were carried out with three separate extracts. Two HPLC methods were developed and used for the analysis of all capsaicinoids:

HPLC-A conditions: column length, 400 mm; mobile phase A, acetonitrile; mobile phase B, 0.5% formic acid in water (45:55, v/v). Because the capsaicin and nonivamide peaks overlapped, HPLC-A was used to quantitate six of the eight capsaicinoids: nordihydrocapsaicin (NDC), dihydrocapsaicin (DHC), homocapsaicin-I (HC-I), homocapsaicin-II (HC-II), homodihydrocapsaicin-I (HDC-I), and homodihydrocapsaicin-II (HDC-II).

HPLC-B. The conditions were the same as for HPLC-A except that a 250 mm column and a mobile phase ratio of 31:59 (v/v) were used. HPLC-B separated capsaicin (CAP) and nonivamide (NON) and revealed the presence of an additional small, unidentified peak (UIP). HPLC-B was used to quantitate these three compounds.

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 were separated using a Zorbax Eclipse XDB-C18 column (4.6 × 150 mm, 3.5 μm) (Agilent Technologies, Palo Alto, CA), with acetonitrile as mobile phase A and distilled water containing 0.5% formic acid as mobile phase B (45:55 or 29:71, v/v) at a flow rate of 1.0 or 0.75 mL/min and at a column temperature of 30 °C. The mass

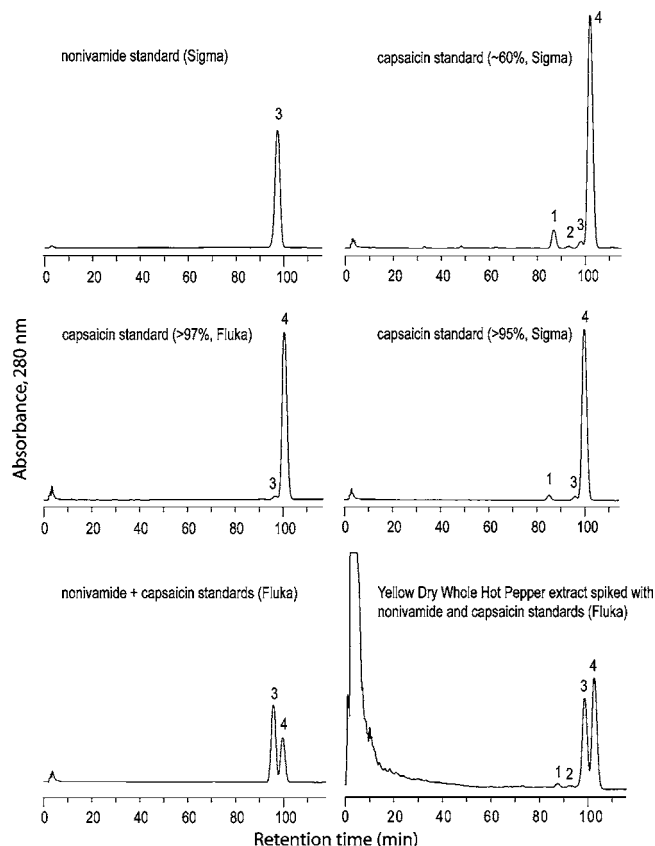


Figure 3. HPLC chromatograms showing separation of nonivamide from capsaicin. Peaks: 1, nordihydrocapsaicin (NDC); 2, unidentified peak (UIP); 3, nonivamide (NON); 4, capsaicin (CAP). Conditions: column; Inertsil ODS-3v (5 μm, 4.0 × 250 mm); column temp, 30 °C; mobile phase; acetonitrile:0.5% formic acid (31:69, v/v); detector; UV at 280 nm; flow rate; 1 mL/min; chart speed, 2.5 mm/min.

spectrometer was equipped with an electrospray ionization source and was operated in the selected-ion monitoring (SIM) mode. The protonated molecules produced from nordihydrocapsaicin (m/z 294), capsaicin (m/z 306), dihydrocapsaicin (m/z 308), homocapsaicin (m/z 320) (two isomers), and homodihydrocapsaicin (m/z 322) (two isomers) and nonivamide (m/z 293.9) were monitored. The optimum conditions for the analysis of the analytes were as follows: flow source voltage, 5.5 kV; source current, 80 μA; capillary voltage, 27 V; tube lens offset, 45 V; multipole RF amplifier, 400 (Vp-p). The Zorbax column of the LC-MS instrument separated the capsaicinoids nearly as well as the HPLC instrument.

Quantification of Capsaicinoids. The concentrations of the other compounds present in a mixture obtained from Sigma and in all pepper preparations were determined as capsaicin (CAP) equivalents. This was done by calculating the relative contribution of each capsaicinoid UV response peak to the sum of all the peaks contained in the capsaicin mixture (~60%) 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 HPLC and LC-MS retention times. In multiple experiments, we found that varying the injection volume was highly reproducible.

Recovery of Capsaicinoids after Spiking. Peppers were analyzed before and after the addition of known amounts of the mixture of capsaicinoids. Recovery (%) = (concentration of each compound in spiked sample)/(concentration of endogenous compound + spike) × 100.

