

Analysis by HPLC and LC/MS of Pungent Piperamides in Commercial Black, White, Green, and Red Whole and Ground Peppercorns

MENDEL FRIEDMAN,^{*,†} CAROL E. LEVIN,[†] SEUNG-UN LEE,[‡] JIN-SHIK LEE,[‡]
MAYUMI OHNISI-KAMEYAMA,[§] AND NOBUYUKI KOZUKUE[‡]

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, California 94710, Department of Food Service Industry, Uiduk University, 780-713, Gangdong, Gyeongju, Gyongbuk, Korea, and Mass Analysis Laboratory, National Food Research Institute, Tsukuba 305-8642, Japan

Pepper plants accumulate pungent bioactive alkaloids called piperamides. To facilitate studies in this area, high-performance liquid chromatography (HPLC) and liquid chromatography/mass spectrometry methods were developed and used to measure the following piperamides in 10 commercial whole (peppercorns) and in 10 ground, black, white, green, and red peppers: piperanine, piperdardine, piperine, piperlonguminine, and piperettine. Structural identification of individual compounds in extracts was performed by associating the HPLC peak of each compound with the corresponding mass spectrum. The piperanine content of the peppers (in mg/g piperine equivalents) ranged from 0.3 for the ground white pepper to 1.4 in black peppercorns. The corresponding range for piperdardine was from 0.0 for seven samples to 1.8 in black peppercorns; for four isomeric piperines, from 0.7 for red to 129 in green peppercorns; for piperlonguminine, from 0.0 in red peppercorns to 1.0 in black peppercorns; and for piperyline, from 0.9 in ground black pepper to 5.9 for red peppercorn. Four well-separated stereoisomeric forms of piperettine with the same molecular weight were present in 19 peppers. The sums of the piperamides ranged from 6.6 for red to 153 for green peppercorns. In contrast to large differences in absolute concentrations among the peppers, the ratios of piperines to total piperamide were quite narrow, ranging from 0.76 for black to 0.90 for white peppercorns, with an average value of 0.84 ± 0.04 ($n = 19$). Thus, on average, the total piperamide content of the peppers consists of 84% piperines and 16% other piperamides. These results demonstrate the utility of the described extraction and analytical methods used to determine the wide-ranging individual and total piperamide contents of widely consumed peppers.

KEYWORDS: Piperanine; piperdardine; piperine; piperlonguminine; piperettine; black pepper; white pepper; green pepper; red pepper; HPLC; LC-MS

INTRODUCTION

Piperamides are secondary pungent metabolites present in the outer part of the fruits and in the seeds of black, white, green, and red peppers (*Piper nigrum* L.) and other cultivated varieties (1–3). Black pepper is produced from green unripe berries of the pepper plant; the fruits are dried after a heat treatment that releases browning enzymes from the cell walls; white pepper is obtained when fully ripe berries are dried with the outer pericarp removed; green peppers are harvested unripe and then air-dried or freeze-dried; and red peppers are harvested when mature (4). Interest in piperamides arises from the fact

that, in addition to its pungent properties that impart the flavor and taste to food, the compounds are reported to possess numerous nonsensory beneficial bioactive (physiological/pharmacological/insecticidal/medicinal) properties that may also benefit human health, as reviewed by Srinivasan (5) and Scott (6). These include antiandrogenic (7), anticarcinogenic (8–12), antidepressant (13–15), anti-diarrheal (16), antimicrobial (17–24), antioxidative (25, 26), anti-ulcer (gastroprotective) (27–29), hypotensive (30), immunomodulatory (10), insecticidal (31, 32), insulin resistant (33), and melanocyte-stimulating properties (34, 35). Piperamides are also reported to inhibit enzymes that catalyze the biotransformation of nutrients and drugs, thus enhancing their bioavailability and effectiveness (36–40).

Piperine is the most prevalent piperamide present in pepper. Piperdardine, the dihydro derivative of piperettine (Figure 1), was isolated from the *Piper tuberculatum* plant widely used in

* To whom correspondence should be addressed. E-mail: mfried@pw.usda.gov.

† U.S. Department of Agriculture.

‡ Uiduk University.

§ National Food Research Institute.

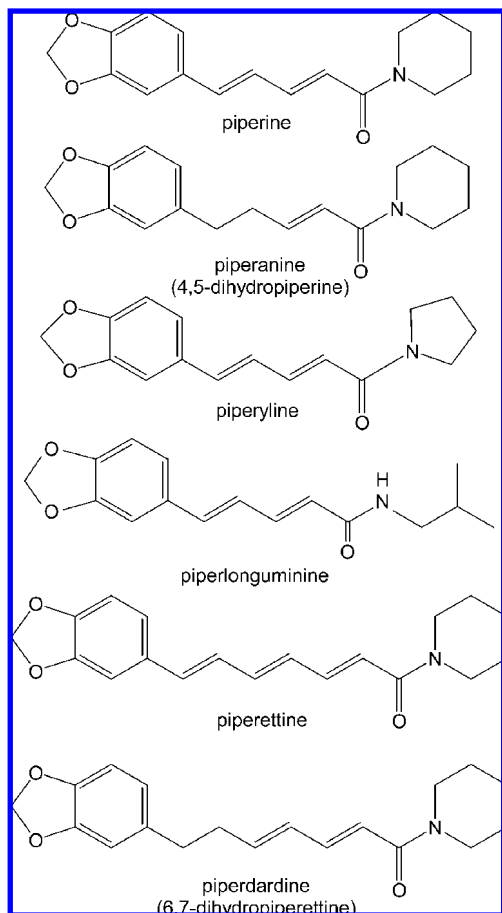


Figure 1. Structures of piperamides evaluated in this study.

Brazil as a sedative and as an antidote for snake bites (41). Piperdardine was also present in Chinese *Piper* species, which were found to inhibit prostaglandin and leukotriene biosynthesis in vitro (42).

Reported analytical methods for piperamides include vibrational spectroscopy (43), UV spectroscopy (44), NMR (32, 45), capillary electrochromatography (46), high-performance liquid chromatography (HPLC) (47, 48), microliquid chromatography (49), liquid chromatography/mass spectrometry (LC/MS) (50, 51), and thin-layer chromatography/gas chromatography (TLC/GC) (52).

We previously developed and validated HPLC and LC/MS methods for the analysis of extracts of fresh peppers containing capsaicinoids and piperines in pepper-containing foods and for analysis and isolation of the following four possible piperine-derived photoinduced isomers: piperine, isopiperine, chavicine, and isochavicine (53–55). To further increase our knowledge about the content of piperamides, the main objective of the present study was to determine by HPLC and LC/MS the structures and contents of additional pepper alkaloids in 20 commercial peppercorns and ground peppers. We report here the wide-ranging concentrations and distributions of the bio-active piperamides in different brands of widely consumed pepper products.

MATERIALS AND METHODS

Materials. Piperine (97.89% pure) was obtained from Aldrich (Milwaukee, WI). Acetonitrile (LC/MS grade) was obtained from Burdick & Jackson (Muskegon, MI), analytical grade formic acid (amino acids sequence analysis grade) was from DC Chemical Co.

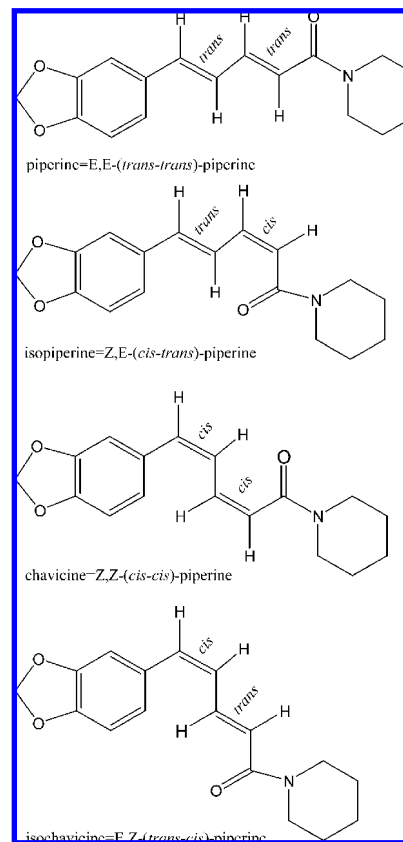


Figure 2. Structures of four theoretically possible and characterized piperine *cis-trans* isomers.

(Seoul, Korea), and ethanol was from Samchum Pure Chemicals (Pyungtack, Korea). The solvents were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA) and then degassed in an ultrasonic bath before use.

