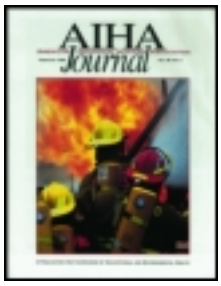

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Determination of Capsaicin and Dihydrocapsaicin in Air in a Pickle and Pepper Processing Plant

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A sampling and analytical method has been developed for measurement of capsaicin and dihydrocapsaicin in air. This method is applicable to measurement of the capsaicinoids in air in pepper processing plants and involves air sampling with a 13-mm glass fiber filter, recovery of the sample with 2 mL of acetonitrile, filtration of the solution, and analysis by high performance liquid chromatography with fluorescence detection. Excitation and emission wavelengths of the detector were 281 and 312 nm, respectively. Average recoveries were 98 to 104% after fortification of glass fiber filters with 0.13- to 2.9-μg quantities of capsaicin. Average recoveries of dihydrocapsaicin were 93 to 99% after fortification of glass fiber filters with 0.11- to 3.0-μg quantities. Detection limits of capsaicin and dihydrocapsaicin were 0.015 and 0.02 μg per sample, respectively. This method was used successfully for determining air concentrations of capsaicin and dihydrocapsaicin in a health hazard evaluation at a large pickle and pepper processing plant. An interesting phenomenon was the fact that the ratio of capsaicin to dihydrocapsaicin in each of the largest air samples was in the range of 0.3:1 to 0.5:1. Generally, capsaicin is the capsaicinoid that occurs in *Capsicum* fruit in the greatest relative abundance.

Keywords: air analysis, capsaicin, capsaicinoids, capsicum fruit, dihydrocapsaicin, capsaicin: dihydrocapsaicin ratio

Capsaicin and dihydrocapsaicin are toxic, are classified as mutagens, and can destroy certain sensory nerve cells.[1,2] These compounds and their analogues are known as capsaicinoids and are pungent components of hot peppers (capsicum fruits). Plants that produce capsaicinoids are in the *Capsicum* genus. The concentration of capsaicin (generally, the most abundant capsaicinoid) in capsicum fruit is variable and ranges from approximately 0.1 to 1% by weight.[1] Dihydrocapsaicin is typically the second most abundant capsaicinoid.[3]

Figure 1 presents molecular structures of four capsaicinoids in decreasing order of relative abundance.[1,4,5] All of these compounds are vanillylamides, which differ only in the aliphatic chain. Although the carbon chain of nordihydrocapsaicin is shorter than that of capsaicin, the carbon chain of homodihydrocapsaicin is longer.

In 1994, researchers at the National Institute for Occupational Safety and Health (NIOSH) conducted environmental surveys at a large pickle and pepper processing plant to investigate whether exposure to capsaicin constitutes a health hazard to workers. These surveys were conducted in response to reports that workers were suffering from irritation of the eyes, nose, and throat; wheezing; chest tightness; shortness of breath; and incessant coughing or sneezing.[6] The potential for exposure increased dramatically at harvest time for peppers (September and October), a time when employment at the plant swelled to 400 workers.

The physiological properties of capsaicinoids in animals and humans are complex and have been described in great detail.[1] Briefly, capsaicinoids can cause pain or a burning sensation at the place of contact by depleting certain sensory nerve cells of Substance P. Intravenous administration of capsaicin to dogs and cats induces apnea, a drop in blood pressure, and bradycardia. Biological research with capsaicinoids continues today.

The original goals of the project reported herein were to (a) develop a sampling and analytical method for capsaicin in air and (b) determine air concentrations of capsaicin in the pickle

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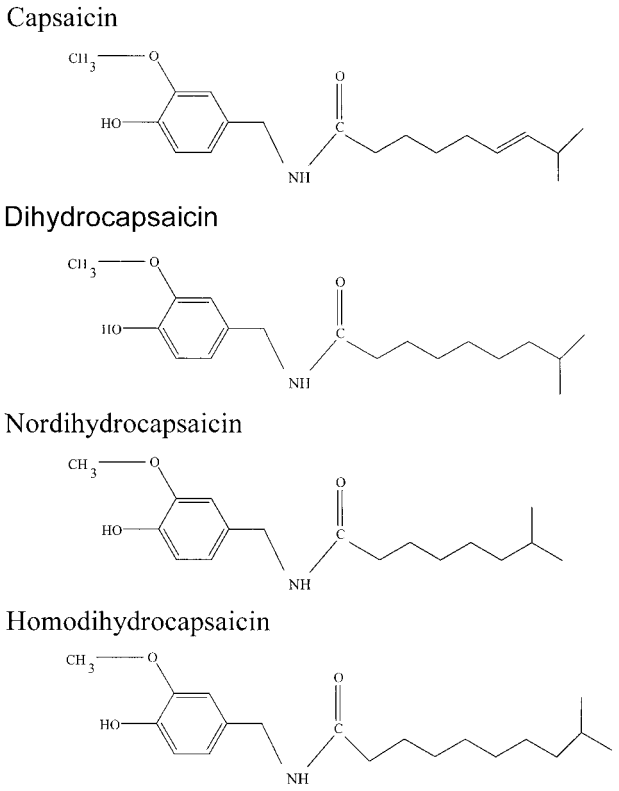

