Simple Method for Quantitation of Capsaicinoids in Peppers Using Capillary Gas Chromatography

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A rapid, simple, safe, and inexpensive technique for extraction and quantitation of capsaicinoids from peppers is presented. Homogenized pepper tissue was extracted with acetone, and the extract was analyzed via capillary gas chromatography/thermoionic selective detection without need of capsaicinoid derivatization. Six of the eight capsaicinoids were positively identified in pepper extracts using gas chromatography/mass selective detection. Isomers for five of the six capsaicinoids were detected. Different varieties of peppers were analyzed for capsaicinoids, and the capsaicinoid concentrations as well as the corresponding Scoville heat values for the peppers are presented.

Keywords: *Peppers; capsaicinoids; chemical analysis*

INTRODUCTION

Capsaicinoids are the group of compounds responsible for the "heat" sensation in *Capsicum* fruits (peppers). The compounds are found in many foods and seasonings. They are also used as pest repellants in agriculture, and there is interest in using them as synergists with organophosphate insecticides. Because of their wide use, rapid methods for quantitating the capsaicinoids are desirable. Several different methods, including organoleptic methods (Scoville, 1912; Govindarajan et al., 1977), spectrophotometry (Awasthi and Singh, 1973; Bajaj and Kaur, 1979), thin layer chromatography (Sankarikutty et al., 1978), high-performance liquid chromatography (HPLC), and gas chromatography (GC) have been used for quantitation of capsaicinoids from peppers and oleoresins. Recently, reversed-phase HPLC has been used most heavily for capsaicinoid analysis because of its rapidity and reliability (Sticher et al., 1978; Saria et al., 1981; Hoffman et al., 1983; Weaver et al., 1984; Chiang, 1986; Weaver and Awde, 1986; Attuquayefio and Buckle, 1987; Cooper et al., 1991; Yao et al., 1994; Collins et al., 1995). Most GC methods in the literature employ packed chromatography columns and require sample cleanup prior to analysis (Todd and Perun, 1961; Morrison, 1967; Holló et al., 1969; Müller-Stock et al., 1970, 1971; Masada et al., 1971; Lee et al., 1976; Iwai et al., 1977, 1979; Todd et al., 1977; DiCecco, 1978; Huffman et al., 1978; Jurenitsch, 1979; Krajewska and Powers, 1987). Many GC methods also require derivatization to increase the volatility of the capsaicinoids.

The purpose of this research was to develop a rapid, simple, and inexpensive capsaicinoid analysis method using capillary GC and thermionic selective detection. In previous capsaicinoid analysis methods, Kosuge and Furuta (1970) and Jurenitsch et al. (1979b) methylated the compounds prior to capillary GC analysis. Saponification followed by methylation of the alkyl side chain for capillary GC analysis was performed by Jurenitsch and Leinmuller (1980). Gannett et al. (1988) used capillary chromatography for both methylated and underivatized capsaicinoids. To chromatograph the underivatized compounds, these researchers used a 100% methyl stationary phase column and a mass selective (MS) detector. For our analyses we chose a more polar column (14% cyanopropylphenyl/86% methyl) for interaction with polar functional groups of the molecules. After completion of this work, a paper was discovered (Hawer et al., 1994) in which the capsaicinoids were gas chromatographed on a similar phase capillary column. Those authors also found this phase to work well in separating the capsaicinoids. However, their method of detection (FID) necessitated sample cleanup before analysis. To eliminate the need for sample cleanup in our study, a thermionic selective detector (TSD) was employed. This procedure allowed GC separation and detection of all five commonly reported capsaicinoids (Figure 1): capsaicin (C), dihydrocapsaicin (DC), nordihydrocapsaicin (NDC), homocapsaicin (HC), and homodihydrocapsaicin (HDC), as well what we believe to be a sixth previously unreported capsaicinoid, nornordihydrocapsaicin (NNDC). Due to the availability of standards, only the three major capsaicinoids, C, DC, and NDC, were quantitated. Measurement of the three compounds was performed on 23 varieties of peppers.