Whole and ground peppercorns were purchased at local stores in the United States (McCormick and Co., Inc., Tone Brothers Inc., and Albertson's store brand) and in Korea (Daesang Co.), as well as from Ponape Island (The Micronesia Union), Miya, Inc. (Japan), S&B Food Inc. (India, Malaysia, and Indonesia), E-tisanes (Japan), Drogheria & Alimentari Co. (Italy), and Ottogi Co. (Korea).

Extraction of Piperamides from Peppers. All operations were carried out in the dark. Each sample of black and white peppercorns was ground in a coffee blender for 2 min and passed through a 100 mesh screen. The resulting powder (0.1–0.15 g) was then placed into a 5 mL vial to which was added 2 mL of 80% ethanol. The suspension was sonicated for 60 min in an ultrasonic bath and then centrifuged at 12000g for 10 min at 5 °C. The supernatant (20 μL) was injected into HPLC for piperamide analysis.

HPLC. HPLC was carried out on a Hitachi liquid chromatograph model 655-II equipped with an autosampler (model 655A-40). The stainless steel column (250 mm \times 4.0 mm inner i.d.) was packed with Inertsil ODS-3v (5 μm particle diameter) (GL Sciences, Tokyo, Japan). The column temperature was maintained at 30 °C with a Shimadzu column oven CTO-10vp (Shimadzu, Kyoto, Japan). Separation was achieved using a mixture of acetonitrile/0.5% formic acid in distilled water with a linear gradient from 31 to 55% acetonitrile for 70 min. The flow rate was adjusted to 1 mL/min. The Shimadzu UV-vis detector (model SPD-10Avp) was set at 280 and 340 nm.

LC-MS/MS. LC/MS experiments were performed by an LCQ mass spectrometer (Thermo Fisher Scientific Inc., MA) equipped with an HPLC system (Agilent model HP-1100, CA) connected with a diode array detector (DAD G1315A). The piperamide solution (5 μL) was applied on an Inertsil ODS-3 column (2.1 mm \times 150 mm, 3 μm , GL Sciences Inc., Tokyo, Japan). Each piperamide was separated using a mixture of acetonitrile/0.5% formic acid in distilled water with a linear gradient at a flow rate of 200 $\mu\text{L}/\text{min}$.

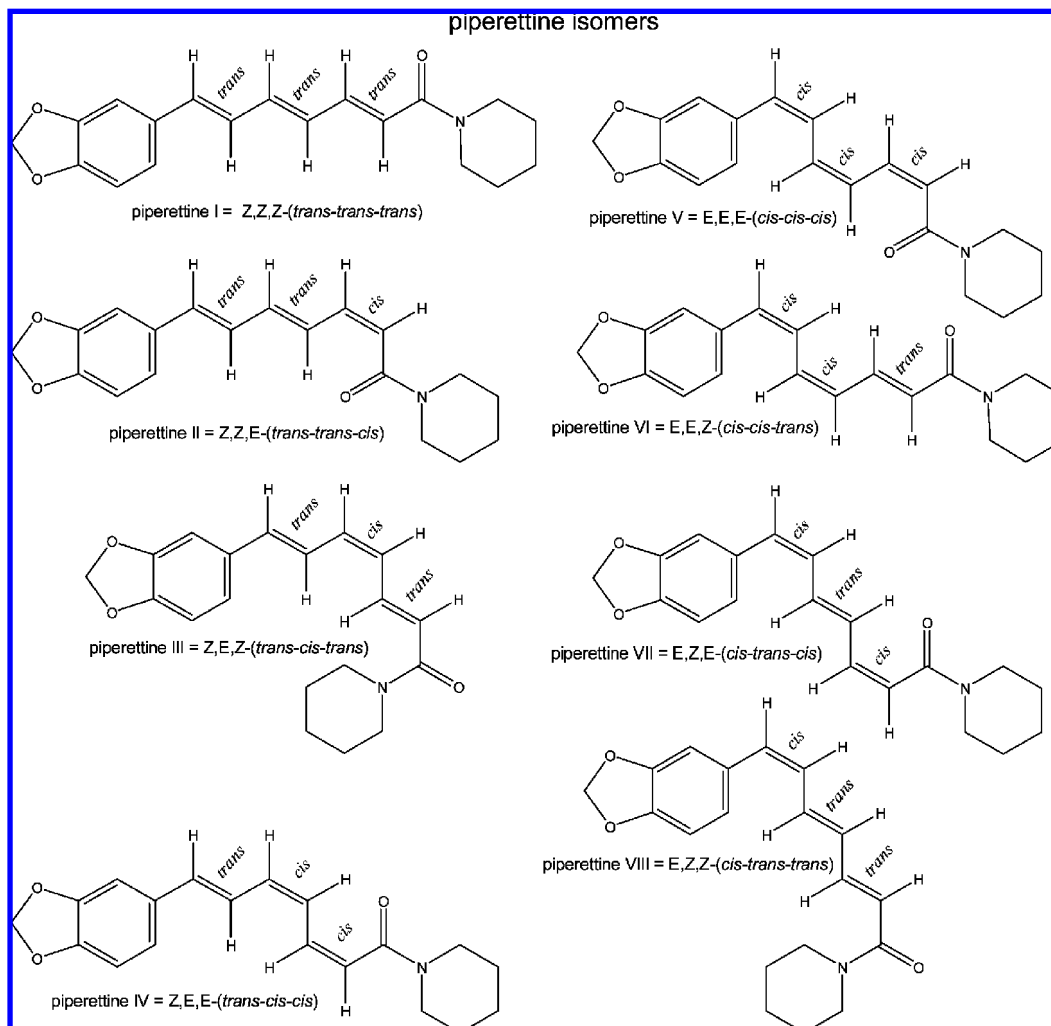


Figure 3. Structures of eight theoretically possible piperettine *cis-trans* isomers, four of which were identified in the present study.

The HPLC method, with a limit of detection (LOD) of $\sim 15\text{--}30$ ng estimated from calibration curves (55), was optimized by determining how the composition of the mobile phase affected retention times. The mobile phase of the (A/B) gradient was (A) acetonitrile and (B) 0.5% formic acid. The content of acetonitrile in the solvent was changed as follows: 31–55% (linear gradient, 0–70); 80% (70.1–80 min); and 31% (80.1–95 min). UV–visible spectra were recorded from 220 to 500 nm.

We reanalyzed the same concentrations of a dilution series of piperine by three HPLC methods: two slightly different isocratic methods (acetonitrile/0.5% formic acid, 31/69 and 30/70, v/v) and a gradient method (31→80%). The same LOD values were obtained by all three methods (55).

The mass spectrometer was initially tuned using a solution containing the mixture of piperines. The HPLC eluate was introduced into the mass spectrometer from 0 to 90 min. Mass and multiple tandem mass spectrometry (MSn) were operated in the positive ion mode in the mass range of m/z 50–400. Helium was used as the collision gas for the MSn spectrometric procedures, followed by the isolation of ions over a selected mass window of 2 Da. MSn represents multiple stage of precursor ion m/z selection followed by product ion detection for successive progeny ions. Mass selection of the analyte by m/z is followed by fragmentation and analysis of the fragments.

Concentrations. All concentrations were automatically calculated as piperine equivalents, taking into account the absolute amount of Sigma piperine of 97.89%. Because only one standard (piperine) was available to us, the parameter that we selected to quantitate the data was the ratio of the integrated peak area for each compound as a percent of the sum of all of the peak areas obtained for each piperamide present in the extract. These values were automatically calculated with the aid of a Hitachi Chromato-integrator model D-2500.

RESULTS AND DISCUSSION

Analytical Aspects. We had previously developed and validated HPLC and LC/MS methods to measure capsaicinoids and piperines in extracts of a large number of peppers and in pepper-containing foods (53–55). In the present study, the methods were further modified and used to analyze the content of piperines and several additional structurally related piperamides in commercial ground peppers and peppercoms.

HPLC retention times, using the gradient system shown, were as follows: piperlyne, 23.27 ± 0.06 min ($n = 3$); piperlonguminine, 31.10 ± 0.04 min; piperanine, 31.83 ± 0.06 min; piperine isomers, 34.52 ± 0.05 min; isochavicine, 36.47 ± 0.05 min; piperdardine, 46.81 ± 0.07 min; piperettine isomer I, 48.26 ± 0.07 min; piperettine isomer II, 49.07 ± 0.07 min; piperettine isomer III, 50.96 ± 0.08 min; and piperettine isomer IV, 52.27 ± 0.08 min.