RESULTS AND DISCUSSION

Analytical Aspects. Figure 1 shows a photograph illustrating the three parts of a whole pepper used for the analysis of

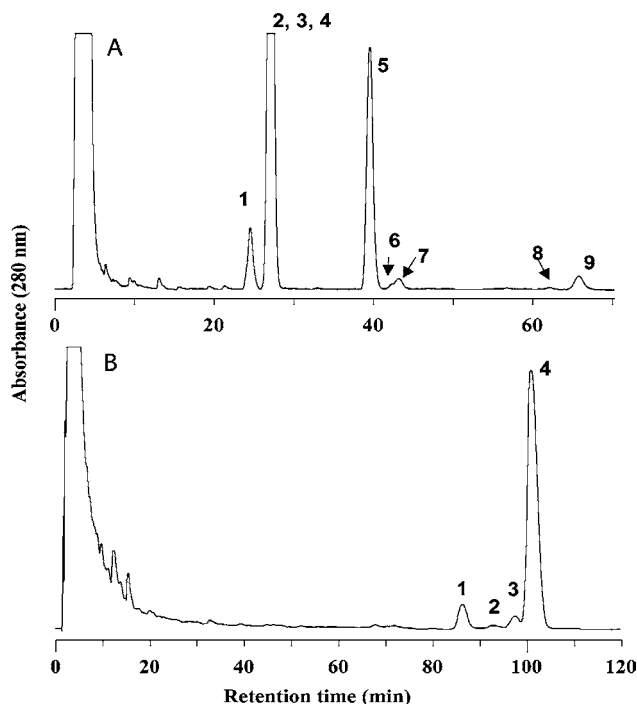


Figure 4. HPLC chromatogram of capsaicinoids in an extract of the placenta of Korean Seongrok hot peppers. Peaks: 1, nordihydrocapsaicin (NDC); 2, unidentified peak (UIP); 3, nonivamide (NON); 4, capsaicin (CAP); 5, dihydrocapsaicin (DHC); 6, homocapsaicin-I (HC-I); 7, homocapsaicin-II (HC-II); 8, homodihydrocapsaicin-I (HDC-I); 9, homodihydrocapsaicin-II (HDC-II). Conditions: column, Inertsil ODS-3v [5 μ m, 4.0 mm \times 400 mm (A); 5 μ m, 4.0 \times 250 mm (B)]; column temp, 30 $^{\circ}$ C; mobile phase, acetonitrile/0.5% formic acid in H₂O [45:55, v/v (A); 31:69, v/v (B)]; detector, UV at 280 nm; flow rate, 1 mL/min; chart speed, 2.5 mm/min.

capsaicinoids. **Figure 2** depicts the structures of the eight capsaicinoids evaluated in this study. **Figures 3** and **4** demonstrate the separation of these compounds present in a pepper extract on HPLC chromatograms. **Figures 5** and **6** show representative selected ion monitoring (SIM) profiles for capsaicinoids obtained by LC-MS and the corresponding mass spectra.

Two HPLC assays (HPLC-A and HPLC-B) were needed to separate all eight capsaicinoids. In the HPLC-A method, a series of studies of the effect of varying the ratios of the mobile phase on retention times of the capsaicinoids suggested that a ratio of 45:55 of acetonitrile/0.5% formic acid in H₂O resulted in separation of seven compounds over a period of \sim 68 min (**Figure 4A** and **Table 1**). Because the HPLC-A method did not separate capsaicin from nonivamide, additional studies (**Table 2**) revealed that the use of a mobile phase ratio of 31:59 of acetonitrile/0.5% formic acid resulted in good separation over a period of \sim 100 min of the peaks associated with these two compounds (HPLC-B method) (**Figure 4B** and **Table 4**). The chromatograms also show the presence of a small, unknown peak.

To quantitatively define to what extent column length influences the retention times, we determined the capacity factor (k) for the separation of capsaicinoids as a function the length of the HPLC column (33). The capacity factor [defined by the equation $k = (t_R - t_{R'})/t_{R'}$, where t_R = the retention time for the sample peak and $t_{R'}$ = the retention time of the solvent peak] is a quantitative measure of a column's ability to separate the peaks associated with the analytes. The data indicated that of the three column lengths investigated, 400 mm appears to be the most

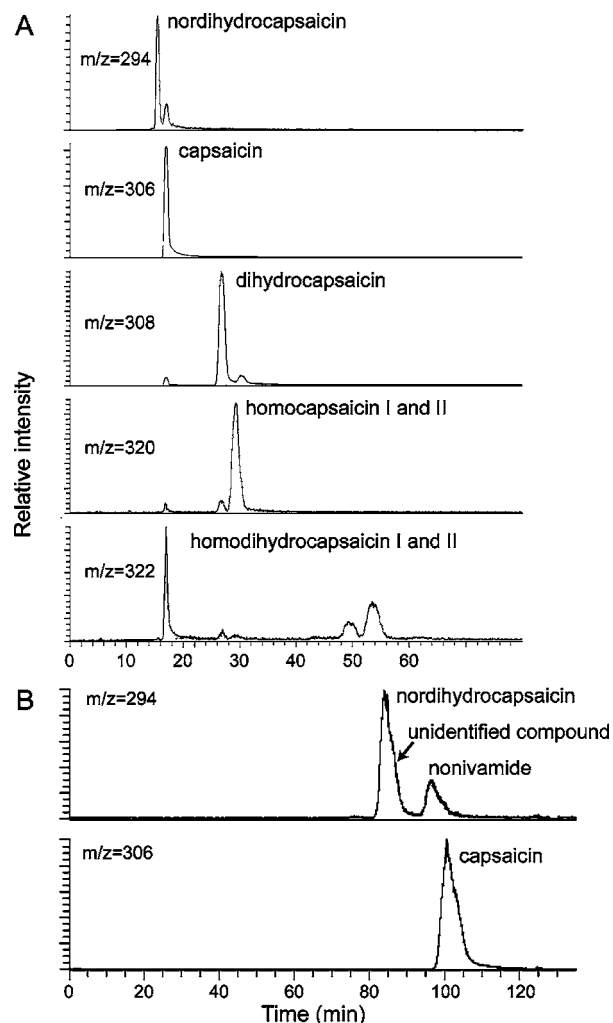


Figure 5. Ion mass chromatograms of capsaicinoids determined by LC-MS. Conditions: column, Zorbax reversed-phase HPLC (150 \times 4.0 mm, 3 μ m); column temp, 30 $^{\circ}$ C; mobile phase, acetonitrile/0.5% formic acid [(45:55 (A) or 29:71 (B), v/v)]; flow rates, 1.0 mL/min (A) or 0.75 mL/min (B).

suitable for the HPLC-A method and a 250 mm for the HPLC-B method (**Table 2**). The cited values permit the selection of columns with different k values for various applications. **Table 4** shows that the linear responses of concentration versus peak areas (calibration plots) ranged from 0 to \sim 2000 ng, the limit of detection of the eight compounds estimated from the calibration plots, and from \sim 15 to 30 ng, and recoveries of spiked samples ranged from 86.4 ± 7.6 to $114.5 \pm 8.0\%$ ($n = 3$). Lack of baseline noise in the chromatograms, linear concentration responses of integrated peak areas, and good recoveries of all eight compounds from spiked peppers support the validity of the HPLC method.

