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Kinetics of Light-Induced *Cis*−*Trans* Isomerization of Four Piperines and Their Levels in Ground Black Peppers as Determined by HPLC and LC/MS

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The pungent compounds piperine and isomers thereof, secondary metabolites present in black and white pepper fruit, undergo light-induced isomerizations. To facilitate studies in this area, an HPLC method has been developed for analysis and isolation of the following four possible piperine-derived photoinduced isomers: piperine, isopiperine, chavicine, and isochavicine. The limits of detection (LOD) estimated from calibration plots were ∼15−30 ng for each isomer. Reproducibilities of the analyses were excellent, and recoveries of spiked samples were as follows (average ± SD; $n = 3$): chavicine, 98.4 ± 2.1%; isopiperine, 96.2 ± 3.2%; piperine, 104 ± 3.8%; isochavicine, 98.9 ± 3.0%. To determine the kinetics of these isomerizations, fluorescent light, sunlight, and UV radiation at 254 nm was used to induce *cis*−*trans* geometric isomerization as a function of light intensities and time of exposure determined with the aid of high-performance liquid chromatography (HPLC) and liquid chromatography with diode array UV detection−mass spectrometry (LC-DAD/MS). HPLC was also used to determine the distribution of the isomers in four commercial ground black pepper products used as spices in culinary practice. Isomerization increased with light intensities and time of exposure and leveled off at the so-called photostationary phases. The piperine levels of the four products were quite similar, ranging (in wt %) from 10.17 to 11.68. The amounts of the other three isomers ranged from 0.01 to 0.07 of the total for chavicine; from 0.15 to 0.23 for isopiperine; and from 0.37 to 0.42 for isochavicine. The results establish the utility of the HPLC method for simultaneous analysis of the four isomers both in pure form and in black pepper extracts. The dietary significance of the results is discussed.

KEYWORDS: Piperine; isopiperine; chavicine; isochavicine; black peppers; photoisomerization; HPLC; LC−MS

INTRODUCTION

Piperines are secondary pungent metabolites present in the outer part of the fruits and in the seeds peppers (*Piper nigrum* L.) (*1*, *2*). Black pepper is produced from green unripe berries of the pepper plant, whereas white pepper is obtained when the seeds of fully ripe berries are dried (*3*). Interest in piperine arises from the fact that, in addition to its sensory properties that impact the taste of food, the compound is reported to possess several nonsensory beneficial biological/pharmacological properties that may also benefit human health. These include antibacterial/antiprotozoan (*4*−*7*), anticarcinogenic/antigenotoxic (*8*−*11*), antidepressant (*12*, *13*), antidiarrheal (*14*), antioxidative (*15*, *16*), insecticidal (*17*), antiulcer (*18*), and insulin-resistance (*19*) activities. Piperine is also reported to inhibit enzymes (cytochrome P450, UDP-glucoronyltransferase) that catalyze the biotransformation of nutrients and drugs, thus enhancing their bioavailability and efficacies *in vivo* (*20*−*23*).

Piperines exist as the following four distinct geometric isomers whose structures are shown in **Figure 1**: *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) (*24*). The *E*,*E*-(*trans*-*trans*)-piperine appears to be the main constituent that is largely responsible for the pungency of black and white peppers. The other three isomers appear to be formed by light-induced and/or enzyme-catalyzed *cis*−*trans* (geometric) isomerizations (photoisomerizations) of the double bonds of the parent piperine molecule.

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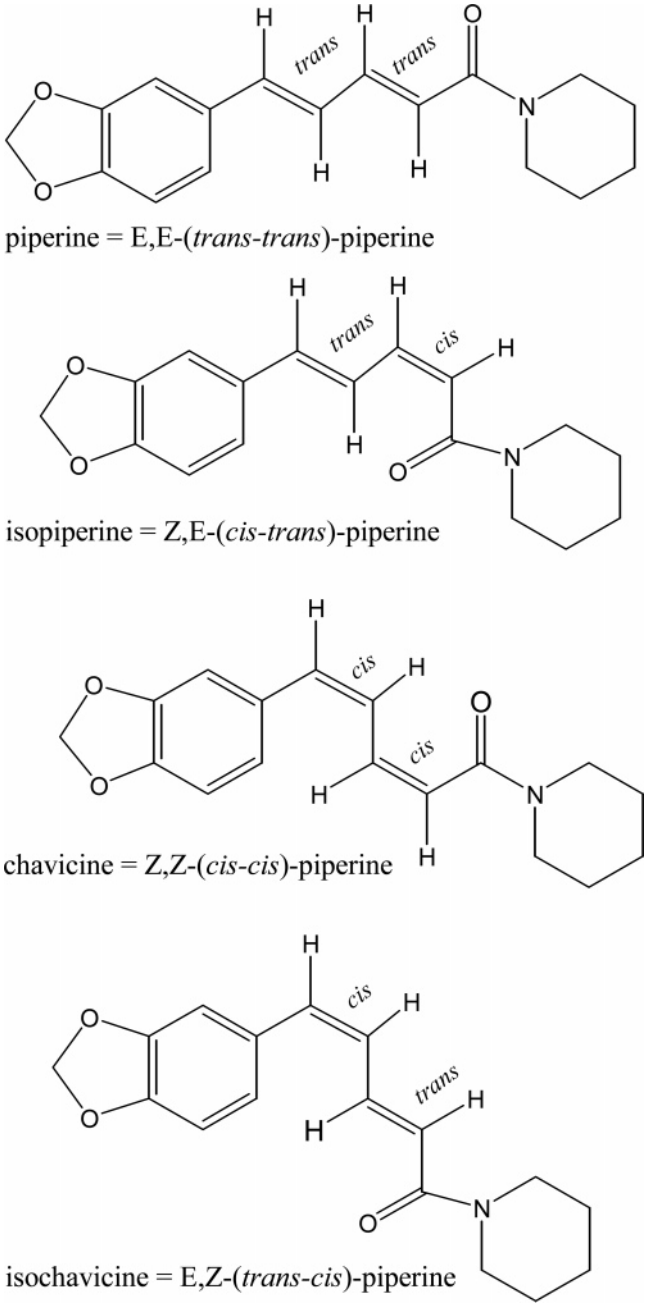

Figure 1. Structure of piperine isomers.