Structural identification of individual compounds in extracts was performed by associating the HPLC peak of each compound with the corresponding mass spectrum. For structural assignment, we used the following literature molecular weights (56) for the piperamides evaluated in the present study (structures are shown in **Figures 1–3**): piperlonguminine, 273.331; piperine and its isomers, 285.342; piperanine (4,5-dihydropiperine), 287.358; piperettine, 311.38; and piperdardine (6,7-dihydropiperettine), 313.396. **Figures 4** and **5** show HPLC chromatograms of several pepper extracts and **Figures 6–8** depict the corresponding ion mass chromatograms and mass spectra as well

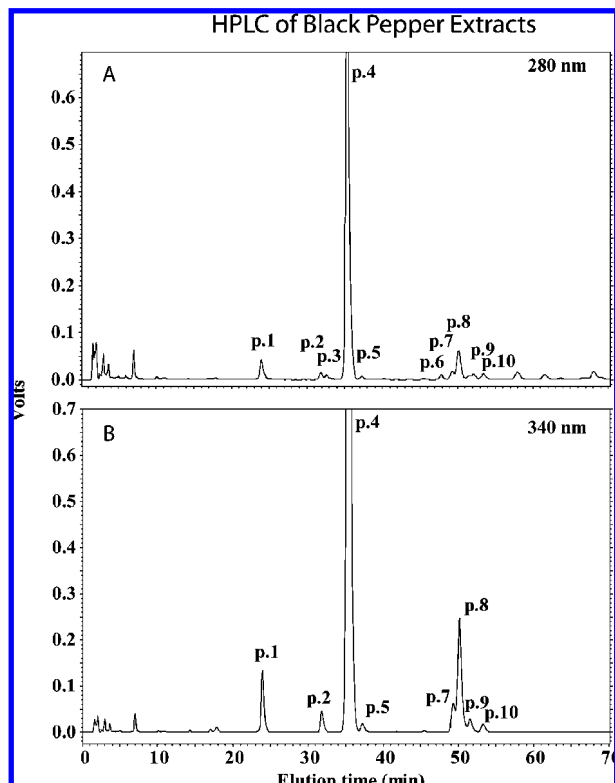


Figure 4. HPLC chromatograms of a black pepper extract. Conditions: column, Inertsil ODS-3v (4.0 mm × 25 cm, 5 μm); column temperature, 30 °C; mobile phase, acetonitrile/0.5% formic acid (gradient mode); flow rate, 1 mL/min; and detector, 280 and 340 nm. Peaks: 1, piperlyline; 2, piperlonguminine; 3, piperanine; 4, piperine isomers (piperine, isopiperine, and chavicine); 5, isochavicine; 6, piperdardine; 7, piperettine I; 8, piperettine II; 9, piperettine III; and 10, piperettine IV.

as the structures of major mass fragments. **Table 3** summarizes retention times and relative intensities of the mass fragments. The data show that the peak patterns of the mass spectra varied widely. Except for the major fragments shown in **Figure 8**, we were unable to assign structures to all of the other mass fragments listed.

Below, we examined trends in total and individual piperamide and ratios of piperine to total piperamide levels for ground and whole peppers.

Piperamide Content of Peppers. **Tables 1** and **2** list the concentrations/distributions of the following piperamides in 20 different types and brands of widely consumed commercial ground peppers and peppercorns: piperlyline, piperlonguminine, piperanine, three piperine isomers (piperine, isopiperine, and chavicine), the piperine isomer isochavicine, piperdardine, and four piperettine isomers. The tables also show the calculated percent contribution of each piperamide to the total (values in parentheses), the sums of all piperamides (total) in mg/g, and the ratios of piperine to total piperamide concentrations.

Total Piperamide Values. The total piperamide values for white and black ground pepper (**Table 1**) ranged from 48.1 (C, black) to 62.8 mg/g (K, black). The corresponding range for peppercorns (**Table 2**) was from 6.6 (K, red) to 152.5 (T, green). The exceptionally low content of piperamides in red peppercorns merits separate consideration from the others. The ratio of piperines to piperamides for ground pepper ranged from 0.81 (F, black) to 0.88 (A, white). The corresponding ratio for peppercorns (excluding red) ranged from 0.76 (M, black) to 0.90 (L, white).

The calculated average value of piperine to total piperamide ratios for 19 ground peppers and peppercorns was 0.84 ± 0.04 ($n = 19$), not including red pepper, which was quite narrow. Evidently, the absolute values but not the ratios vary widely among the evaluated peppers.

Because the various piperamides have been shown to exhibit a variety of biological/pharmacological effects in insects, animals, and humans, it is useful to examine the trends in the contents of the individual piperamides in the different pepper products. This aspect is examined below.

Piperlyline. **Table 1** shows that the piperlyline content of the 10 white and black ground peppers (in mg/g) ranged from 0.9 (F) to 1.9 (G). For peppercorns, the corresponding range was from 1.8 (M, black) to 5.2 (T, green) (**Table 2**). The average for all peppers was 2.5 ± 1.42 ($n = 20$). Moreover, unlike the other peppers to which piperlyline contributes from 1.6 (F, black) to 4.6% (P, black) to the total, the corresponding value for red peppercorns (5.9 mg/g) was 89.4% of the total. The remaining 10.6% consisted of piperine isomers. Red peppercorns contained no other piperamides. Because red peppercorns contained low amounts of only two piperamides, this brand was omitted from the following description of observed trends for 19 other peppers.

Piperlonguminine. **Table 1** shows that the piperlonguminine content (in mg/g) of ground peppers ranged from 0.1 (B) to 0.9 (G). The corresponding range for peppercorns was from 0 (R) to 1.0 (P) (**Table 2**). The average value for all ground peppers and peppercorns equaled 0.5 ± 0.29 ($n = 19$). The percent contribution of piperlonguminine to the total value averaged 0.8 ± 0.46 ($n = 19$). These results indicate that on average piperlonguminine contributes ~1% to the total piperamide content of the peppers.

Piperanine. **Table 1** shows that the piperanine content (in mg/g) for ground peppers ranged from 0.3 (B) to 0.6 (F, H, and J). The corresponding range for peppercorns was from 0.5 (L, M) to 1.4 (S) (**Table 2**). The average for all peppers equaled 0.7 ± 0.29 ($n = 19$). The percent contribution of piperanine to the total value of all peppers averaged 0.9 ± 0.22 ($n = 19$). These results indicate that piperanine also contributes ~1% to the total piperamide content of the peppers. Piperanine has been found to be present in several pepper species including *Piper chaba* (29), *Piper guineense* (57), and *Piper longum* L. (48).

Piperine Isomers. Four *cis-trans* piperine stereochemical isomers have been isolated from black peppers (**Figure 2**). In a previous study, we reported that the isopiperine, chavicine and isochavicine isomers together contributed ~1% to the total content of the piperine isomers in black peppers (55). In the present study, the values for the three piperine isomers, piperine, isopiperine, and chavicine were combined because the minor peaks were not well-resolved from piperine in this system. However, it was possible to measure the amounts of isochavicine, the fourth piperine isomer.

Table 1 shows that the content of three piperine isomers (piperine + isopiperine + chavicine) in ground peppers (in mg/g) ranged from 33.2 (F) to 52.2 (H, J). The corresponding range for peppercorns was from 40.2 (M, black) to 128.6 (T, green) (**Table 2**). The percent contribution to the total piperamides for all 19 peppers ranged from 58.9% for ground black pepper (F) to 89.2% for white peppercorns (L).