Isomeric Capsaicinoids. The pairs of 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 weights (**Figure 2**). LC-MS analysis of the mass spectra does not tell us which chromatographic peak is associated with which isomer. The intensity of m/z 320 of HC-II relative to that of m/z 320 of HC-I equals 0.33, and the corresponding ratio of m/z 322 of the HDC-II peak to that of m/z 322 of the HDC-I peak is 1.71. These data suggest that the mass fragmentation patterns of the isomers differ from each other.

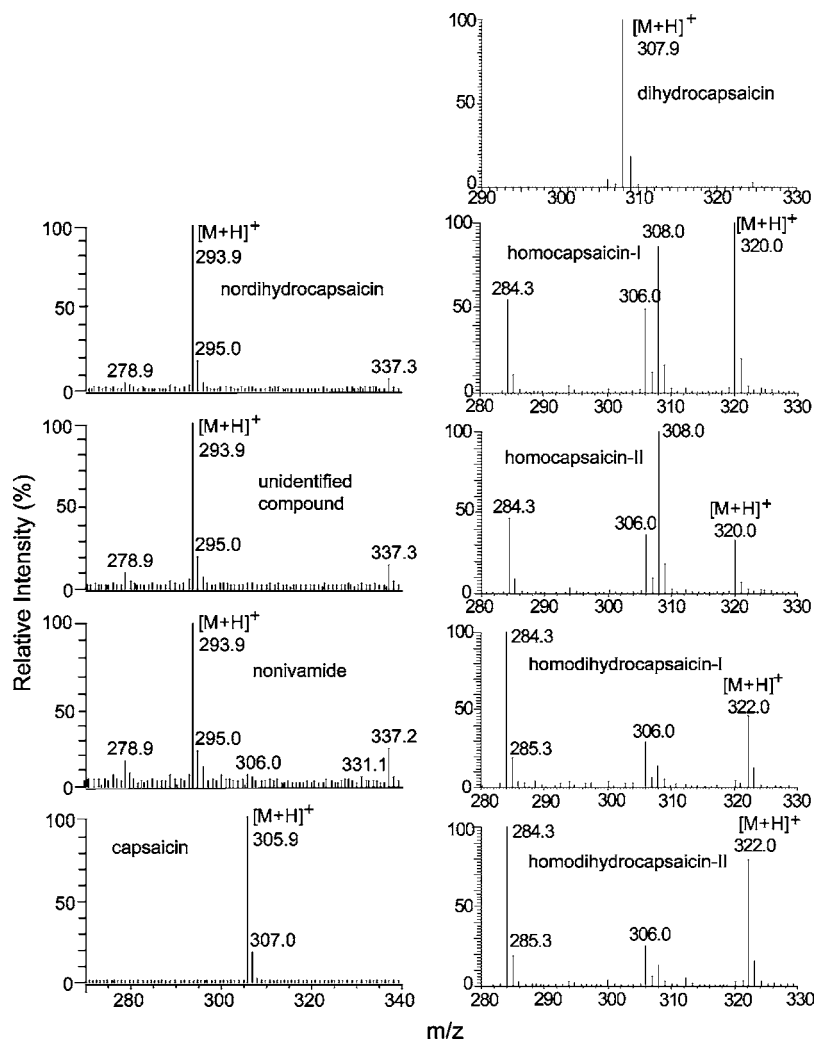


Figure 6. Mass spectra of capsaicinoids. The protonated positively charged molecular ion peaks are designated by $[M + H]^+$. Note spectra of isomeric homocapsaicin-I and homocapsaicin-II and homodihydrocapsaicin-I and homodihydrocapsaicin-II, respectively, each derived from individual peaks of the LC chromatograms.

Table 1. Effects of the Mobile Phase Ratio for Acetonitrile (A) to 0.5% Formic Acid in Water (B) on HPLC Retention Times of Seven Capsaicinoids^a

capsaicinoid	retention time (min) at a ratio of acetonitrile to formic acid (A/B) of				
	45:55	47:53	50:50	52:48	55:45
nordihydrocapsaicin (NDC)	25.24 ± 0.04 ^b	22.73 ± 0.10	21.93 ± 0.20	18.45 ± 0.02	15.09 ± 0.03
capsaicin (CAP)	27.54 ± 0.03	24.60 ± 0.05	23.73 ± 0.22	19.79 ± 0.01	16.12 ± 0.08
dihydrocapsaicin (DHC)	39.98 ± 0.05	35.46 ± 0.36	33.67 ± 0.19	27.55 ± 0.01	21.70 ± 0.07
homocapsaicin-I (HC-I)	42.76 ± 0.03	37.86 ± 0.04	35.79 ± 0.47	29.16 ± 0.12	23.37 ± 0.09
homocapsaicin-II (HC-II)	43.67 ± 0.04	38.51 ± 0.35	36.53 ± 0.51	29.81 ± 0.11	23.37 ± 0.09
homodihydrocapsaicin-I (HDC-I)	61.94 ± 0.02	53.38 ± 0.03	51.61 ± 0.72	40.69 ± 0.07	31.32 ± 0.18
homodihydrocapsaicin-II (HDC-II)	68.32 ± 0.62	56.37 ± 0.01	55.17 ± 0.79	42.68 ± 0.12	32.39 ± 0.03

^a Conditions: column, Inertsil ODS-3v (5 μ M particle size); column temp, 30 °C; column length, 4 × 400 mm; flow rate, 1 mL/min; detector, UV at 280 nm. ^b Average ± SD; *n* = 3.