Reported analytical methods for piperine include NMR (*25*), vibrational spectroscopy (*26*), UV spectroscopy (*27*), HPLC (*28*, *29*), LC/MS (*30*, *31*), and TLC/GC (*32*). Verzele et al. (*33*) found that piperine in solution is the principal pungent substance of black peppers; the other three isomers have low pungency. These authors used the internal standard *p*-bromoacetanilide to achieve good separation of the four isomers on a nitro-silica gel stationary phase, but with poor precision. However, the analytical results were difficult to reproduce. A later study (*34*) describes improved separation by micro-liquid chromatography.

Ternes and Krause (*24*) developed two HPLC methods for the piperine isomers based on separation on ODS-Hypersil and on a second column, whose packing consisted of silver-modified cation-exchange ligand-covered spherical silica material. These authors related the structures of the individual isomers to the respective observed UV, NMR, and FT-IR spectra. The method was successfully applied to the analysis of piperine isomers in eggs produced by Leghorn laying hens fed 80 mg of piperine/100 g of diet. Although the hen diet contained only piperine, and precautions were taken to avoid light-induced isomerization during the trial, an egg yolk sample contained all four isomers. These observations suggest that after consumption, piperine probably undergoes enzyme-catalyzed *cis-trans* isomerizations *in vivo*.

Although several studies have described the photoisomerization of piperine to form a mixture of *cis-trans* isomers (*24*, *31*, *33*, *34*), to our knowledge, no previous study has investigated the analogous individual photoinduced isomerizations of pure isomers of piperine, namely, isopiperine, chavicine, and isochavicine exposed to light over a long time period. Therefore, the main objectives of the present study were (a) to validate an HPLC method for the analysis of the composition of mixtures of four piperine isomers; (b) to determine whether LC−MS can be used to characterize the isomers; (c) to determine the time-course (kinetics) of conversion of individual pure isomers to mixtures of all four isomers as function of light intensities; (d) to define the time-course of analogous isomerization of isopiperine, chavicine, and isochavicine induced by fluorescent light of a single intensity; and (e) to demonstrate the applicability of the HPLC method to measure the content of the four isomers in commercial ground black peppers products.

MATERIALS AND METHODS

Materials. Piperine (97.89% pure) was obtained from Aldrich (Milwaukee, WI). HPLC grade acetonitrile, methanol, and analytical grade formic acid were obtained from commercial sources. The solvents were filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA) and then degassed in an ultrasonic bath before use. Because chavicine, isopiperine, and isochavicine standards were not available from commercial sources, these three compounds were isolated by HPLC from photoisomerized piperine as described below. Four commercial ground black peppers were obtained from local stores, two in the United States and two in Korea.

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 × 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 constant 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 (30:70, v/v) as eluent with an isocratic flow rate with 0.8 mL/min at 25 °C. A Shimadzu UV−vis detector (model SPD-10Avp) was set at 220 to 340 nm.

Photoisomerization of Piperine by White Fluorescent and UV Light. Commercial piperine (3.14 mg/mL of methanol) was placed in a 5 mL glass vial. The vials were irradiated with the three fluorescent light intensities (1000, 3000, and 6000 lux) and UV light (2 lux at 254 nm) for nine photoperiods (from 5 to 2880 min) at 25 °C. After irradiation, each solution (10 μL) was directly injected into HPLC for the separation of isomers produced by photochemical isomerization. A Hitachi Chromato-integrator model D-2500 recorded the peak areas of each individual piperine isomer. The glass vial absorbed 3.4% of the incident UV light.

LC−MS/MS. Liquid chromatography/mass spectrometry experiments were performed by an LCQ mass spectrometer (Thermo Fisher Scientific Inc., MA) equipped with an HPLC system (Agilent, CA) connected with a DAD (G1315A). The piperine mixture solution (5 μL) was applied on an Inertsil ODS-3 column (2.1 × 150 mm, 3 μm, GL Sciences Inc., Tokyo, Japan) and was separated to piperine isomer peaks using isocratic solvent system consisting of 30% acetonitrile (LC/MS grade, Wako Pure Chemical Industries, Ltd., Osaka, Japan) containing 0.5% formic acid (amino acids sequence analysis grade) at the flow rate of 200 μL/min. UV−vis spectra were recorded in the wavelength range from 220 to 500 nm. The mass spectrometer was initially tuned using a solution containing the mixture of piperine isomers. The HPLC eluate was introduced into the mass spectrometer

from 5 to 115 min. Mass and multiple tandem mass spectrometry (MSn) were operated in the positive-ion mode in the mass range of *m*/*z* 50−500.

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.

Preparative Conversion of Piperine to Chavicine, Isopiperine, and Isochavicine. Chavicine, isopiperine, and isochavicine were produced from commercial piperine (3.14 mg/mL in methanol) by light exposure (6000 lux for 72 h). The individual compounds were isolated by repetitive high performance liquid chromatography using UV detection (280 nm). The isomer solutions (20 μL) produced by light exposure were each applied to HPLC column and eluted with acetonitrile/0.5% formic acid (30:70, v/v) at 25 °C. The individual isomers were monitored at 280 nm and collected from the UV detector. This procedure was repeated four times. Solutions containing each of the isolated isomers were stored in the dark at −30 °C. Because piperine isomers are sensitive to light, all experimental operations were performed in the dark.