FIGURE 1. Molecular structures of selected capsaicinoids

and pepper processing plant to support the NIOSH health hazard evaluation. Capsaicin is known to be the most potent and, generally, the most abundant capsaicinoid in hot peppers. However, chromatograms of air samples collected at the plant exhibited peaks for dihydrocapsaicin that were larger than the peaks for capsaicin. Dihydrocapsaicin was considered to be a health hazard also. Thus, the project was extended to evaluate the method for dihydrocapsaicin and to measure dihydrocapsaicin in air samples collected from the processing plant.

A literature search at the time of the surveys revealed that no sampling and analytical method was available for capsaicin or dihydrocapsaicin in air. However, several high performance liquid chromatography (HPLC) methods for one or both analytes in solution had been published.[3,5,7–10]

In the present research a sampling and analytical method for measurement of capsaicin and dihydrocapsaicin in air has been developed and evaluated. This method has been published in the *NIOSH Manual of Analytical Methods* as method 5041 and involves air sampling with a 13-mm glass fiber filter at 1 to 3 L/min, recovery of the sample with 2 mL of acetonitrile in an ultrasonic bath, filtration of the solution, and analysis by HPLC with fluorescence detection.[11,12] Excitation and emission wavelengths are 281 and 312 nm, respectively. This method is available on the NIOSH home page at http://www.cdc.gov/niosh/nmam/nmammenu.html.

MATERIALS AND METHODS

Standard solutions of capsaicin in acetonitrile were prepared from 98% pure capsaicin (Sigma Chemical Co., St. Louis, Mo.). Standard solutions of dihydrocapsaicin in acetonitrile were prepared from dihydrocapsaicin that was 90% pure (Sigma Chemical Co.); an impurity in the dihydrocapsaicin was identified as capsaicin by HPLC retention time. Capsicum extract (about 60% capsaicin) was purchased from Sigma Chemical Co. Glass fiber filters without organic binders, 13 mm in diameter, type A/E, were purchased from Gelman Sciences (Ann Arbor, Mich., through Fisher Scientific, Cincinnati, Ohio). Air sampling was conducted with glass fiber filters housed in Swinnex filter holders (Millipore Corp., Bedford, Mass.).

Recoveries of capsaicin from 13-mm glass fiber filters were determined. Filters in 4-mL vials were fortified with capsaicin in acetonitrile solution (0.025 to 1.49 μg/μL; volumes of solution were 10.7 μL and less). Solvent was allowed to evaporate from filters. Uncapped vials containing filters were stored overnight. Afterward, vials in two sets (to be stored for a total of 28 days) were capped. Recovery was accomplished by addition of 2 mL of acetonitrile and treatment in an ultrasonic bath for 10 min. Solutions were filtered through PTFE filters (Acrodisc 3 CR filter units, 0.45-μm porosity, Gelman Sciences) prior to analysis. Recoveries of dihydrocapsaicin from 13-mm glass fiber filters were determined in the same fashion.

Air sampling was conducted at 2 L/min for 7 hours at various locations in the pickle and pepper processing plant. Area samples and personal samples were collected, for a total of 70 samples. Swinnex filter holders were positioned in a downward direction to prevent large particles from falling into the filter holders during sampling. Field samples were kept cool with "blue ice" (Ice-Pak®, Cole-Parmer, Chicago, Ill.) during shipment to the analytical laboratory.

All except one of the sample solutions were analyzed with external standard solutions by HPLC with fluorescence detection (see next paragraph for the exception). The analytical column (3.9 × 150 mm) was packed with μBondapak C_{18} (10-μm particles, Waters Chromatography Division of Millipore Corp., Milford, Mass.). The mobile phase was 48:52 acetonitrile:water (v/v), and the flow rate was 1 mL/min. The injection volume was 25 μL. Excitation and emission wavelengths were 281 and 312 nm, respectively.