The exact stereochemistry of the side chains associated with the isomeric capsaicinoids could probably be defined by nuclear magnetic resonance (NMR) spectroscopy, which can provide information about three-dimensional structures of isomeric compounds (34, 35). This is a challenging problem, which may require quantum chemical calculations and the use of an external chiral element to assign experimental chemical shifts in the ¹H, ²H, and ¹³C NMR spectra of the isomeric capsaicinoids (36–38).

Percent Relative Abundance of Capsaicinoids in Three Commercial Preparations. Figures 3 and 4 illustrate the elution positions on HPLC chromatograms of the eight capsaicinoids present in commercial pepper extracts.

Identification and quantitation of each compound were determined by LC-MS of elution profiles of each peak associated with each of the protonated molecular ions peaks $[M + H]^+$ (Figures 5 and 6). Table 5 shows the measured triplicate values for each constituent of three commercial pepper extracts. The two capsaicinoids with the highest concentration were capsaicin (CAP, 60.51%) and dihydrocapsaicin (DHC, 24.91%). The mixture also contained 4.41% of nordihydrocapsaicin (NDC), 2.79% of homocapsaicin-II (HC-II), 1.82% of nonivamide (NON), 1.31% of homocapsaicin-I (HC-I), 1.22% of homodihydrocapsaicin-II (HDC-II), 0.59% of homodihydrocapsaicin-I (HDC-I), and

Table 2. Effects of Acetonitrile/0.5% Formic Acid Ratio of the Mobile Phase and Column Temperature on the HPLC Retention Times of Nonivamide and Capsaicin^a

capsaicinoid	retention time (min) at a ratio of acetonitrile to 0.5% formic acid in H ₂ O of				
	31:69	33:67	35:65	40:60	45:55
20 °C Column					
nonivamide (NON)	96.33 ± 0.08 ^b	70.88 ± 0.08	53.07 ± 0.00	28.82 ± 0.15	17.27 ± 0.04
capsaicin (CAP)	100.28 ± 0.02	73.44 ± 0.08	54.71 ± 0.01	29.90 ± 0.11	17.27 ± 0.04
30 °C Column					
nonivamide (NON)	96.73 ± 0.11	68.47 ± 0.04	53.80 ± 0.18	28.77 ± 0.01	16.71 ± 0.02
capsaicin (CAP)	100.72 ± 0.16	70.94 ± 0.03	55.75 ± 0.01	29.93 ± 0.07	16.73 ± 0.02
40 °C Column					
nonivamide (NON)	95.15 ± 0.03	69.00 ± 0.36	51.93 ± 0.03	27.50 ± 0.18	16.87 ± 0.01
capsaicin (CAP)	98.96 ± 0.00	71.50 ± 0.38	53.56 ± 0.04	27.88 ± 0.17	16.87 ± 0.01

^a Conditions: column, Inertsil ODS-3v (5 μm particle size); column length, 4 × 250 mm; flow rate, 1 mL/min; detector, UV at 280 nm. ^b Average ± SD; n = 3.

Table 3. Capacity Factors (*k*) for the Separation of Capsaicinoids by Two HPLC Methods

capsaicinoid	<i>k</i> for column length of		
	150 mm	250 mm	400 mm
HPLC-A ^a			
nordihydrocapsaicin (NDC)	6.20 ± 0.04 ^d	6.53 ± 0.02	8.04 ± 0.18
capsaicin (CAP) ^b	6.84 ± 0.03	7.20 ± 0.02	8.89 ± 0.20
dihydrocapsaicin (DHC)	10.27 ± 0.01	10.84 ± 0.01	13.44 ± 0.28
homocapsaicin-I (HC-I)	11.23 ± 0.01	11.90 ± 0.01	14.17 ± 0.31
homocapsaicin-II (HC-II)	11.23 ± 0.01	11.90 ± 0.01	14.78 ± 0.31
homodihydrocapsaicin-I (HDC-I)	16.23 ± 0.04	17.26 ± 0.04	21.50 ± 0.48
homodihydrocapsaicin-II (HDC-II)	17.21 ± 0.03	18.28 ± 0.01	22.75 ± 0.57
HPLC-B ^c			
nordihydrocapsaicin (NDC)	26.15 ± 0.72	31.67 ± 0.75	40.55 ± 0.65
unidentified (UIP)	27.96 ± 0.74	34.07 ± 0.63	43.34 ± 0.77
nonivamide (NON)	30.76 ± 0.83	35.64 ± 0.36	45.91 ± 0.75
capsaicin (CAP)	30.76 ± 0.83	37.17 ± 0.32	48.38 ± 0.07

^a HPLC-A conditions: column, 400 mm Inertsil ODS-3v (5 μm particle size); column temp, 30 °C; mobile phase, acetonitrile/0.5% formic acid (45:55, v/v); flow rate, 1 mL/min; detector, UV at 280 nm. ^b Sum of capsaicin and nonivamide. ^c HPLC-B conditions are the same as for HPLC-A except that the mobile phase was acetonitrile/0.5% formic acid (31:59, v/v) and column length was 250 mm. ^d Average ± SD, n = 3.

0.49% of an unidentified peak (UIP). The Sigma capsaicin (≥95%) sample contained 95.25% capsaicin, 2.42% nordihydrocapsaicin, and 2.03% nonivamide. The Fluka capsaicin (≥97%) sample contained 98.10% capsaicin and 1.87% nonivamide. The reproducibility of triplicate analyses and the ability of the method to measure nanograms of a capsaicinoid demonstrate the sensitivity of the HPLC procedures.