Photoinduced Isomerization Kinetics of Chavicine, Isopiperine, and Isochavicine. Each solution (1 mL) of pure chavicine, isopiperine, and isochavicine isolated by HPLC from irradiated piperine was placed into a 5 mL vial. Each sample was then irradiated at a photointensity of 3000 lux for eight photoperiods ranging from 15 to 4320 min at 25 °C. After irradiation, each sample (80 μL) was injected into the HPLC column.

Quantification of Piperines. To establish the linearity of the response by HPLC, piperine (17.2 mg) was placed into a 10 mL vial to which was added to 5 mL of ethanol. The sample was then irradiated with white fluorescent light at an intensity of 3000 lux for 4 days at 25 °C. The reaction mixture containing the four isomers was then subject to 9-fold serial dilution series with ethanol. Each of the nine diluents (10 μL) was then directly injected onto the HPLC column in order to determine the concentration-dependent linear response of the integrated peak areas for each isomer. The calibration plot of each compound was obtained by plotting the integrated peak area against the amount injected.

Because only one standard (piperine) was available to us, the parameter 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 the peak areas obtained for each mixture of the four isomers. These values were automatically calculated with the aid of a Hitachi Chromato-integrator model D-2500.

Recovery of Piperine Isomers after Spiking. Piperine isomers were analyzed before and after addition of known amounts of each isomer to the mixture of isomers after exposure to fluorescent light of 3000 lux for 30 h at 25 °C. Recovery (%) = (concentration of each compound in spiked sample)/concentration of endogenous compound + spike) × 100.

Extraction-Analysis of Piperines from Commercial Ground Black Peppers. All operations were carried out in the dark. Each dry black pepper powder (0.1−0.15 g) was 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 18000*g* for 10 min at 1 °C. The supernatants were then passed through a 0.45 μm Millipore nylon filter (Bedford, MA). The filtrates were analyzed by HPLC.

RESULTS AND DISCUSSION

Analytical Aspects. *Identification of Chavicine, Isopiperine, Piperine, and Isochavicine.* We had previously developed and validated HPLC methods for analysis of extracts of fresh peppers containing capsaicinoids and of both capsaicinoids and piperines in pepper-containing foods (*35*, *36*). This method was used to quantify the distribution of capsaicinoids in 11 Korean whole peppers and in 12 commercial pepper-containing foods.

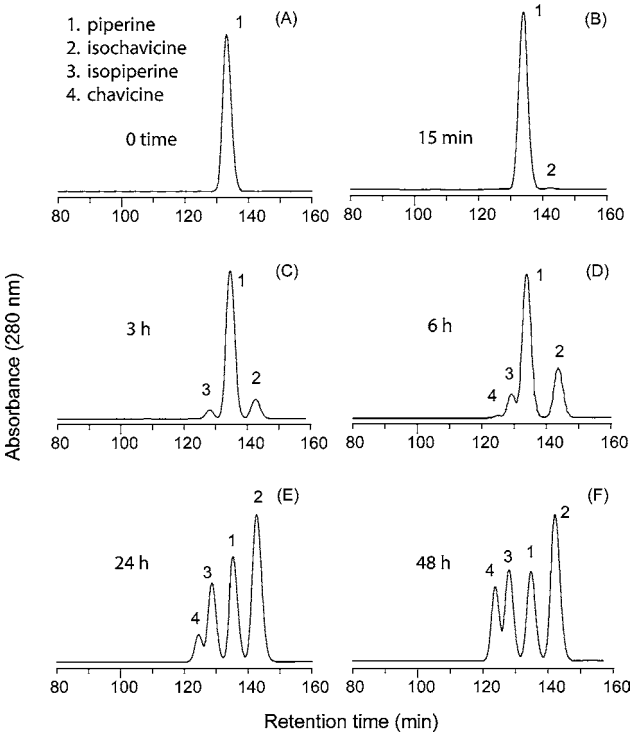

Figure 2. Time course of the photoisomerization of piperine at 3000 lux: HPLC chromatograms of piperine isomers produced from piperine before and after light exposure. Column: Inertsil ODS-3v (5 μm, 4.0 × 250 mm). Detector: UV at 280 nm. Flow rate, 0.8 mL/min. Chart speed: 1.25 mm/min.

The HPLC system we previously used to determine both capsaicinoid levels derived from red peppers and piperine levels derived from black peppers in pepper-containing foods was further refined and adapted in the present study to the analysis of the four piperine isomers by defining how composition of the mobile phase and column temperature affected retention times. The elution times of the four isomers on HPLC chromatograms were determined with ratios of the mobile phase of acetonitrile/0.5% formic ranging from 28/72 (v/v) to 40/60 (v/v), each at four temperatures ranging from 25 °C to 40 °C. The separation of peaks and retention times decreases with increasing acetonitrile/0.5% formic acid ratios and column temperatures. We selected the 30/70 (v/v) ratio for the mobile phase at 25 °C as conditions for optimum separation of the isomers, as illustrated in **Figure 2**. The retention times ranged as follows: chavicine, 129.5 min; isopiperine, 133.9 min; piperine, 141.0 min; isochavicine, 148.8 min.

Plots of concentration versus peak area (calibration plots) were linear from 0 to 5000 ng. The limit of detection (LOD) estimated from calibration plots was ∼15−30 ng. Recoveries of spiked samples were as follows (average ± SD; $n = 3$): chavicine, 98.4 ± 2.1%; isopiperine, 96.2 ± 3.2%; piperine, 104 ± 3.8%; isochavicine, 98.9 ± 3.0%.