An acetonitrile solution of capsicum extract (30 μg/mL) was analyzed by HPLC with ultraviolet (UV) detection at 281 nm. The analytical column was a Waters Symmetry C_{18} column (3.9 × 150 mm) packed with 5-μm particles. The mobile phase and injection volume were the same as before, but the flow rate was 1.3 mL/min.

RESULTS AND DISCUSSION

A laboratory evaluation of the method for capsaicin and dihydrocapsaicin was performed prior to field testing to determine analytical limits, recoveries from 13-mm glass fiber filters, precision of measurement, and storage stabilities.

The limit of detection (LOD) and lower limit of quantitation (LOQ) of capsaicin were 0.015 and 0.050 μg per sample, respectively. The LOD is defined as three times the standard error of the least squares calibration curve divided by the slope, and the LOQ is defined as ten times the standard error of the calibration curve divided by the slope.[13,14] Six standards were employed for the determination of these limits, and the lowest two standards were below the calculated LOD.

By the same definitions as those indicated above, the LOD and LOQ of dihydrocapsaicin were 0.02 and 0.067 μg per sample,

TABLE I. Recoveries of Capsaicin from 13-mm Glass Fiber Filters

Quantity Applied (μg)	Storage Time (days)	Storage Temperature (°C)	Average Recovery (%)	Relative Standard Deviation
0.13	1	25	102	0.018
0.58	1	25	98	0.032
0.99	28	25	92	0.052
0.99	28	5	96	0.023
2.9	1	25	104	0.037

Note: N = 6/level

respectively. Six standards were employed for determination of these limits, and the lowest two standards were below the calculated LOD.

Average recoveries of capsaicin from glass fiber filters were 98 to 104% after fortification with 0.13-, 0.58-, and 2.9-μg quantities and storage for 1 day at 25°C; these three levels are approximately 2, 10, and 60 times the LOQ, levels that could be encountered in air samples from a pepper processing plant (see Table I). The pooled relative standard deviation of measurement for 18 samples was 3.0%. Capsaicin was found to be stable on glass fiber filters during 28 days of storage at both 25 and 5°C (average recoveries were 92 and 96%, respectively, after storage).

Average recoveries of dihydrocapsaicin from glass fiber filters were 93 to 99% after fortification with 0.11-, 1.1-, and 3.0-μg quantities and storage for 1 day at 25°C (see Table II); the pooled relative standard deviation of measurement for 17 samples was 5.6%.

The recovery of dihydrocapsaicin after 26 days of storage at 5°C was somewhat lower than expected (88%), but recoveries greater than 75% after extended storage are considered acceptable.

For a sampling and analytical method to be included in the *NIOSH Manual of Analytical Methods,* it must be evaluated against certain criteria.[15,16] One of those criteria is that recoveries should be in the range of 75 to 125%; the data for this method (Tables I and II) clearly meet that criterion. Another criterion is 25% method accuracy, which means that results should be within 25% of the correct value 95% of the time, as determined from precision and bias estimates. The precision and bias estimates for this method are both below 10%; thus, the accuracy criterion is met.[15]

The sampling and analytical method developed for capsaicin and dihydrocapsaicin in air was used successfully in a NIOSH health hazard evaluation at a large pickle and pepper processing plant. This plant processed cucumbers, hot peppers, and mild peppers in addition to vinegar, fruit juices, and apple cider. Most of the pickle and pepper processing operations were located in one large three-floor production building. Pickle and pepper packing operations took place on the second floor, where steaming hot brine was added to glass jars. Third floor operations included brine preparation, slicing/cutting, and blanching operations.

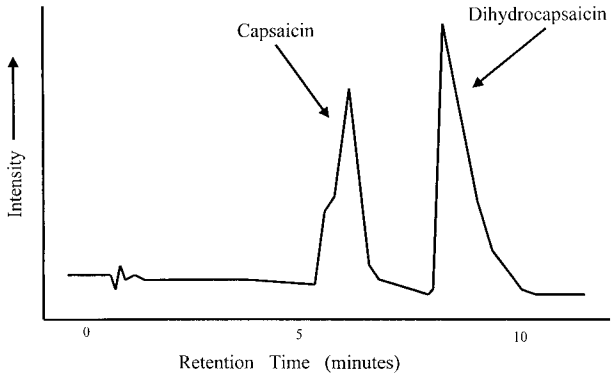

FIGURE 2. Chromatogram of representative air sample from pepper plant

The sampling and analytical method developed was sensitive enough for the measurement of capsaicin and dihydrocapsaicin only in the highest exposure areas of the pepper processing plant (areas that were point source locations). Capsaicin was detected in only 11 of 70 air samples (16% of samples), and dihydrocapsaicin was detected in only 28 of 70 air samples (40% of samples). The high exposure areas included the brine preparation area, vacuum briner, filling and packing area, and preparation line operations. Workers on the filling/packing and preparation lines were exposed to capsaicinoids directly from handling hot peppers. In these locations, air concentrations of capsaicin ranged from 0.07 to 0.13 μg/m³, and air concentrations of dihydrocapsaicin ranged from 0.15 to 0.33 μg/m³.