Capsaicinoids in Different Parts of the Pepper Fruit. Table 6 lists the determined values of the eight capsaicinoids in the placenta, seeds, and pericarp of the hot Korean pepper variety Seongrok. The placenta contained a total of 1849.8 μg of CAP equiv/g of fresh wt. The corresponding amounts in the seeds was 179.8 μg/g, and that in the pericarp was 138.0 μg/g.

These results show that the placenta contained 85.34% of the total amount of capsaicinoids, the seeds 8.29%, and the pericarp 6.37%, respectively. These findings support the view that capsaicinoids are synthesized in the placenta and that the presence of capsaicinoids in other parts of the fruit appears to be the result of leakage and diffusion from the placenta to the periderm and seeds (18, 39–42).

Table 6 also lists capsaicinoid levels present in the lower, top, and middle parts of the whole Korean mild pepper variety Hansuwi (Figure 1). The lower part contained 10.7 μg of CAP

Table 4. 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 (%)
HPLC-A				
nordihydrocapsaicin (NDC)	25.24 ± 0.04	23.15	0–2000	92.5 ± 1.2 ^b
capsaicin (CAP) ^c	27.54 ± 0.03	19.95	0–2500	96.4 ± 3.5 ^b
dihydrocapsaicin (DHC)	39.98 ± 0.05	19.74	0–1800	106.3 ± 1.6 ^b
homocapsaicin-I (HC-I)	42.76 ± 0.03	17.74	0–500	105.7 ± 5.8 ^b
homocapsaicin-II (HC-II)	43.67 ± 0.04	15.25	0–1100	114.5 ± 8.0 ^b
homodihydrocapsaicin-I (HDC-I)	61.94 ± 0.02	30.35	0–500	103.6 ± 6.5 ^b
homodihydrocapsaicin-II (HDC-II)	68.32 ± 0.62	23.90	0–500	86.4 ± 7.6 ^b
HPLC-B				
nordihydrocapsaicin (NDC)	84.99 ± 0.35	19.74	0–2000	96.8 ± 2.9 ^d
unidentified (UIP)	91.19 ± 0.10	15.36	0–2000	97.1 ± 3.0 ^d
nonivamide (NON)	96.73 ± 0.11	18.60	0–2000	95.6 ± 3.2 ^d
capsaicin (CAP)	100.72 ± 0.16	17.28	0–2000	97.6 ± 2.5 ^d

^a Linear plots of concentration versus peak area in μV; n = 3; r² = 0.99 for all compounds. ^b Korean Hansuwi red pepper was spiked with a commercial mixture of capsaicinoids from Sigma (~60%) before extraction. ^c Sum of capsaicin and nonivamide. ^d Yellow whole hot peppers was spiked with a commercial mixture of capsaicinoids from Sigma (~60%) before extraction.

Table 5. Analyses of Individual Capsaicinoids in Three Commercial Capsaicin Preparations by HPLC

capsaicinoid	capsaicin (Sigma, ≥95%)	capsaicin powder (Sigma, ~60%)	capsaicin (Fluka, ≥97%)
nordihydrocapsaicin (DHC)	2.42 ± 0.01 ^a	4.41 ± 0.11	nd
unidentified peak (UIP)	nd	0.49 ± 0.01	nd
nonivamide (NON)	2.03 ± 0.04	1.82 ± 0.12	1.87 ± 0.04
capsaicin (CAP)	95.25 ± 0.10	60.51 ± 0.13	98.10 ± 0.04
dihydrocapsaicin (DHC)	nd	24.91 ± 0.10	nd
homocapsaicin-I (HC-I)	nd	1.31 ± 0.06	nd
homocapsaicin-II (HC-II)	nd	2.79 ± 0.20	nd
homodihydrocapsaicin-I (HDC-I)	nd	0.59 ± 0.13	nd
homodihydrocapsaicin-II (HDC-II)	nd	1.22 ± 0.18	nd

^a Average ± SD; n = 3. Integrated areas of all peaks = 100%; nd = not detected.

equiv/g of fresh wt, the top part, 9.7 μg/g, and the middle part, 3.3 μg/g, respectively. These results indicate that the capsaicinoids are not distributed uniformly across all parts of a whole

Table 6. Capsaicinoids in Parts of Seongrok (Hottest) and Hansuwi (Hot) Red Korean Peppers Determined by HPLC^a

pepper part	NDC ^b	IUP ^c	NON	CAP	DHC	HC-I	HC-II	HD-I	HD-II	total
Seongrok pepper										
placenta	94.4 ± 3.9	21.0 ± 0.5	55.9 ± 1.4	1258.6 ± 30.8	363.9 ± 8.9	19.9 ± 1.7 ^d	trace	trace	36.1 ± 2.6	1,849.8
seeds	10.8 ± 0.4	nd	30.4 ± 1.0	96.4 ± 3.4	32.3 ± 1.2	trace	trace	nd	9.9 ± 0.4	179.8
pericarp	4.6 ± 0.5	nd	33.0 ± 0.8	59.2 ± 1.6	28.0 ± 0.7	2.4 ± 0.0 ^d	nd	nd	10.8 ± 1.2	138.0
Hansuwi pepper										
lower	1.6 ± 0.2	nd	3.9 ± 0.4	0.5 ± 0.1	4.0 ± 0.6	0.7 ± 0.1 ^d	nd	nd	nd	10.7
top	0.7 ± 0.1	nd	1.5 ± 0.2	2.9 ± 0.3	4.6 ± 0.9	trace	trace	nd	nd	9.7
middle	trace	nd	0.6 ± 0.0	0.7 ± 0.1	1.5 ± 0.1	0.5 ± 0.1 ^d	nd	nd	nd	3.3

^a Values in $\mu\text{g/g} \pm \text{SD}$ of fresh of wt; $n = 3$; nd = not detected. ^b See **Table 5** for names of compounds shown as abbreviations. ^c IUP (unidentified peak) expressed as capsaicin equivalent. ^d Sum of HC-I and HC-II.