MATERIALS AND METHODS

Peppers. Peppers used for this study were grown in two different locations. The main location was a single plot at the Rosa Unit of the Irrigated Agricultural Research and Extension Center in Prosser, WA. The second location was the Othello Agricultural Research and Extension Unit in Othello, WA. Both are part of Washington State University. Peppers in both locations were grown under similar climatic conditions and harvested in September 1994. Analyses were performed in April and May of 1995. The varieties examined in this study are listed in Table 3.

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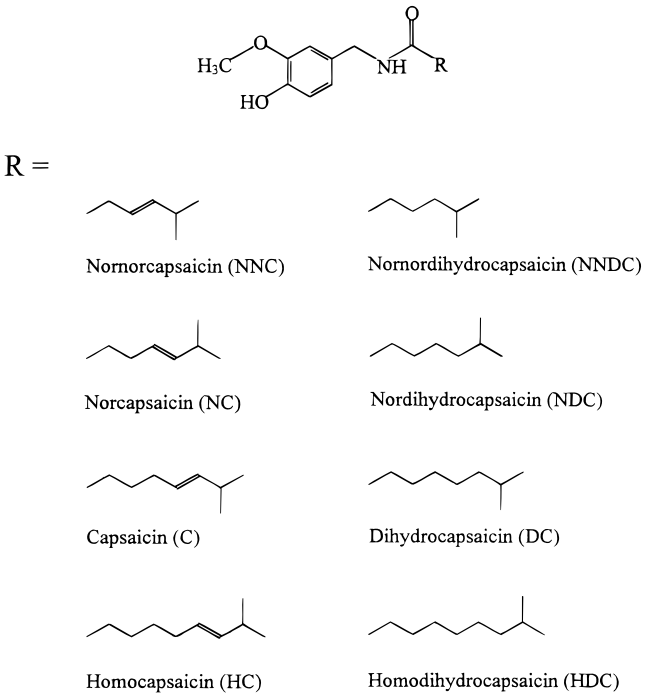

Figure 1. Generally accepted structures of the capsaicinoids.

Pepper Extraction. Frozen (−20 °C) pepper samples were thawed and the stems and seeds removed. Some samples had been allowed to air-dry before freezing. The number of peppers used for each sample varied from 2 to >30, depending on the size of the peppers and how many were required to produce at least 50 g (wet tissue) or 10 g (air-dried tissue) of sample. During seed removal, no visible amounts of the placenta were removed since the epidermis of the placenta is the site of capsaicinoid production and storage (Suzuki et al., 1980; Rowland et al., 1983). The peppers were then finely chopped in a food processor (RSI 6V, Robot Coupe, Ridgeland, MS), which provided a uniform mix of pepper tissue. Subsamples weighing 5−20 g were weighed into 125 mL Erlenmeyer flasks, and 25 mL of capillary GC grade acetone (EM Industries, Gibbstown, NJ) was added. This mixture was homogenized (PRO300D, PRO Scientific, Monroe, CT) for 3−5 min until all of the tissue was macerated. The homogenate was filtered through No. 40 Whatman filter paper into a 50 mL volumetric flask. The filter and solids were rinsed three times with acetone to collect any residual capsaicinoid. The filtrate was brought to volume with acetone and mixed, and 1 μL was injected into the GC. A portion of the chopped pepper was weighed into a drying pan, dried to constant weight at 80 °C (~3 days), and reweighed for dry weight determination.