Figure 2 is a chromatogram of a typical air sample collected at the pepper processing plant. Separation was accomplished with 10-μm particles in the HPLC analytical column (μBondapak C_{18}). The shoulder on the capsaicin peak was probably due to nordihydrocapsaicin.[5]

Figure 3 is a chromatogram of capsicum extract that was run more recently (1998) as a supplement to the major laboratory work performed in 1994; separation was accomplished with 5-μm particles in the HPLC analytical column (Waters Symmetry C_{18}). The sample of capsicum extract, which contained a natural mixture of capsaicinoids, was an excellent sample for illustrating the superior resolving power of the Symmetry C_{18} column. Note that the resolution is much improved when 5-μm particles are employed in the HPLC column instead of 10-μm particles; baseline separation of nordihydrocapsaicin is observed in Figure 3. Although a UV detector was employed for Figure 3, a fluorescence detector could have been employed with a more dilute solution of capsicum extract for similar results. Use of a high resolution HPLC column, such as the Waters Symmetry C_{18} column, would be recommended for future work when samples containing capsaicinoids are analyzed.

TABLE II. Recoveries of Dihydrocapsaicin from 13-mm Glass Fiber Filters

Quantity Applied (μg)	n	Storage Time (days)	Storage Temperature (°C)	Average Recovery (%)	Relative Standard Deviation
0.11	5	1	25	94	0.016
0.88	6	26	5	88	0.047
1.1	6	1	25	99	0.069
3.0	6	1	25	93	0.062

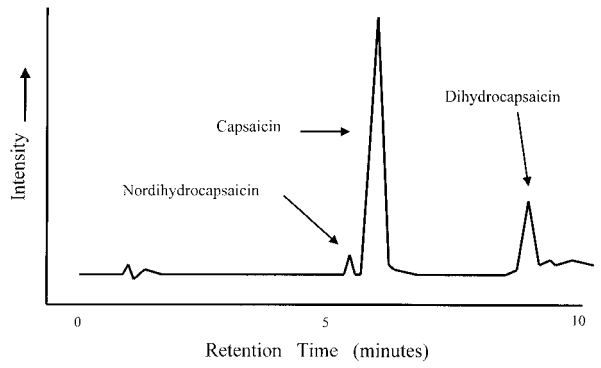

FIGURE 3. Chromatogram of capsicum extract

An unexpected observation was made about ratios of capsaicin to dihydrocapsaicin in air samples collected at the pickle and pepper processing plant. Ratios of capsaicin to dihydrocapsaicin in seven air samples ranged from 0.3:1 to 0.5:1. (Of the 70 air samples collected at the plant, only 7 contained sufficient capsaicin and dihydrocapsaicin for accurate measurement.)

These small ratios were unexpected because Weaver and coworkers[3] reported that the average distribution of capsaicin, dihydrocapsaicin, and nordihydrocapsaicin in 22 samples of oleoresins and whole spices was 58, 37, and 5%, respectively, and that these distributions were fairly constant. This distribution gives an average ratio of capsaicin to dihydrocapsaicin of 1.6:1. They reported that ratios as small as 1:1 occurred only 2.5% of the time or less. In fact, oleoresin used by the plant in this study to fortify food products with capsaicinoids had an abundance of 1.2:1. The ratio of capsaicin to dihydrocapsaicin for this oleoresin was larger than that for the air samples, but this oleoresin was not the only source of capsaicinoids in the processing plant. Other sources were not analyzed, but small ratios have been found in some pepper samples.[17,18] It is assumed that these source materials had small ratios of capsaicin to dihydrocapsaicin because other hypotheses (conversion reactions, differential volatility) did not offer a good explanation.

CONCLUSIONS

A sampling and analytical method was developed for capsaicin and dihydrocapsaicin that has the following features: recoveries of capsaicin and dihydrocapsaicin from glass fiber filters are essentially quantitative; the capsaicinoids are stable on glass fiber filters during storage at 5°C and at room temperature for short periods; the pair of wavelengths of light used for detection by fluorescence imparts high specificity to the method for both analytes; and capsaicin and dihydrocapsaicin exhibit very reproducible retention times by HPLC. The method was used successfully to analyze air samples from a pickle and pepper processing plant.

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