For the isochavicine piperine isomer, the values for ground peppers (in mg/g) ranged from 0.3 (D) to 12.7 (F). For peppercorns, the corresponding range was from 0.2 (O) to 0.9 (N). We do not know whether the very high content of

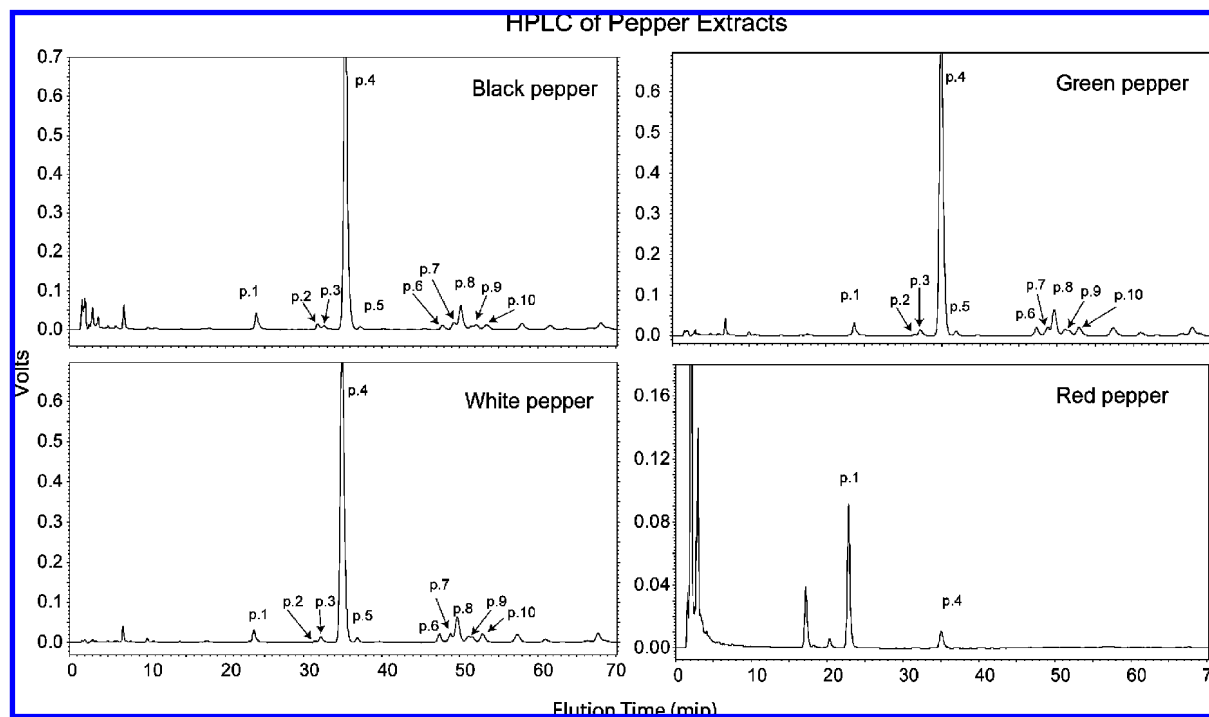


Figure 5. HPLC chromatograms of extracts of black, white, green, and red peppers. Conditions: column, Inertsil ODS-3v (4.0 mm \times 25 cm, 5 μ m); column temperature, 30 $^{\circ}$ C; mobile phase, acetonitrile/0.5% formic acid (gradient mode); flow rate, 1 mL/min; and detector, 280 nm. The assignment of peak numbers to specific piperamides is the same as shown in **Figure 4**.

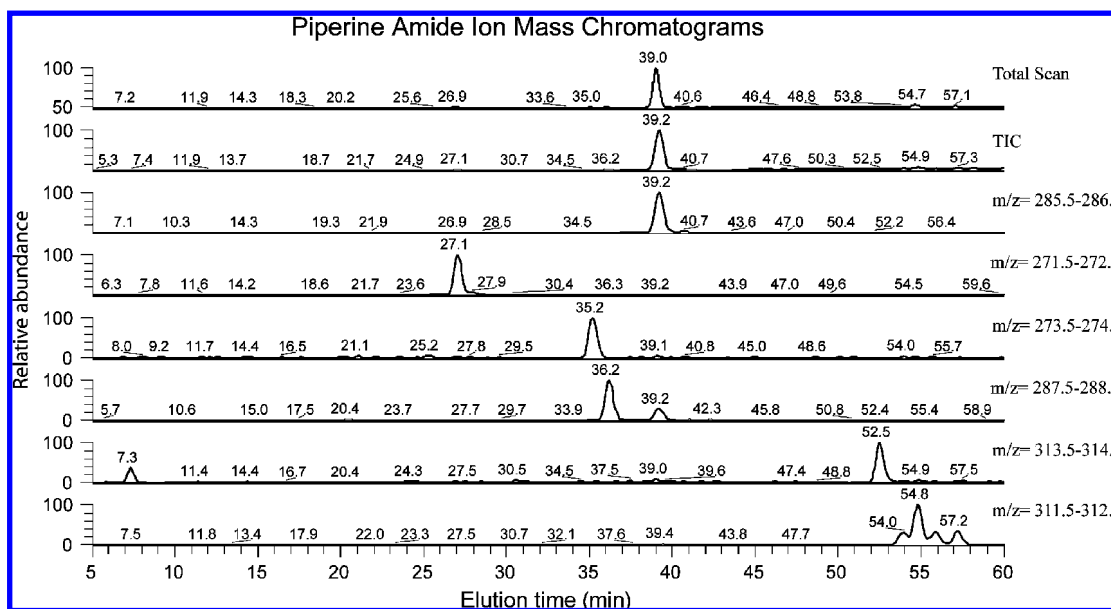


Figure 6. Ion mass chromatograms of piperine amides determined by LC-MS. Conditions: column, Inertsil ODS-3 (3.0 mm \times 15 cm, 3 μ m); column temperature, 30 $^{\circ}$ C; mobile phase, acetonitrile/0.5% formic acid (gradient mode); and flow rate, 0.2 mL/min. The indicated m/z values of the parent molecular ions correspond to the individual piperamides shown in the next two figures.

isochavicine in black ground pepper F (22.5% of the total) is the result of preharvest biosynthesis in the plant, postharvest isomerization of piperine, or a coeluting peak.

Piperdardine. **Table 1** shows that the piperdardine content of ground peppers (in mg/g) ranged from 0 (A, C, D, G, J) to 0.2 (I). The corresponding range for peppercorns was from 0 (N) to 1.8 (Q) (**Table 2**). The piperdardine content (in mg/g) of all peppers averaged 0.3 ± 0.48 ($n = 19$). The percent contribution of piperdardine to the total value averaged 0.4 ± 0.49 ($n = 19$). These results indicate that black peppercorn (Q) contains significant amounts of piperdardine.

Piperettine Isomers. With the exception of red peppercorns, our analytical data revealed the presence of four isomeric forms of piperettine in all of the other peppers. The four isomers separated by HPLC had the same molecular weight in the mass spectra. In analogy with piperine isomers, it is likely that these are *cis-trans* stereoisomers (**Figure 3**). However, data based on HPLC and LC/MS used in the present study do not permit assigning specific stereoisomeric structures to each isomer. Such assignment requires the use of detailed NMR spectroscopy. To our knowledge, this is the first report suggesting the presence of four piperettine isomers in commercial peppers.

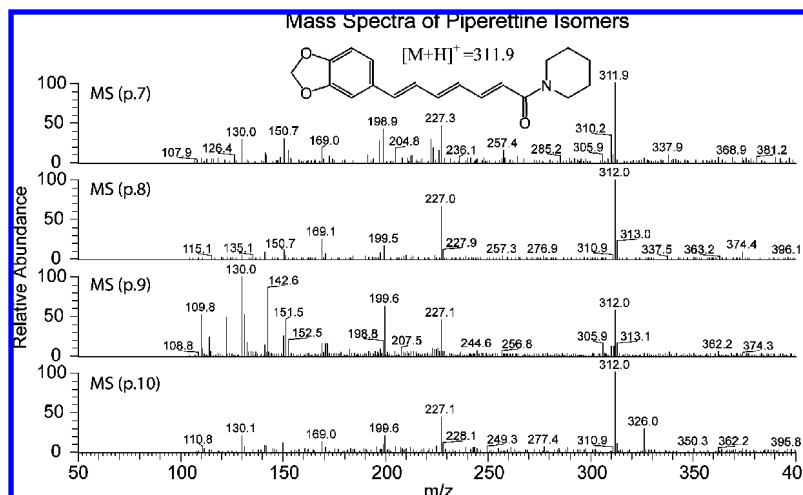


Figure 7. Mass spectra of piperettine isomers I–IV (peaks 7–10). Protonated molecular ion peaks are designated by $[M + H]^+$.