Ultraviolet and Mass Spectra of Piperines. In the present study, structural identification of individual piperine isomers following irradiation was performed by associating the HPLC peak for each isomer with its corresponding UV spectrum that was previously associated by Ternes and Krause (*24*) with the corresponding NMR spectrum. We also assessed the potential of LC/MS to differentiate among the isomers.

Figures 3 and **4** depict the UV and mass spectra of the four piperine isomers determined in the present study by LC-DAD/MS. The UV absorbance shown in the three-dimensional plot

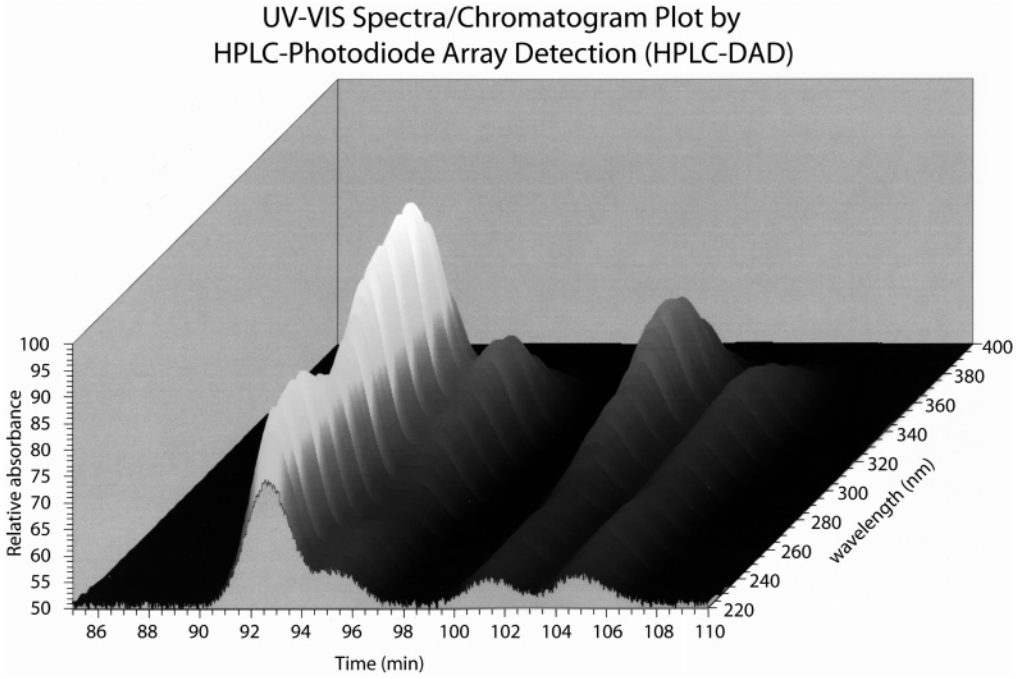

Figure 3. UV–vis spectra/chromatogram plot by HPLC-photodiode array detection (HPLC-DAD): Three-dimensional chromatogram/spectra plot of four piperine isomers determined by diode array UV detection (DAD). The UV absorbance was monitored at wavelengths 220–400 nm throughout the chromatographic run.