Table 7. Capsaicinoid Content of Red Korean Peppers^a

pepper variety	NDC ^b	IUP	NON	CAP	DHC	HC-I	HC-II	HD-I	HD-II	total
Seongrok	4.6 ± 0.5	nd	35.3 ± 0.1	59.2 ± 1.65	28.0 ± 0.7	2.4 ± 0.1 ^c	nd	nd	10.8 ± 1.2	140.3
Cheongryang	24.1 ± 0.3	nd	29.8 ± 1.0	54.1 ± 1.88	25.9 ± 0.8	nd	nd	nd	nd	135.9
Buja	5.3 ± 0.7	nd	15.9 ± 0.2	26.3 ± 0.4	25.1 ± 0.7	nd	nd	nd	nd	72.6
Gumsang	3.5 ± 0.3	nd	8.9 ± 1.5	18.1 ± 3.17	21.8 ± 1.9	nd	nd	nd	nd	52.3
Cheonhatongil	3.1 ± 0.1	nd	8.0 ± 0.2	15.4 ± 0.4	13.1 ± 0.5	nd	nd	nd	nd	39.6
Hansuwi	trace	nd	0.5 ± 0.0	0.8 ± 0.1	1.5 ± 0.1	0.5 ± 0.1 ^c	nd	nd	nd	3.3
Gichan	nd	nd	0.4 ± 0.0	0.7 ± 0.1	0.6 ± 0.0	nd	nd	nd	nd	1.7
Teajangbu	nd	nd	0.5 ± 0.0	0.9 ± 0.0	nd	nd	nd	nd	nd	1.4

^a Pericarps of middle part of the fruits excluding placenta and seeds were used for analyses. Values in $\mu\text{g/g} \pm \text{SD}$ of fresh wt; $n = 3$; nd = not detected. Capsaicinoids were not detected in the mild Jeongsang and Taewang peppers. ^b See **Table 5** for names of compounds shown as abbreviations. ^c Sum of HC-I and HC-II.

Table 8. Individual and Total Capsaicinoid Contents of Whole Fresh and Canned Peppers Sold in California Determined by HPLC^a

pepper	NDC	UIP	NON	CAP	DHC	HC-I	HC-II	DHC-I	DHC-II	total
habanero (86.6)	105.4 ± 12.6	11.4 ± 0.4	97.2 ± 3.5	1187.4 ± 42.2	771.9 ± 8.5	nd	44 ± 2.7	12.3 ± 0.6	31.3 ± 1.1	2,260.9
Thai (80.8)	187.6 ± 7.1	6.8 ± 0.2	32.6 ± 0.9	509.7 ± 12.7	501.4 ± 21.6	8.4 ± 0.8	34.8 ± 3.1	11.7 ± 1.3	39.7 ± 2.4	1,332.7
chipotle whole, canned ^b	34.6 ± 2.9	nd	nd	196.8 ± 23.5	163.5 ± 17.5	5.2 ± 2.0 ^c	nd	0.9 ± 0.3	14.4 ± 2.5	415.5
hot chili, canned (89.1) ^d	30.4 ± 5.1	3.6 ± 0.6	4.0 ± 0.7	179.9 ± 30.7	68.2 ± 11.3	1.7 ± 0.1	6.4 ± 0.5	2.7 ± 0.1	8.6 ± 0.5	305.5
jalapeno green whole pickled, canned ^b	24.4 ± 2.4	nd	nd	105 ± 14.7	72.0 ± 9.2	2.1 ± 0.3 ^c	nd	0.38 ± 0.1	5.6 ± 0.6	209.5
serrano green whole pickled, canned ^b	32.0 ± 0.4	nd	nd	61.7 ± 0.6	59.5 ± 1.8	2.8 ± 0.3 ^c	nd	0.6 ± 0.1	7.5 ± 0.3	164.1
jalapeños whole, canned ^e	11.0 ± 0.8	nd	nd	33.1 ± 0.7	38.7 ± 2.4	nd	nd	trace	1.8 ± 0.5	84.6
pepperoncini, canned (88.4) ^f	10.6 ± 0.1	nd	2.5 ± 0.1	30.3 ± 0.5	33.3 ± 0.3	1.7 ± 0.3	1.9 ± 0.2	nd	1.7 ± 0.1	82.0
serrano (88.8)	14.0 ± 1.6	nd	3.0 ± 0.1	16.2 ± 0.5	35.2 ± 0.8	nd	3.3 ± 0.7	2.2 ± 0.2	3.0 ± 0.2	76.9
green jalapeño (90.7)	11.2 ± 0.3	nd	2.0 ± 0.0	27.6 ± 0.2	29.6 ± 0.2	nd	2.3 ± 0.1	nd	3.1 ± 0.6	75.8
red jalapeño (86.9)	5.6 ± 0.9	nd	3.0 ± 0.2	22.4 ± 1.2	11.0 ± 0.3	nd	1.9 ± 0.2	nd	2.4 ± 0.5	46.3
sliced jalapeño, canned (92.5) ^d	6.0 ± 0.01	nd	0.5 ± 0.0	2.6 ± 0.1	8.2 ± 0.2	nd	0.5 ± 0.02	nd	0.9 ± 0.1	18.7

^a See **Table 5** for names of compounds shown as abbreviations. Values are in μg of CAP equivalents/g of fresh weight $\pm \text{SD}$ of fresh wt calculated by taking into account moisture content (values in parentheses if freeze-dried before analysis; others were not freeze-dried); $n = 3$; nd = not detected. Capsaicinoids were not detected in the following peppers: green bell (92.7), red bell (90.8), yellow bell (90.5), and roasted red, canned (92.3). ^b La Costena brand imported from Mexico by Vilore Foods, Vilore, TX. ^c Sum of HC-I and HC-II. ^d G. L. Mezzetta, Inc., Napa Valley, CA. ^e Imported from Mexico by Authentic Specialty Food, Chino, CA. ^f Imported from Greece by Star Fine Foods, Fresno, CA.

pepper because the lower part contained 45.15% of the total, the top part, 40.93%, and the middle part, 13.92%, respectively. These results are consistent with those reported by other investigators (39, 43).