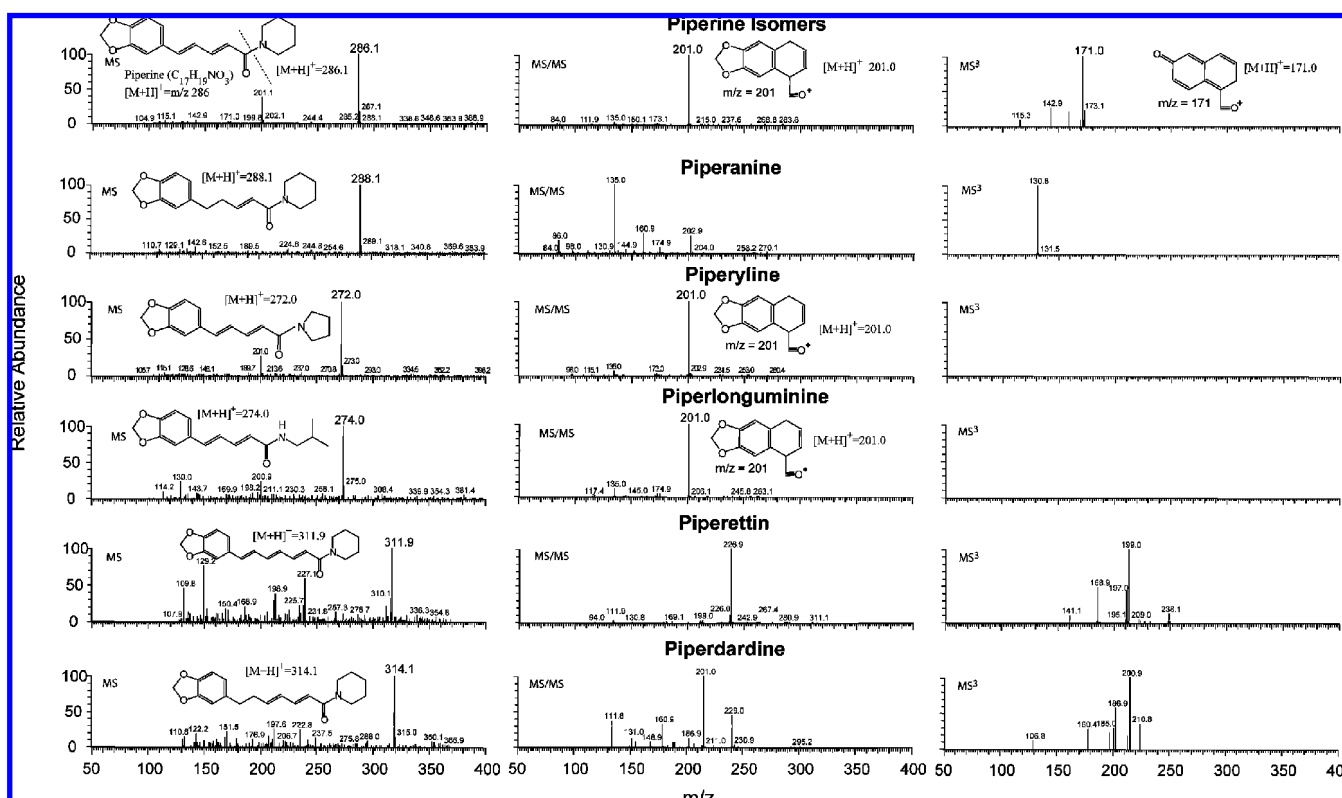


Figure 8. Mass spectra of piperamides. Note also the m/z values and postulated structures of major mass fragments.

Table 1 shows that the content of piperettine isomer (I) (in mg/g) in ground peppers ranged from 0.4 (A, C, H) to 0.9 (F). The corresponding range for peppercorns was from 0.3 (N) to 2.3 (P) (Table 2). The content of piperettine (I) in all peppers averaged 0.9 ± 0.63 ($n = 19$). The percent contribution of piperettine isomer (I) to the total value for all peppers averaged 1.2 ± 0.52 ($n = 19$). These results indicate that some peppers contain significant amounts of piperettine isomer (I).

Table 1 shows that the content of piperettine isomer (II) (in mg/g) for ground peppers ranged from 1.3 (A) to 4.2 (I). The corresponding range for peppercorns was from 2.7 (N, black) to 12.4 (T, green) (Table 2). The content of piperettine isomer (II) for all peppers (in mg/g) averaged 4.5 ± 2.96 ($n = 19$). The percent contribution of the content of isomer (II) to the total value for all peppers averaged 5.8 ± 2.20 ($n = 19$). These results indicate that peppercorns P, Q, R, and T contain high amounts of piperettine isomer (II).

Table 1 shows that the content of piperettine isomer (III) (in mg/g) for ground peppers ranged from 1.1 (A, C) to 2.8 (F). The corresponding range for peppercorns was from 0.5 (L) to 3.1 (R) (Table 2). The content of piperettine isomer (III) for all peppers (in mg/g) averaged 1.8 ± 0.68 ($n = 19$). The percent contribution of piperettine isomer (III) to the total value of all peppers averaged 2.6 ± 1.02 ($n = 19$). Black peppercorns (M) contributed a relatively high amount (4.3%) of the piperettine isomer (III) to the total.

Table 1 shows that the content of piperettine isomer (IV) (in mg/g) for ground peppers ranged from 0.9 (C) to 2.0 (F). The corresponding range for peppercorns was from 0.2 (P) to 1.7 ((M, Q) (Table 2). The piperettine isomer (IV) content for all peppers (in mg/g) averaged 1.2 ± 0.46 ($n = 19$). The percent contribution of piperettine isomer (IV) to the total value of all peppers averaged 1.8 ± 0.94 ($n = 19$).

Table 1. Concentration of Individual Piperamides Listed in Order of Elution Times, Total Piperamide Content, and Ratios of Piperine Isomers to Total Piperamide Levels in Extracts of Commercial Black and White Ground Peppers^a

pepper source	pepper type	piperlyline	piperlonguminine	piperanine	piperine + isopiperine + chavicine	isochavicine (piperine isomer)	piperdardine	piperettine isomer (I)	piperettine isomer (II)	piperettine isomer (III)	piperettine isomer (IV)	total piperamides	% piperines
A	white	1.2 ± 0.0 (2.6)	0.4 ± 0.0 (0.8)	0.4 ± 0.0 (0.8)	42.0 ± 0.1 (85.9)	1.0 ± 0.0 (2.0)	0.0 ± 0.0 (0)	0.4 ± 0.0 (0.8)	1.3 ± 0.0 (2.7)	1.1 ± 0.0 (2.2)	1.1 ± 0.0 (2.2)	48.9	88
B	white	1.1 ± 0.0 (2.1)	0.1 ± 0.0 (0.2)	0.3 ± 0.0 (0.6)	40.2 ± 0.5 (77.5)	4.5 ± 0.1 (8.7)	0.1 ± 0.0 (0.2)	0.6 ± 0.0 (1.2)	2.1 ± 0.1 (4.0)	1.5 ± 0.1 (2.8)	1.4 ± 0.1 (2.7)	51.9	86
C	black	1.6 ± 0.0 (3.3)	0.9 ± 0.0 (1.9)	0.4 ± 0.0 (0.8)	40.4 ± 0.1 (84.0)	0.5 ± 0.0 (1.0)	0.0 ± 0.0 (0)	0.4 ± 0.0 (0.8)	1.9 ± 0.0 (4.0)	1.1 ± 0.0 (2.3)	0.9 ± 0.0 (1.9)	48.1	85
D	black	1.4 ± 0.1 (2.7)	0.4 ± 0.0 (0.8)	0.5 ± 0.0 (1.0)	43.5 ± 0.4 (83.3)	0.3 ± 0.0 (0.6)	0.0 ± 0.0 (0)	0.6 ± 0.0 (1.1)	2.9 ± 0.1 (5.5)	1.3 ± 0.0 (2.5)	1.3 ± 0.0 (2.5)	52.2	84
E	black	1.4 ± 0.1 (2.7)	0.5 ± 0.0 (1.0)	0.5 ± 0.1 (1.0)	42.9 ± 0.4 (81.8)	0.5 ± 0.1 (1.0)	0.1 ± 0.0 (0.2)	0.6 ± 0.0 (1.1)	3.0 ± 0.1 (5.6)	1.5 ± 0.1 (2.9)	1.4 ± 0.0 (2.7)	52.4	83
F	black	0.9 ± 0.0 (1.6)	0.3 ± 0.0 (0.5)	0.6 ± 0.0 (1.1)	33.2 ± 0.1 (58.9)	12.7 ± 0.0 (22.5)	0.1 ± 0.0 (0.2)	0.9 ± 0.2 (1.6)	2.9 ± 0.0 (5.1)	2.8 ± 0.0 (5.0)	2.0 ± 0.0 (3.5)	56.4	81
G	black	1.9 ± 0.0 (3.1)	0.9 ± 0.0 (1.5)	0.5 ± 0.0 (0.8)	50.4 ± 0.4 (83.2)	0.5 ± 0.0 (0.8)	0.0 ± 0.0 (0)	0.6 ± 0.0 (1.0)	3.0 ± 0.0 (5.0)	1.4 ± 0.0 (2.3)	1.4 ± 0.1 (2.3)	60.6	84
H	black	1.8 ± 0.0 (2.9)	0.4 ± 0.0 (0.6)	0.6 ± 0.0 (1.0)	52.2 ± 0.0 (84.6)	0.7 ± 0.0 (1.1)	0.1 ± 0.0 (0.2)	0.4 ± 0.0 (0.6)	2.8 ± 0.0 (4.5)	1.6 ± 0.0 (2.6)	1.1 ± 0.1 (1.9)	61.7	86
I	black	1.8 ± 0.1 (2.9)	0.7 ± 0.0 (1.1)	0.5 ± 0.1 (0.8)	50.7 ± 1.2 (81.8)	0.4 ± 0.0 (0.6)	0.2 ± 0.0 (0.3)	0.6 ± 0.0 (1.0)	4.2 ± 0.0 (6.8)	1.6 ± 0.0 (2.6)	1.3 ± 0.0 (2.1)	62.0	82
J	black	1.7 ± 0.0 (2.7)	0.5 ± 0.0 (0.8)	0.6 ± 0.0 (1.0)	52.2 ± 0.0 (83.1)	0.7 ± 0.0 (1.1)	0.0 ± 0.0 (0)	0.5 ± 0.0 (0.8)	3.3 ± 0.1 (5.3)	1.9 ± 0.1 (3.0)	1.4 ± 0.0 (2.2)	62.8	84