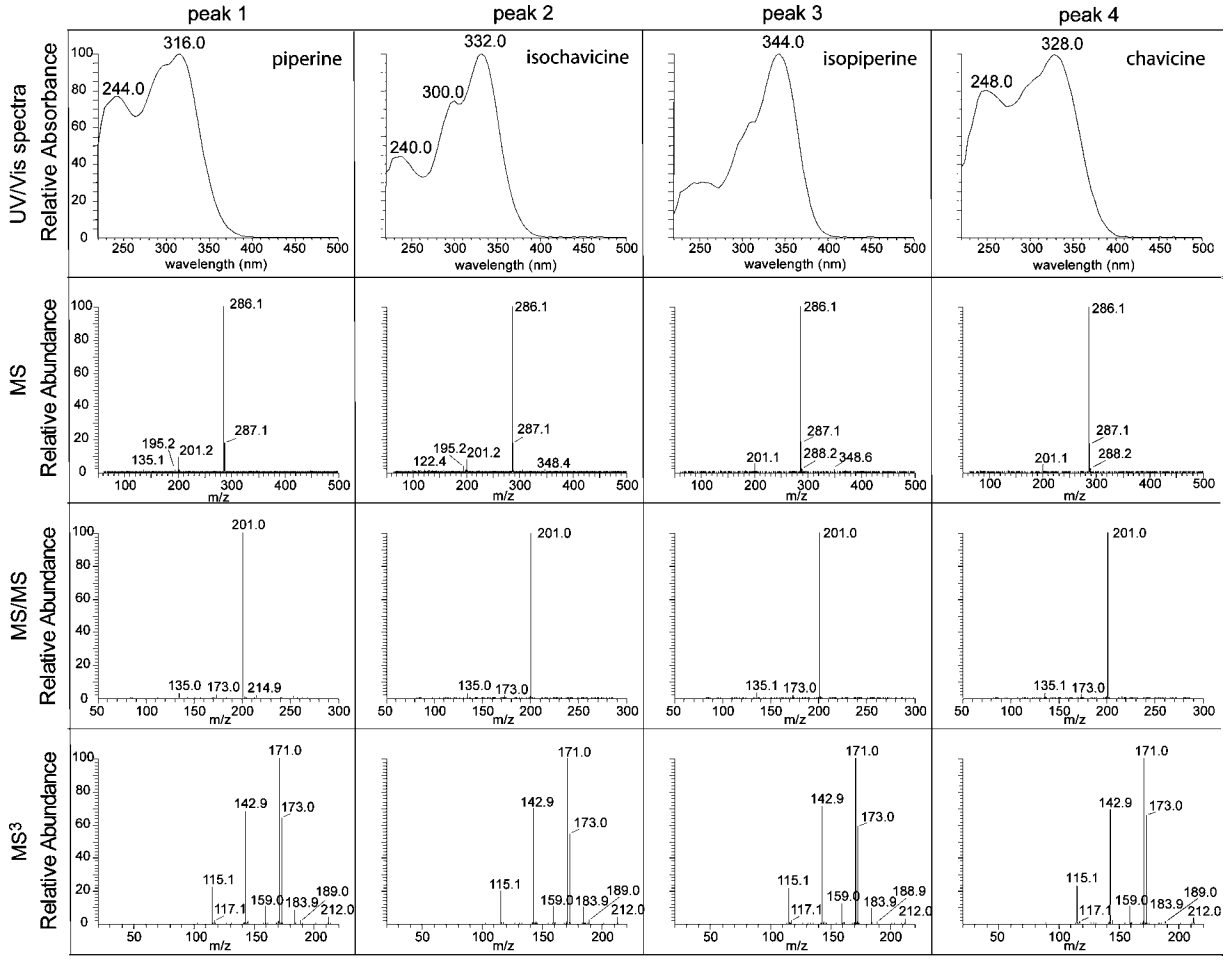

Figure 4. Identification of HPLC/LC peaks **1**–**4** by UV and mass spectra: UV and mass spectra of four piperine isomers. Peak **1**: piperine. Peak **2**: isochavicine. Peak **3**: isopiperine. Peak **4**: chavicine. MS spectra show only identical parent ion peaks at 286.1 Da. The major peaks in the MS/MS and MS^3 spectra may be due to the mass fragments shown in **Figure 8**. **Table 2** summarizes relative abundance of peaks in the MS^3 spectra.

of **Figure 3** was monitored at wavelengths 220−400 nm throughout the chromatographic run. To visualize the chromatogram, look along the *x*-axis: note gray trace. To visualize the UV spectrum, take a slice at any particular time (for example, 92 min for the first peak) along the *z*-axis (wavelength) and mentally rotate that clockwise. These spectra can be checked

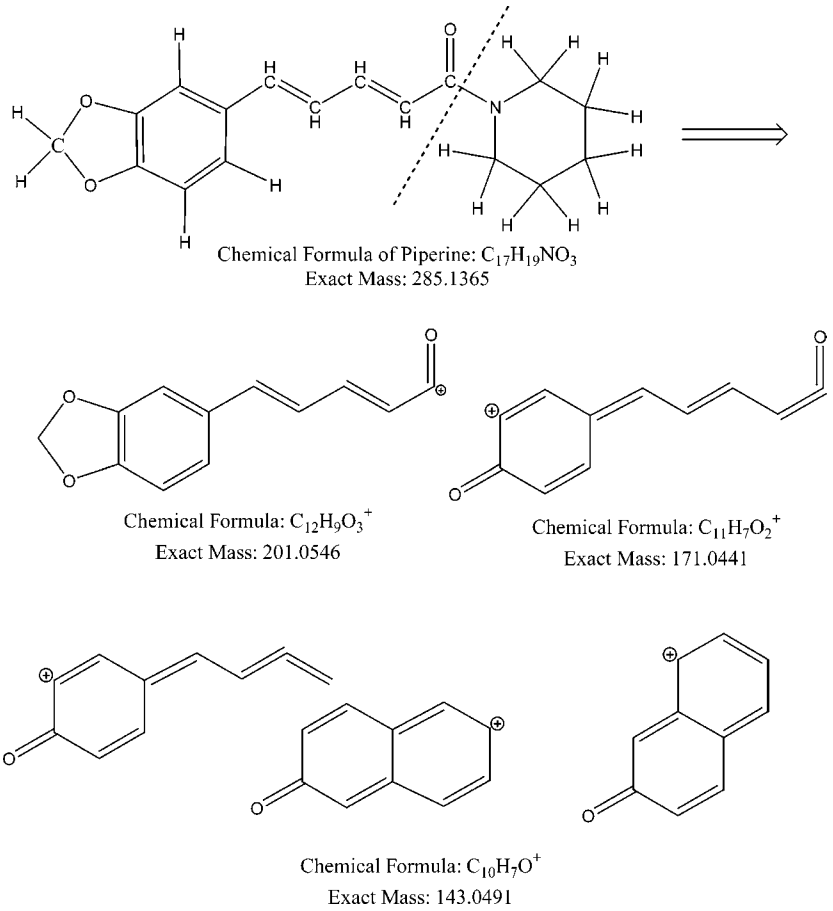

Figure 5. Piperine mass fragments: Possible structures of major mass spectral fragments of piperines.

Table 1. Reproducibility as Determined by HPLC of Four Separate Light-Induced Isomerizations[a]

	retention time (min)				peak area (μV)			
experiment	chavicine	isopiperine	piperine	isochavicine	chavicine	isopiperine	piperine	isochavicine
1	122.70	126.43	132.40	139.92	406128	1213859	1927683	2249932
2	122.63	126.59	132.67	140.19	404816	1202798	1936908	2243921
3	123.23	127.07	133.10	140.62	396294	1175316	1900063	2174519
4	123.28	126.50	132.96	140.28	399139	1189382	1887995	2225244
average	122.96	126.70	132.72	140.24	401594	1195339	1913162	2223404
SD	0.34	0.33	0.35	0.35	4657	16684	22947	34243
RSD (%)	0.28	0.26	0.27	0.25	1.16	1.40	1.20	1.54

[a] Piperine was exposed to 3000 lux for 30 h.

against known spectra for purity. The following observed λ_{max} values in the UV spectra (in nm) are consistent with corresponding values reported in the literature (*24*, *33*): chavicine, 316; isopiperine, 332; piperine, 340; isochavicine, 332.