Capsaicinoids in the Pericarp of Red Korean Peppers. To demonstrate the applicability of the analytical method to fresh peppers, we analyzed the capsaicinoid levels of the pericarp of the middle part of the fruits of 10 Korean peppers ranging from very hot to very mild (**Table 7**). We chose to analyze the pericarp because it is the most commonly eaten part of the pepper, when eaten as a vegetable.

The data show that the levels of total capsaicinoids ranged from 1.4 μg of CAP equiv/g of fresh wt for the Teajangbu pepper to 140.3 $\mu\text{g/g}$ for the very hot Seongrok pepper. The pericarp of two pepper varieties contained no capsaicinoids. On the basis of the relative distribution of capsaicinoids in the different parts

of the pepper fruit mentioned above, it is possible to estimate the approximate content of capsaicinoids in the placenta and seeds of these peppers from the values listed for the pericarps.

Capsaicinoids in Fresh and Canned Peppers Sold in California. Fresh whole and canned peppers sold in California were freeze-dried and then shipped to Korea for analysis. **Table 8** shows that the capsaicinoid content of 17 fresh and canned peppers tested varied widely (in CAP equivalents, $\mu\text{g/g}$ of fresh wt). The capsaicinoid levels for 12 peppers that had measurable levels ranged from 18.7 for canned jalapeño sliced peppers to 2260.9 for the habanero variety. The second highest amount (1332.7) was present in the Thai peppers; the third highest, in canned chipotle whole peppers (415.5); the fourth highest, in canned hot chili peppers (305.5); the fifth highest, in canned jalapeno whole pickled peppers (209.5); and the sixth highest, in canned serrano whole pickled green peppers (164.1). The

Table 9. Individual and Total Capsaicinoid Contents of Commercial Dry Peppers and Pepper-Containing Foods Sold in California Determined by HPLC^a

pepper/food	NDC	UIP	NON	CAP	DHC	HC-I	HC-II	HDC-I	HDC-II	total
red pepper, ground cayenne ^b	302.2 ± 7.1	12.4 ± 0.2	190.2 ± 2.4	1,471.5 ± 18.8	1,422.0 ± 6.4	22.8 ± 0.1	43.8 ± 1.6	34.7 ± 0.1	88.2 ± 0.6	3587.8
red dry whole ^c	111.7 ± 7.9	49.3 ± 1.1	20.5 ± 0.5	1,328.8 ± 29.2	508.4 ± 10.9	10.0 ± 1.6 ^d	4.1 ± 0.8	3.3 ± 0.8	2036.1	
red pepper, ground cayenne ^b	138.4 ± 6.8	10.6 ± 0.1	41.5 ± 0.4	912.1 ± 8.4	690.6 ± 21.0	16.6 ± 2.5	23.5 ± 3.6	trace	77.5 ± 12.5	1910.8
yellow dry whole hot ^e	98.9 ± 3.6	23.8 ± 1.7	20.0 ± 1.4	1,059.2 ± 75.9	438.3 ± 20.4	nd	nd	nd	3.9 ± 0.2	1644.1
cayenne pepper, 100% organic ^f	76.6 ± 5.8	5.6 ± 0.1	53.0 ± 0.7	474.7 ± 5.5	302.5 ± 2.0	5.4 ± 0.6	10.7 ± 1.3	trace	35.4 ± 3.1	963.9
original chili seasoning ^b	124.3 ± 22.1	nd	173.0 ± 7.5	532.3 ± 23.2	nd	nd	nd	nd	nd	829.6
Tabasco Hot Habanero Sauce ^g	74.1 ± 9.5	nd	nd	329.9 ± 4.9	125.8 ± 0.8	16.6 ± 1.8 ^d	nd	trace	546.5	
Hungarian hot paprika ^h	48.2 ± 0.8	4.0 ± 0.5	17.2 ± 2.1	139.7 ± 16.9	217.8 ± 3.7	0.7 ± 0.1 ^d	1.1 ± 0.0	10.6 ± 3.3	439.3	
hot taco seasoning ^b	43.0 ± 0.1	nd	87.6 ± 3.3	186.8 ± 7.0	76.3 ± 2.5	nd	nd	nd	393.7	
Choula Hot Sauce (red and piquin peppers) ⁱ	34.2 ± 4.4	nd	trace	92.0 ± 3.8	59.9 ± 5.1	3.9 ± 1.2 ^d	7.8 ± 0.4	3.06 ± 0.5	200.8	
Tabasco Original Flavor Sauce ^g	9.0 ± 0.3	0.4 ± 0.01	5.8 ± 0.1	110.0 ± 0.5	66.6 ± 1.2	3.0 ± 0.6	nd	nd	194.7	
mild taco seasoning ^b	38.1 ± 7.5	nd	74.1 ± 5.7	73.3 ± 5.6	nd	nd	nd	nd	185.5	
Crystal Hot Sauce (extra hot) ^j	nd	nd	trace	90.1 ± 4.0	72.3 ± 5.3	7.4 ± 0.9	trace	4.3 ± 0.5	174.1	
Tabasco New Flavor Chipotle Sauce ^g	6.1 ± 0.6	nd	1.5 ± 0.2	28.2 ± 0.9	33.6 ± 1.0	2.3 ± 0.02	nd	nd	71.6	
hot taco spices and seasoning ^k	nd	nd	2.4 ± 0.1	40.4 ± 1.7	22.5 ± 6.3	nd	nd	nd	65.3	
Safeway Hot Pepper Sauce ^l	nd	nd	2.9 ± 0.5	23.4 ± 0.1	14.4 ± 0.1	3.8 ± 0.6 ^d	nd	nd	44.5	
Ashanti Louisiana Hot Sauce ^m	2.1 ± 0.1	nd	nd	21.9 ± 0.1	4.7 ± 0.3	trace	tracs	nd	28.7	
kettle-cooked jalapeno extra crunchy potato chips ⁿ	4.1 ± 0.4	nd	nd	7.1 ± 2.5	9.3 ± 0.2	nd	nd	nd	20.6	
sun-dried tomato habanero tortillas ^o	trace	nd	1.4 ± 0.1	4.1 ± 0.1	2.4 ± 0.2	nd	nd	nd	7.9	
Miss Vickie's Jalapeño Potato Chips ⁿ	trace	nd	2.1 ± 0.1	2.6 ± 0.1	3.2 ± 0.3	nd	nd	nd	7.9	