^aListed numbers [mean ± SD in mg/g (*n* = 3)] rounded off to one decimal point represent piperine equivalents; 0 = not detected. Values in parentheses show the percent of total for each piperamide. Total piperamides = sum of concentrations of piperlyline, piperlonguminine, piperanine, piperine isomers (piperine, isopiperine, chavicine, and isochavicine), piperdardine, and piperettine isomers I–IV.

Table 2. Concentration of Individual Piperamides Listed in Order of Elution Times, Total Piperamide Content, and Ratios of Piperine Isomers to Total Piperamide Levels in Extracts of Commercial Black, White, Green, and Red Peppers^a

pepper source	pepper type	piperlyline	piperlonguminine	piperanine	piperine + isopiperine + chavicine	isochavicine (piperine isomer)	piperdardine	piperettine isomer (I)	piperettine isomer (II)	piperettine isomer (III)	piperettine isomer (IV)	total piperamides	% piperines
K	red	5.9 ± 0.0 (89.4)	0	0	0.7 ± 0.0 (10.6)	0	0	0	0	0	0	6.6	11
L	white	2.4 ± 0.1 (2.7)	0.7 ± 0.0 (0.8)	0.5 ± 0.0 (0.6)	77.4 ± 0.1 (89.2)	0.3 ± 0.0 (0.3)	0.4 ± 0.0 (0.5)	0.5 ± 0.0 (0.6)	3.3 ± 0.1 (3.8)	0.5 ± 0.0 (0.6)	0.8 ± 0.0 (0.9)	86.8	90
M	black	1.8 ± 0.1 (3.4)	0.5 ± 0.0 (0.9)	0.5 ± 0.0 (0.9)	40.2 ± 0.2 (75.3)	0.4 ± 0.0 (0.7)	0.1 ± 0.0 (0.2)	0.7 ± 0.0 (1.3)	5.2 ± 0.1 (9.8)	2.3 ± 0.2 (4.3)	1.7 ± 0.4 (3.2)	53.4	76
N	black	1.9 ± 0.0 (2.9)	0.5 ± 0.0 (0.7)	0.8 ± 0.0 (1.2)	58.2 ± 0.5 (84.7)	0.9 ± 0.0 (1.3)	0.0 ± 0.0 (0)	0.3 ± 0.0 (0.4)	2.7 ± 0.0 (3.9)	2.3 ± 0.0 (3.3)	1.1 ± 0.2 (1.6)	68.7	86
O	black	3.2 ± 0.1 (3.5)	0.8 ± 0.0 (0.9)	0.6 ± 0.0 (0.7)	78.2 ± 0.1 (84.8)	0.2 ± 0.0 (0.2)	0.6 ± 0.0 (0.7)	1.2 ± 0.0 (1.3)	5.4 ± 0.0 (5.9)	1.1 ± 0.1 (1.1)	0.8 ± 0.0 (0.9)	92.1	85
P	black	4.3 ± 0.1 (4.6)	1.0 ± 0.1 (1.1)	1.3 ± 0.1 (1.4)	73.3 ± 0.0 (79.2)	0.3 ± 0.0 (0.3)	0.5 ± 0.0 (0.5)	2.3 ± 0.1 (2.5)	7.1 ± 0.0 (7.3)	2.3 ± 0.1 (2.5)	0.2 ± 0.0 (0.2)	92.6	79
Q	black	3.0 ± 0.1 (3.1)	0.1 ± 0.0 (0.1)	1.0 ± 0.0 (1.0)	77.5 ± 0.1 (80.5)	0.4 ± 0.0 (0.4)	1.8 ± 0.0 (1.9)	1.7 ± 0.0 (1.8)	7.0 ± 0.1 (7.3)	2.0 ± 0.0 (2.1)	1.7 ± 0.0 (1.8)	96.2	81
R	black	3.4 ± 0.1 (3.5)	0.0 ± 0.0 (0)	0.8 ± 0.1 (0.8)	74.4 ± 0.1 (76.2)	0.5 ± 0.0 (0.5)	1.3 ± 0.0 (1.3)	2.0 ± 0.0 (2.0)	10.8 ± 0.1 (11.1)	3.1 ± 0.0 (3.2)	1.4 ± 0.0 (1.4)	97.7	77
S	black	4.1 ± 0.1 (3.3)	0.9 ± 0.0 (0.7)	1.4 ± 0.1 (1.1)	107.4 ± 0.2 (86.2)	0.8 ± 0.0 (0.6)	0.4 ± 0.0 (0.3)	1.7 ± 0.0 (1.4)	4.6 ± 0.1 (3.8)	2.8 ± 0.1 (2.2)	0.5 ± 0.0 (0.4)	124.6	87
T	green	5.2 ± 0.1 (3.4)	0.5 ± 0.0 (0.4)	0.8 ± 0.0 (0.5)	128.6 ± 0.1 (84.2)	0.4 ± 0.0 (0.2)	0.5 ± 0.0 (0.4)	1.8 ± 0.1 (1.2)	12.4 ± 0.1 (8.1)	1.8 ± 0.0 (1.2)	0.5 ± 0.0 (0.4)	152.5	85

^aListed numbers [mean ± SD in mg/g (*n* = 3)] rounded off to one decimal point represent piperine equivalents; 0 = not detected. Values in parentheses show percent of total for each piperamide. Total piperamides = sum of concentrations of piperlyline, piperlonguminine, piperanine, piperine isomers (piperine, isopiperine, chavicine, and isochavicine), piperdardine, and piperettine isomers I–IV.

Table 3. Retention Time and Mass Spectra for Piperamides Identified Using the LC-MS Method

piperamides	LC-MS retention time (min)	mass spectra M ⁺ m/z (% relative intensity)
piperlyline	26.72–27.16	273.0 (14.5); 272.0 (100); 201.0 (27.2)
piperlonguminine	34.73–35.41	275.0 (18.6); 274.0 (100); 200.9 (24.0); 130.0 (23.1)
piperanine	35.85–36.34	289.1 (11.2); 288.1 (100)
piperine (isopiperine, chavicine, and isochavicine)	38.59–39.36	287.1 (18.1); 286.1 (100); 201.1 (37.4)
piperdardine	52.19–52.70	315.0 (15.4); 314.1 (100); 237.5 (13.6); 222.8 (24.5); 206.7 (9.3); 197.6 (25.6); 176.9 (10.7); 151.5 (21.9); 122.2 (18.4); 110.8 (14.4)
piperettine (I)	53.20–53.81	311.9 (100); 310.2 (38.1); 257.4 (15.7); 227.3 (45.5); 204.8 (18.3); 198.9 (41.8); 169.0 (14.9); 150.7 (30.6); 130.0 (26.9)
piperettine (II)	54.55–55.01	313.0 (22.7); 312.0 (100); 227.0 (67.4); 199.5 (17.4); 169.1 (25.0); 150.7 (10.6)
piperettine (III)	55.66–56.11	313.1 (15.1); 312.0 (100); 227.1 (45.7); 199.6 (63.4); 169.0 (14.3); 151.5 (46.0); 142.6 (86.0); 130.0 (100); 109.8 (52.8)
piperettine (IV)	56.98–57.40	326.0 (29.1); 312.0 (100); 227.1 (44.8); 199.6 (20.5); 169.0 (11.9); 130.1 (20.5)

These cited observations show that piperettine isomers contribute significantly to the total piperamide content of the peppers. In terms of both absolute values and the percent of total, the relative contribution appears to be in the following order: piperettine isomer (II) > piperettine isomer (III) > piperettine isomer (IV) > piperettine isomer (I).