Previously, Ternes and Krause (*24*) found that the fragment patterns of the MS spectra of the piperine isomers did not differ [(*m*/*z* 285 (M^+), 201, 173, 143, 115, 84)]. Scott et al. (*30*) observed the following mass spectra M^+ *m*/z (relative intensities) for piperine: 287.1 (20), 286.1 (100), 201.0 (35), 135.0 (5). Hashimoto (*31*) found that the spectra of the four isomers determined by HPLC/APCIMS appeared identical, each with a single major [MH^+] ion at *m*/*z* 286. By monitoring the increased relative abundance of the ions, these authors attempted to identify piperine isomers in natural pepper before and after exposure to sunlight. Finally, Marutolu et al. (*32*) identified piperine and chavicine in black pepper by TLC and GC−MS.

The results of the present study are shown in **Figures 4** and **5** and in **Table 2**. **Figure 4** shows that the peak patterns of the MS and MS/MS spectra of the piperine isomers appear identical.

Table 2. Relative Intensities (in %) of the Four Major Mass Spectra Peaks of the Four Piperine Isomers Detected by MS^3 (Positive Ionization Mode)

	mass spectra (MS^3), M^+ *m*/*z*:			
isomer	115.1	142.9	171.0	173.0
chavicine	22.4	67.4	100.0	63.5
isopiperine	19.3	69.1	100.0	54.3
piperine	21.8	70.9	100.0	59.2
isochavicine	23.5	68.9	100.0	65.0

By contrast, **Figure 4** and **Table 2** also show that the fragment patterns in terms of relative abundance obtained with MS^3 (positive ionization mode) appear somewhat different from each other. Thus, setting the intensity of the 171.0 *m*/*z* peak at 100%, the relative intensities (in %) for the 115.1 *m*/*z* peak for the four isomers ranged from 19.3 to 23.5; for the 142.9 *m*/*z* peak, from 67.4 to 70.9; and for the 173.0 peak, from 54.3 to 65.0. Because the differences in relative abundances of the fragments

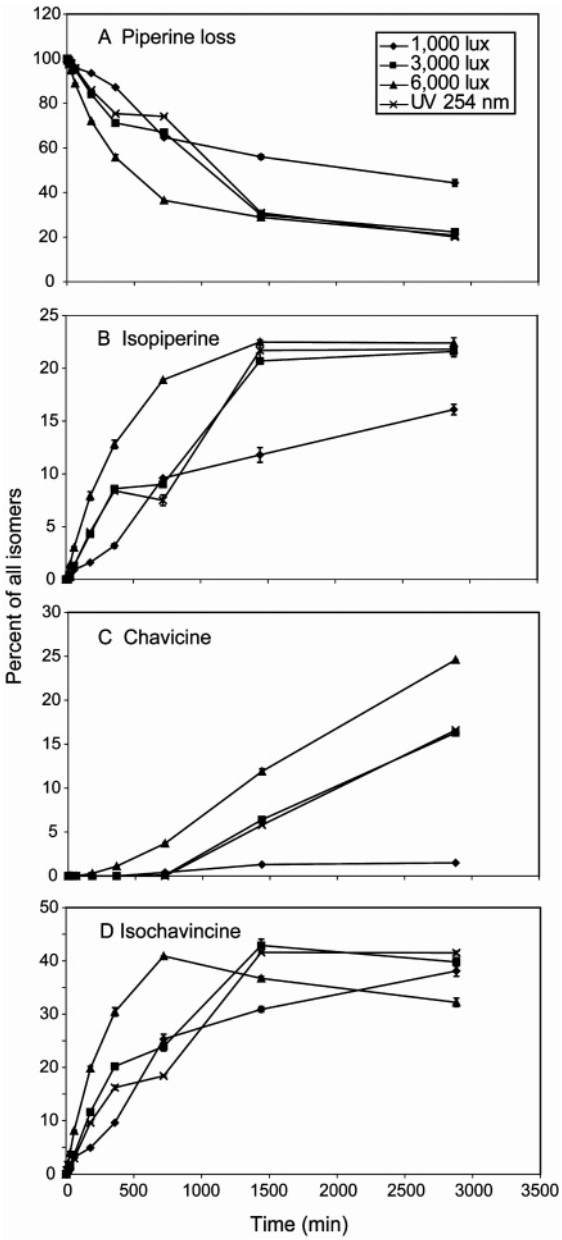

Figure 6. Effect of quality of light on piperine degradation and isomer formation: Effect of light intensities and exposure times on losses of piperine **(A)** and formation of isopiperine **(B)**, chavicine **(C)**, and isochavicine **(D)**. Plotted values are averages from two separate experiments ± SD ($n = 2$). SD values for most points are too small to be visible.

are probably within the method's normal variation, the electrospray MS/MS data may not show significant enough differences to differentiate between the isomers.

Figure 5 shows postulated structures of mass spectral fragments of the isomers. Although the fragment of mass 201 must be formed by the indicated cleavage of the carboxyamide moiety, the mechanisms of formation of the structures with masses of 171 and 143 are not obvious to us. Note that three possible structures can account for the 143 *m*/*z* peak. Because piperine and its isomers possess the same molecular weight and similar mass fragments in the mass spectra, our results suggest that it may not be possible to concurrently identify all four isomers by LC/MS.