GC Analysis. Analyses were performed on a Varian Star 3400 CX GC equipped with a 1079 temperature programmable on-column injector (Varian, Walnut Creek, CA). Injector temperature program was as follows: 50 °C for 0 min, 100 °C/min to 280 °C for 27.7 min (30 min total). The injection liner was a Varian high-performance liner, silanized with DCDMS (Supelco, Bellefonte, PA). Injections were performed by a Varian 8200 CX autosampler. The column was an AT-1701 (14% cyanopropylphenyl/86% methyl stationary phase), 30 m × 0.25 mm i.d., with a 0.25 μm film (Alltech Associates, Deerfield, IL). A Supelco 1 m × 0.32 mm i.d. untreated fused silica guard column was connected to the injector end with a fused silica column connector. The column temperature program was as follows: 160 °C for 1 min, 20 °C/min to 250 °C for 0 min, 1 °C/min to 260 °C for 7 min, 5 °C/min to 270 °C for 5.50 min (30 min total). The carrier gas was helium with a flow rate of 1.8 mL/min at 160 °C and a septum purge of 2.2 mL/min. The detector was a thermionic selective detector (TSD) operated at 290 °C.

Structural confirmation of the capsaicinoids in several pepper extracts was performed by GC/MS. The GC was a Fisons 8000, equipped with a Fisons 800 autosampler (Fisons Instruments, Manchester, U.K.). The injector was split/splitless, 1:4 split, held at 260 °C. The column, oven temperature program, and column gas and flow rates were the same as for TSD analyses, above. The detector was a Fisons Trio 1000 quadrapole mass spectrometer with EI^+ ionization at 70 eV in full scan mode.

Standards and Quantitation. Quantitations were performed from integrated TSD peak area measurements using Varian 4.0 software and mixed external standards. The C and DC standards (98 and 90%, respectively), along with a lesser characterized capsaicinoid mix, were obtained from Sigma (St. Louis, MO). The NDC standard (98%) was donated by Dr. Thomas Cooper at Kalsec, Inc. Purity was verified by area sum using GC/MS. The well-characterized standards of C, DC, and NDC were used to characterize a solution of the capsaicinoid mix, and dilutions of this mix were used as external standards. Compound identification in extracts was performed by comparison of retention times of peaks in the extracts with those of external standards. The five-point standard curves were linear ($r^2 = 1.000$) and ranged from 4 to 100 ng/μL total capsaicinoid (60% C, 30% DC, 5% NDC). Samples with higher capsaicinoid content were diluted to this range for quantitation.

Method Evaluation. Three approaches for examining the efficiency of the method were taken. The first was a fortification/recovery study using green bell peppers (also *Capsicum annuum*), which had been previously extracted and found to contain no detectable capsaicinoids. The second was a triplicate extraction of two varieties of peppers, a mild and a pungent, to check for reproducibility. The third was an extraction of three different amounts of one variety to examine the effect of sample size on extraction efficiency.

For the fortification/recovery study, triplicate portions of chopped fresh bell pepper were fortified with mixed capsaicinoid stock solutions at 500 μL of 1 μg/μL stock in 20 g of fresh tissue, 100 μL of 8 μg/μL stock in 2 g of fresh tissue, and 1000 μL of 8 μg/μL stock in 2 g of fresh tissue. This mimicked dry weight pepper concentrations of approximately 200, 3000, and 30 000 ppm of total capsaicinoids and covered the range of concentrations found in this study. The fortifications were then extracted as previously outlined.

The triplicate extraction was performed on the varieties Hot Cherry Pepper (mild) and Habanero (very pungent). Approximately 8 g of pepper and a 50 mL acetone final volume were used per replicate.

The extraction of different amounts of tissue was performed with the most pungent pepper variety, the Habanero. For the low, medium, and high amounts, 2.6, 10.4, and 20.1 g, respectively, were each extracted using a final acetone volume of 50 mL.

Scoville Heat Unit Conversions. Capsaicinoid concentrations were converted to Scoville heat units (SHU) by multiplying the pepper dry weight capsaicinoid concentration in parts per million by the coefficient of the heat value for each compound as given in Todd et al. (1977). The coefficients are 9.3 for NDC and 16.1 for both C and DC. Individual capsaicinoid SHUs were summed for each pepper to give the Scoville heat value (SHV) for each pepper.