^a See Table 5 for names of compounds shown as abbreviations. Values in μg of CAP equivalents/g of fresh weight \pm SD; $n = 3$; nd = not detected; tr = trace. The following condiments contained no or trace amounts of capsaicinoids: chili powders (McCormick; Grandma's Spanish), fajita seasoning (McCormick), ground white pepper (McCormick), paprika (McCormick), seasoned pepper blends (McCormick; Lawry's Foods). ^b McCormick, Hunt Valley, MD. ^c Aji Panca Rojo Seco, Peru. ^d Sum of HC-I and HC-II. ^e Aji Ammarillo Mirasol Seco, Peru. ^f Morton & Bassett, San Francisco, CA. ^g McIlhenny Co., Avery Island, LA. ^h Product of Szeged, Hungary; Spiceco, Avenel, NJ. ⁱ Imported from Mexico by Tipcon, El Paso, TX. ^j Lawry's Foods. ^k Safeway Co., CA. ^m Bridge Foods, New Orleans, LA. ⁿ Frito-Lay. ^o Trader Joe's.

following peppers contained $<100 \mu\text{g/g}$ of capsaicinoids: canned jalapeño whole (84.6), canned pepperoncini (82.0), fresh serrano (76.9), fresh green jalapeño (75.8), fresh red jalapeño (46.3), and canned sliced jalapeño (18.7). The following five varieties contained no detectable amounts of capsaicinoids: green bell, red bell, yellow bell, roasted red, and green chiles whole canned peppers. All eight capsaicinoids in the pepper extracts could be detected at concentrations as low as $0.38 \mu\text{g/g}$. These data permit the consumer to select peppers with undetectable, low, or high amounts of capsaicinoids.

Capsaicinoids in Pepper-Containing Foods Sold in California. Table 9 lists the distribution of the capsaicinoids in 23 dry pepper products and pepper-containing foods sold in California and analyzed in Korea. The total capsaicinoid levels in CAP equivalents of micrograms per gram of original product ranged from 7.9 for Miss Vickie's Jalapeño Potato Chips and sun-dried tomato habanera tortillas to 3587.8 in ground cayenne red pepper. The total amounts in the other products were as follows: red dry whole peppers, 2036.1; red ground cayenne peppers, 1910.8; yellow dry whole hot peppers, 1644.1; organic cayenne peppers, 963.9; original chili seasoning, 829.6; Tabasco Hot Habanero Sauce, 546.5; Hungarian hot paprika, 439.3; hot taco seasoning, 393.7; Choula Hot Sauce, 200.8; Tabasco Original Flavor Sauce, 194.7; mild taco seasoning, 185.5; Crystal Hot Sauce, 174.1; Tabasco New Flavor Chipotle Sauce, 71.6; hot taco spices and seasoning, 65.3; Safeway Hot Pepper Sauce, 45.5; Ashanti Louisiana Hot Sauce, 28.7; and kettle-cooked jalapeno extra crunchy potato chips, 20.6. The following condiments and seasonings contained no detectable amounts of capsaicinoids: chili powder, fajita seasoning, ground white pepper, paprika, and seasoned pepper blends.

Related studies analyzed capsaicinoid levels in fresh peppers (16, 27, 30, 44), paprikas (45), and salsa seasonings (31). There is wide variation in the capsaicinoid levels among fresh peppers, ground and whole dry peppers, and pepper-containing foods.

The word "pepper" in the name of a spice or a food does not always indicate the presence of capsaicinoids.

Water Content of Peppers. The water content of 12 fresh peppers ranged from 80.8% for the Thai variety to 92.7% for the green bell peppers [average \pm SD of $89.2 \pm 3.3\%$ (Table 8)]. Moisture levels of most peppers and parts of the pepper fruit should fall within this range.

Conclusions. The described procedures for the extraction, analysis, and distribution of eight capsaicinoids present in various parts of the pepper fruit, in fresh and dry peppers, and in capsaicinoid-containing processed dry and liquid foods suggest that the method merits further evaluation and application in agricultural and biomedical sciences. Extending the HPLC method to large-scale isolation of individual compounds may facilitate determining the structures as well as biological activities of major and minor capsaicinoids, both individually and in combination. An unresolved question is whether some combinations (mixtures) of capsaicinoids will exhibit synergistic biological effects, as do potato glycoalkaloids (46) and tea flavonoids (47–49). This aspect merits study.

ACKNOWLEDGMENT

We thank Journal reviewers for constructive suggestions.

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Received for review March 1, 2005. Revised manuscript received September 9, 2005. Accepted September 13, 2005.

JF050469J