Piperine Photoisomerizations. A main objective of the present study was to determine the kinetics of the photoisomerizations of the four individual isomers as a function of light intensities and sources. The data are plotted in **Figures 6**−**8** for a visual depiction of the observed trends.

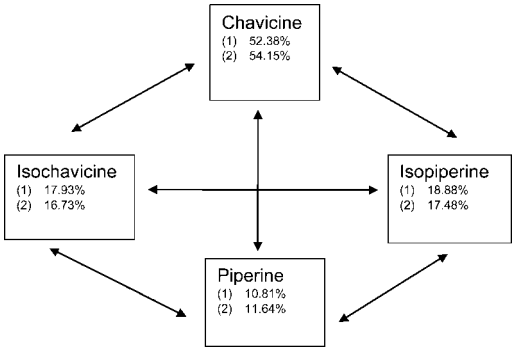

Figure 7. Identical composition of four isomers resulting from the photochemical isomerization of piperine induced by (**1**) fluorescent light (3000 lux) for 20 days at 25 °C; and (**2**) sunlight for 20 days at room temperature.

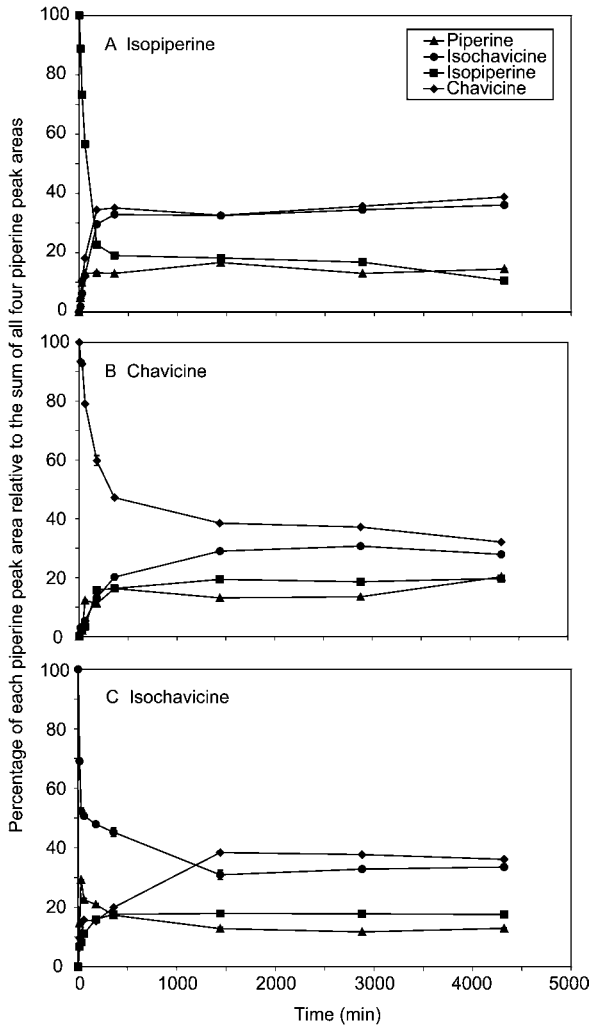

Figure 8. Percentage of each piperine isomer produced from isolated piperine isomers by timed exposure to white fluorescent light (3000 lux). Time-course of the photoisomerization by fluorescent light (3000 lux) of three isolated isomers: isopiperine **(A)**, chavicine **(B)**, and isochavicine **(C)**. Plotted values are averages from two separate experiments ± SD. SD values for most points are too small to be visible.

Figure 6 shows the percent decrease in piperine concentrations and the concurrent formation of the other three isomers as a function of both intensity of light source and exposure time

Table 3. Piperine Isomer Content in Four Commercial Brands of Pure Ground Black Peppers[a–c]

pepper		piperine	isopiperine	chavicine	isochavicine	total (sum)
A	μg/g	116,807 ± 1338	270 ± 45	36 ± 6	555 ± 13	117,669 ± 1402
	% total	99.27	0.23	0.03	0.47	
B	μg/g	105,158 ± 1245	158 ± 2	70 ± 12	455 ± 6	105,841 ± 1240
	% total	99.35	0.15	0.07	0.43	
C	μg/g	107,727 ± 594	194 ± 32	12 ± 2	401 ± 4	108,335 ± 556
	% total	99.44	0.18	0.01	0.37	
D	μg/g	101,724 ± 1005	177 ± 42	40 ± 3	426 ± 12	102,367 ± 1038
	% total	99.37	0.17	0.04	0.42	

[a] Pepper source: A, Albertson's (USA); B, McCormick (USA); C, Daesaug (Korea); D, Ottogi (Korea). [b] Values in μg/g ± SD of pepper powder; $n = 2$. [c] Values in % of total of all four isomers.

up to 2880 min (2 days). At 1000 lux, the decrease in piperine starts at about 60 min and progresses with time, resulting in a final loss of 55.7% after 2 days. At the end of the experiment, these decreases are accompanied by concurrent increases in the concentrations of the three isomers in the following order: isochavicine (38.1%) > isopiperine (16.1%) > chavicine (1.5%). Exposure at 3000 lux for 2 days induced a 77.7% decrease in the piperine level. The concentration of isochavicine (39.8%) is similar to that observed at 1000 lux, and that of isopiperine (21.6%) approaches that chavicine (16.3%). At 6000 lux, the final loss of piperine (79.2%) is similar to that observed at 3000 lux, as are the levels of the other three isomers. Isomer distribution resulting from exposure to UV (254 nm) radiation parallels that observed with 3000 lux. This observation is reinforced by our observation that identical mixtures of isomers are formed after 20 days following exposure of piperine to both fluorescent light at 3000 lux and sunlight that provides UV radiation (**Figure 7)**.