We do not know whether some or all of the isomers are synthesized in the plant or whether they are formed postharvest by light-induced and/or enzyme-catalyzed *cis-trans* isomerization. Activities of the isomers in cells, microbes, insects, animals, and humans merit further study.

In summary, to our knowledge, there are no other recently reported studies on the piperamide contents of commercial peppers. The cited data show that there is wide variation in both individual and total amounts of piperamides among the peppers sold in Korea and the United States. Green peppers have the highest amounts. The percent contribution of piperine to total piperamide content appears to be relatively constant.

Although piperine is known to exhibit multiple physiological and pharmacological effects in animals and humans mentioned earlier, further studies are needed to determine biological potencies of the other piperamides described in the present study. Knowledge of the wide-ranging content of these alkaloids in different peppers should facilitate ascertaining their significance in plant physiology, agriculture, host–plant resistance, entomology, the diet, and medicine.

Finally, our data on the content of piperamides in commercial peppers may also help consumers select pepper products with desirable piperamide contents. Labeling of such products for piperamide content would undoubtedly benefit consumers.

LITERATURE CITED

- Semler, U.; Gross, G. G. Distribution of piperine in vegetative parts of *Piper nigrum*. *Phytochemistry* **1988**, *27*, 1566–1567.
- Teuscher, E. Pepper. *Medicinal Spices*; GmbH Scientific Publishers: Stuttgart, Germany, 2006; pp 290–302.
- Parmar, V. S.; Jain, S. C.; Gupta, S.; Talwar, S.; Rajwanshi, V. K.; Kumar, R.; Azim, A.; Malhotra, S.; Kumar, N.; Jain, R.; Sharma, N. K.; Tyagi, O. D.; Lawrie, S. J.; Errington, W.; Howarth, O. W.; Olsen, C. E.; Singh, S. K.; Wengel, J. Polyphenols and alkaloids from piper species. *Phytochemistry* **1998**, *49*, 1069–1078.
- Szallasi, A. Piperine: Researchers discover new flavor in an ancient spice. *Trends Pharmacol. Sci.* **2005**, *26*, 437–439.
- Srinivasan, K. Black pepper and its pungent principle-piperine: A review of diverse physiological effects. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 735–748.
- Scott, I. M.; Jensen, H. R.; Philogène, B. J. R.; Arnason, J. T. A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. *Phytochem. Rev.* **2008**, *7*, 65–75.
- Hirata, N.; Tokunaga, M.; Naruto, S.; Iinuma, M.; Matsuda, H. Testosterone 5- α -reductase inhibitory active constituents of *Piper nigrum* leaf. *Biol. Pharm. Bull.* **2007**, *30*, 2402–2405.
- Bezerra, D. P.; Castro, F. O.; Alves, A. P.; Pessoa, C.; Moraes, M. O.; Silveira, E. R.; Lima, M. A.; Elmiro, F. J.; Costa-Lotufo, L. V. In vivo growth-inhibition of sarcoma 180 by piperine and piperine, two alkaloid amides from *Piper*. *Braz. J. Med. Biol. Res.* **2006**, *39*, 801–807.
- Selvendiran, K.; Banu, S. M.; Sakthisekaran, D. Protective effect of piperine on benzo(a)pyrene-induced lung carcinogenesis in Swiss albino mice. *Clin. Chim. Acta* **2004**, *350*, 73–78.
- Sunila, E. S.; Kuttan, G. Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine. *J. Ethnopharmacol.* **2004**, *90*, 339–346.
- Reen, R. K.; Wiebe, F. J.; Singh, J. Piperine inhibits aflatoxin B1-induced cytotoxicity and genotoxicity in V79 Chinese hamster cells genetically engineered to express rat cytochrome P4502B1. *J. Ethnopharmacol.* **1997**, *58* (3), 165–173.
- Das, B.; Kundu, J.; Bachar, S. C.; Uddin, M. A.; Kundu, J. K. Antitumor and antibacterial activity of ethylacetate extract of *Ludwigia hyssopifolia* Linn. and its active principle piperine. *Pak. J. Pharm. Sci.* **2007**, *20*, 128–131.
- Li, S.; Wang, C.; Li, W.; Koike, K.; Nikaido, T.; Wang, M. W. Antidepressant-like effects of piperine and its derivative, antiepilepsirine. *J. Asian Nat. Prod. Res.* **2007**, *9*, 421–430.
- Li, S.; Wang, C.; Wang, M.; Li, W.; Matsumoto, K.; Tang, Y. Antidepressant like effects of piperine in chronic mild stress treated mice and its possible mechanisms. *Life Sci.* **2007**, *80*, 1373–1381.
- Lee, S. A.; Hong, S. S.; Han, X. H.; Hwang, J. S.; Oh, G. J.; Lee, K. S.; Lee, M. K.; Hwang, B. Y.; Ro, J. S. Piperine from the fruits of *Piper longum* with inhibitory effect on monoamine oxidase and antidepressant-like activity. *Chem. Pharm. Bull. (Tokyo)* **2005**, *53*, 832–835.
- Bajad, S.; Bedi, K. L.; Singla, A. K.; Johri, R. K. Antidiarrhoeal activity of piperine in mice. *Planta Med.* **2001**, *67*, 284–287.
- Hiwale, A. R.; Dhuley, J. N.; Naik, S. R. Effect of co-administration of piperine on pharmacokinetics of beta-lactam antibiotics in rats. *Indian J. Exp. Biol.* **2002**, *40*, 277–281.
- Khan, I. A.; Mirza, Z. M.; Kumar, A.; Verma, V.; Qazi, G. N. Piperine, a phytochemical potentiator of ciprofloxacin against *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2006**, *50*, 810–812.
- Kumble, K. D. Anti-bacterial drug discovery using systems biology. *Mini Rev. Med. Chem.* **2006**, *6*, 1275–1283.
- Huhtanen, C. N. Inhibition of *Clostridium botulinum* by spice extracts and aliphatic alcohols. *J. Food Prot.* **1980**, *43*, 195196, 200.
- Veerareddy, P. R.; Vobalaboina, V.; Nahid, A. Formulation and evaluation of oil-in-water emulsions of piperine in visceral leishmaniasis. *Pharmazie* **2004**, *59*, 194–197.
- Ghoshal, S.; Prasad, B. N.; Lakshmi, V. Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica* in vitro and in vivo. *J. Ethnopharmacol.* **1996**, *50*, 167–170.
- Ghoshal, S.; Lakshmi, V. Potential antiamoebic property of the roots of *Piper longum* Linn. *Phytother. Res.* **2002**, *16*, 689–691.