The cited observations suggest, as expected, not only that both light intensity and exposure time favor isomerizations but also that the formation of isopiperine reaches a maximum level and then levels off (**Figure 6B)**. Chavicine formation is slow initially but then progresses linearly with time under all four light conditions evaluated. Also note that isochavicine formation parallels that of isopiperine (**Figure 6B**,**D**).

Isopiperine Isomerization. Loss of piperine exposed to fluorescent and UV light is more rapid than the loss of the other isomers. Isomerization of isopiperine is also more rapid than that of the other isomers, reaching 77.3% after 3 h and 79.4% after 3 days (**Figure 8A**). The data also show that the concurrent formation of piperine over the prolonged time period is slower than the formation of chavicine and isochavicine. The latter are formed at nearly identical rates. The trends in the formation of isomers from isopiperine as a function of light quality are similar to those mentioned earlier for piperine.

Chavicine Isomerization. To better define the dynamics of the photoisomerizations, **Figure 8B** shows trends for the fluorescent light (3000 lux)-induced isomerization of chavicine. Chavicine levels decrease progressively with time up to 3 days (67.9% loss). The decreases are accompanied by formation of isochavicine (19.7%), piperine (20.3%), and isochavicine (19.7%). The decrease in chavicine levels off reaching the photostationary state at ~500 min, as do the corresponding increases in the amounts of three isomers formed. The leveling-off effect suggests that none of the isomers formed is degraded during the long exposure time to light.

Isochavicine Isomerization. The initial loss of isochavicine exposed to fluorescent light at 3000 lux is more rapid than losses observed with piperine, isopiperine, and chavicine (**Figure 8C)**. The reduction in isochavicine concentrations level off at ~1000 min, roughly paralleling those of isopiperine and chavicine. Again, beginning at about that time, the photostationary phase remains constant up to 3 days.

Piperine Content of Ground Black Peppers. In the present study, we determined levels of all four piperine isomers in four commercial ground black pepper products, two originating from the United States and the other two from Korea. **Table 3** shows that (a) the piperine levels of the four products were quite similar, ranging from 101,724 μg/g (10.17% by wt) to 116,807 μg/g (11.68% by wt), a small 13% difference from highest to lowest level; (b) the levels of the other three isomers were quite low, ranging from 0.01 to 0.07% of the total for chavicine; from 0.15 to 0.23% for isopiperine; and from 0.37 to 0.42% for isochavicine; and (c) piperines comprise about 10−12% of the weights of the dry black pepper products. These results suggest that the described HPLC method can be used to measure very low (0.01 wt %) to very high (11.68 wt %) levels of all four isomers in dry pepper products.

Previously (*36*), we found that 4 of 12 pepper-containing foods evaluated contained, in addition to capsaicinoids, two piperine isomers. The piperine levels of the foods ranged from about 0.1% to 0.8% of the levels cited above for the black peppers. Because these foods contained both capsaicinoids and piperines, our results suggest that they were prepared with both red and black pepper products.

Related Photoisomerizations. Light-induced excitation of the π-electrons of the double bond of olefin compounds (alkenes) results in the formation of a nonplanar excited π*-transition state of either *cis* or *trans* olefins due to promotion of an electron, which then returns to the ground state (*37*). Thus, continued exposure to light absorbed by piperine isomers over time results in a constant ratio of the excited state being generated and leads to a constant ratio of isomers on decay of the light-induced excited state. Although our data show that the individual isomers varied in their susceptibilities to light-induced isomerization, in principle, this so-called "photostationary steady state" is usually independent of the initial isomer or mixture of isomers. The trends illustrated in **Figures 6−8** appear generally consistent with this concept.

As is well-known, the described photoisomerizations of piperines are not unique in nature. Other light-induced events include *cis*−*trans* isomerization of carotenes (β-carotene, lutein, lycopene, phytoein, zeaxanthin), natural polyene pigments with 11 conjugated double bonds (*38*, *39*); *cis*−*trans* isomerizations of double bonds of fatty acids such linoleic acid (*40*); *cis*−*trans* isomerization of the 11-*cis*-retinal chromophore, a primary event in human vision (*41*); conversion of 7-dehydrocholesterol located under the skin to pre-vitamin D_3 (*42*); and postharvest synthesis of chlorophyll and glycoalkaloids in potatoes (*43*−*45*). The dietary consequences of such light-induced changes in food ingredients are not always apparent and merit further study.

Research Needs. The above-cited observations on light-induced, time-dependent isomerization of each piperine isomer can serve as a guide to facilitate and/or to minimize isomerizations, depending on need. Although, as mentioned earlier, dietary, pharmacological, and medicinal aspects of piperine have been extensively explored (*21*, *46*), this is apparently not the case for the other three isomers. For example, it would be of interest to compare the relative insecticidal and antimicrobial properties of isopiperine, chavicine, and isochavicine to those reported for piperine.

Our data on the content of piperines in commercial pepper-containing foods and in commercial black pepper products may help consumers to select foods with desirable piperine content. Because light may influence both the nature and amounts of piperine isomers present, which in turn may affect sensory and biological properties, labeling for piperine content would undoubtedly benefit consumers.

It is not known whether the piperine isomers we found to be present in the four commercial ground peppers are formed naturally or by light-induced postharvest isomerizations. We also do not know whether and to what extent light-induced isomerizations of pure piperines also occur with piperine(s) located within the complex matrices of vegetative plant tissues during growth and/or postharvest (*38*). Will exposure of freshly harvested peppers to light induce racemization? These aspects merit further study.

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