- (24) Ribeiro, T. S.; Freire-de-Lima, L.; Previato, J. O.; Mendonca-Previato, L.; Heise, N.; de Lima, M. E. Toxic effects of natural piperine and its derivatives on epimastigotes and amastigotes of *Trypanosoma cruzi*. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3555–3558.
- (25) Vijayakumar, R. S.; Surya, D.; Nalini, N. Antioxidant efficacy of black pepper (*Piper nigrum* L.) and piperine in rats with high fat diet induced oxidative stress. *Redox Rep.* **2004**, *9*, 105–110.
- (26) Vijayakumar, R. S.; Nalini, N. Efficacy of piperine, an alkaloidal constituent from *Piper nigrum* on erythrocyte antioxidant status in high fat diet and antithyroid drug induced hyperlipidemic rats. *Cell Biochem. Funct.* **2006**, *24*, 491–498.
- (27) Bai, Y. F.; Xu, H. Protective action of piperine against experimental gastric ulcer. *Acta Pharmacol. Sin.* **2000**, *21*, 357–359.
- (28) Bajad, S.; Bedi, K. L.; Singla, A. K.; Johri, R. K. Piperine inhibits gastric emptying and gastrointestinal transit in rats and mice. *Planta Med.* **2001**, *67*, 176–179.
- (29) Morikawa, T.; Matsuda, H.; Yamaguchi, I.; Pongpiriyadacha, Y.; Yoshikawa, M. New amides and gastroprotective constituents from the fruit of *Piper chaba*. *Planta Med.* **2004**, *70*, 152–159.
- (30) Duarte, C. D. M.; Verli, H.; Araújo, J. X. D., Jr.; Medeiros, I. A. D.; Barreiro, E. J.; Fraga, C. A. M. New optimized piperamide analogues with potent in vivo hypotensive properties. *Eur. J. Pharm. Sci.* **2004**, *23*, 363–369.
- (31) Scott, I. M.; Gagnon, N.; Lesage, L.; Philogene, B. J.; Arnason, J. T. Efficacy of botanical insecticides from *Piper* species (Piperaceae) extracts for control of European chafer (Coleoptera: Scarabaeidae). *J. Econ. Entomol.* **2005**, *98*, 845–855.
- (32) Yang, Y. C.; Lee, S. G.; Lee, H. K.; Kim, M. K.; Lee, S. H.; Lee, H. S. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. *J. Agric. Food Chem.* **2002**, *50*, 3765–3767.
- (33) Vijayakumar, R. S.; Nalini, N. Piperine, an active principle from *Piper nigrum*, modulates hormonal and apo lipoprotein profiles in hyperlipidemic rats. *J. Basic Clin. Physiol. Pharmacol.* **2006**, *17*, 71–86.
- (34) Lin, Z.; Liao, Y.; Venkatasamy, R.; Hider, R. C.; Soumyanath, A. Amides from *Piper nigrum* L. with dissimilar effects on melanocyte proliferation in-vitro. *J. Pharm. Pharmacol.* **2007**, *59*, 529–536.
- (35) Soumyanath, A.; Venkatasami, R.; Joshi, M.; Faas, L.; Adejuyigbe, B.; Drake, A. F.; Hider, R. C.; Young, A. R. UV irradiation affects melanocyte stimulatory activity and protein binding of piperine. *Photochem. Photobiol.* **2006**, *82*, 1541–1548.
- (36) Shoba, G.; Joy, D.; Joseph, T.; Majeed, M.; Rajendran, R.; Srinivas, P. S. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med.* **1998**, *64*, 353–356.
- (37) Pattanaik, S.; Hota, D.; Prabhakar, S.; Kharbanda, P.; Pandhi, P. Effect of piperine on the steady-state pharmacokinetics of phenytoin in patients with epilepsy. *Phytother. Res.* **2006**, *20*, 683–686.
- (38) Platel, K.; Srinivasan, K. Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. *Nahrung* **2000**, *44*, 42–46.
- (39) Hu, Z.; Yang, X.; Ho, P. C.; Chan, S. Y.; Heng, P. W.; Chan, E.; Duan, W.; Koh, H. L.; Zhou, S. Herb-drug interactions: A literature review. *Drugs* **2005**, *65*, 1239–1282.
- (40) Kasibhatta, R.; Naidu, M. U. R. Influence of piperine on the pharmacokinetics of nevirapine under fasting conditions: A randomised, crossover, placebo-controlled study. *Drugs R&D* **2007**, *8*, 383–391.
- (41) De Araújo, J. X., Jr.; Da-Cunha, E. V. L.; De O. Chaves, M. C.; Gray, A. I. Piperdardine, a piperidine alkaloid from *Piper tuberculatum*. *Phytochemistry* **1997**, *44*, 559–561.
- (42) Stöhr, J. R.; Xiao, P. G.; Bauer, R. Constituents of Chinese *Piper* species and their inhibitory activity on prostaglandin and leukotriene biosynthesis in vitro. *J. Ethnopharmacol.* **2001**, *75*, 133–139.
- (43) Schulz, H.; Baranska, M.; Quilitzsch, R.; Schutze, W.; Losing, G. Characterization of peppercorn, pepper oil, and pepper oleoresin by vibrational spectroscopy methods. *J. Agric. Food Chem.* **2005**, *53*, 3358–3363.
- (44) Lupina, T.; Cripps, H. UV spectrophotometric determination of piperine in pepper preparations: Collaborative study. *J. AOAC Int.* **1987**, *70*, 112–113.
- (45) Catchpole, O. J.; Grey, J. B.; Perry, N. B.; Burgess, E. J.; Redmond, W. A.; Porter, N. G. Extraction of chili, black pepper, and ginger with near-critical CO₂, propane, and dimethyl ether: analysis of the extracts by quantitative nuclear magnetic resonance. *J. Agric. Food Chem.* **2003**, *51*, 4853–4860.
- (46) Musenga, A.; Mandrioli, R.; Ferranti, A.; D’Orazio, G.; Fanali, S.; Raggi, M. A. Analysis of aromatic and terpenic constituents of pepper extracts by capillary electrochromatography. *J. Sep. Sci.* **2007**, *30*, 612–619.
- (47) Wood, A. B.; Barrow, M. L.; James, D. J. Piperine determination in pepper (*Piper nigrum* L.) and its oleoresins—A reversed-phase high-performance liquid chromatographic method. *Flavour Fragr. J.* **1988**, *3*, 55–64.
- (48) Wu, S.; Sun, C.; Pei, S.; Lu, Y.; Pan, Y. Preparative isolation and purification of amides from the fruits of *Piper longum* L. by upright counter-current chromatography and reversed-phase liquid chromatography. *J. Chromatogr. A* **2004**, *1040*, 193–204.
- (49) Verzele, M.; van Damme, F.; Schuddinck, G.; Vyncke, P. Quantitative microscale liquid chromatography of piperine in pepper and pepper extracts. *J. Chromatogr. A* **1989**, *471*, 335–346.
- (50) Scott, I. M.; Puniani, E.; Jensen, H.; Livesey, J. F.; Poveda, L.; Sanchez-Vindas, P.; Durst, T.; Arnason, J. T. Analysis of *Piperaceae* germplasm by HPLC and LCMS: A method for isolating and identifying unsaturated amides from *Piper* spp. extracts. *J. Agric. Food Chem.* **2005**, *53*, 1907–1913.
- (51) Hashimoto, K.; Yaoi, T.; Koshihara, H.; Yoshida, T.; Maoka, T.; Fujiwara, Y.; Yamoto, Y.; Mori, K. Photochemical isomerization of piperine, a pungent constituent in pepper. *Food Sci. Technol. Int. (Tokyo)* **1996**, *2*, 24–29.
- (52) Marutoiu, C.; Gogoasa, I.; Oprean, I.; Marutoiu, O.-F.; Moise, M.-I.; Tigae, C.; Rada, M. Separation and identification of piperine and chavicine in black pepper by TLC and GC-MS. *J. Planar Chromatogr.—Mod. TLC* **2006**, *19*, 250–252.
- (53) Kozukue, N.; Han, J. S.; Kozukue, E.; Lee, S. J.; Kim, J. A.; Lee, K. R.; Levin, C. E.; Friedman, M. Analysis of eight capsaicinoids in peppers and pepper-containing foods by high-performance liquid chromatography and liquid chromatography-mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 9172–9181.
- (54) Choi, S. H.; Suh, B. S.; Kozukue, E.; Kozukue, N.; Levin, C. E.; Friedman, M. Analysis of the contents of pungent compounds in fresh Korean red peppers and in pepper-containing foods. *J. Agric. Food Chem.* **2006**, *54*, 9024–9031.
- (55) Kozukue, N.; Park, M. S.; Choi, S. H.; Lee, S. U.; Ohnishi-Kameyama, M.; Levin, C. E.; Friedman, M. Kinetics of light-induced *cis-trans* isomerization of four piperines and their levels in ground black peppers as determined by HPLC and LC/MS. *J. Agric. Food Chem.* **2007**, *55*, 7131–7139.
- (56) Yannai, S. *Dictionary of Food Compounds*; Chapman Hall/CRC: Boca Raton, FL, 2004.
- (57) Ekanem, A. P.; Wang, M.; Simon, J. E.; Obiekezie, A. I.; Morah, F. *In vivo* and *in vitro* activities of the seed extract of *Piper guineense* Schum. and Thonn. against skin and gill monogenean parasites of gold fish (*Carassius auratus auratus*). *Phytother. Res.* **2004**, *18*, 793–797.

Received for review December 19, 2007. Revised manuscript received February 14, 2008. Accepted February 15, 2008.

JF703711Z