RESULTS AND DISCUSSION

The percent moisture content of the fresh pepper tissue was significantly greater than that of the air-dried pepper tissue. Average [standard deviation (SD)] values were 85 (4)% for the fresh pepper tissue and 7 (1)% for the air-dried tissue.

Except for the anomalous recovery of 150% for the 3000 ppm fortification of NDC (Table 1), the recoveries and associated coefficients of variation (CVs) for the bell pepper fortifications were acceptable. The recoveries for all three compounds from the 200 ppm fortifications were appreciably lower and the CVs higher than for the

Table 1. Mean Capsaicinoid Recovery from Fortified Bell Pepper

level	NDC[a] (CV%)	C[a] (CV%)	DC[a] (CV%)
low	90 (20)	90 (10)	90 (10)
medium	150 (10)	110 (4)	100 (4)
high	110 (2)	110 (3)	110 (3)

[a] NDC, nordihydrocapsaicin; C, capsaicin; DC, dihydrocapsaicin.

Table 2. Amounts of Capsaicinoids Extracted from Differing Amounts of Habanero Tissue

amount extracted (g)	NDC, ppm dry wt	C, ppm dry wt	DC, ppm dry wt
2.6	630	14000	7400
10.4	720	12000	6400
20.1	710	12000	6100
av (CV%)	680 (7)	13000 (8)	6600 (10)

3000 and 30000 ppm fortifications. More tissue (20 g) was used in the 200 ppm fortification than in the others, and 50 mL of acetone may not achieve complete extraction from this much pepper tissue.

To test the efficiency of extraction for different amounts of pepper tissue, three different weights of a chopped Habanero sample were extracted. The results are presented in Table 2. In general, the extraction appears to be more complete the less pepper tissue used. The CVs were greater for these Habanero analyses than those of the Habanero analyses in the precision evaluation in which equal weights were used. Whether this difference is real or not is uncertain, however, because the efficiency evaluation was not replicated at each level. To assure adequate extraction of the capsaicinoids, a maximum of 10 g (wet weight) of pepper tissue to a 50 mL final volume of acetone is recommended. This would result in limits of quantitation of 12 ppm (C), 6 ppm (DC), and 1 ppm (NDC) wet weight in the pepper tissue with the mixed standard used. This corresponds to a total capsaicinoid detection limit of 130 ppm dry weight in the pepper, which is less concentrated than the least pungent pepper analyzed (Anaheim M; Table 3). These detection limits were determined for extraction from wet tissue. Extraction from dried tissues might differ in efficiency. The instrumental detection limit ($3 \times S/N$) for each capsaicinoid is 0.2 ng/μL in the final extract. This corresponds to 1 ppm wet weight, or 6 ppm dry weight, for 10 g of fresh pepper and 50 mL of acetone final extract volume. There may also be a quenching of the detector response at higher matrix concentrations. Smaller sample sizes would also reduce this problem.

To examine method precision, a mild and a hot variety were each extracted in triplicate. The means in ppm dry wt (CV%) were as follows: for Hot Cherry Pepper, NDC = 150 (10), C = 390 (10), DC = 440 (10); for Habanero, NDC = 770 (1), C = 12100 (1), DC = 6730 (2). The Hot Cherry Pepper is a firmer pepper than the Habanero and thus did not chop quite as finely as the Habanero. This may have resulted in a less homogenous sample, which could have caused the higher variability.

The sample chromatogram (Figure 2A) of a 1:5 dilution of Bulgarian Carrot extract illustrates both the baseline separation of the capsaicinoids and the selectivity of GC/TSD for these compounds. Bulgarian Carrot displayed medium pungency (Table 3). Chromatograms of extracts of the least pungent varieties, such as the undiluted extract of Sandia Hot (Figure 2B), show that even with a large amount of coextractives and no dilution, there was no interference in quantitation of the capsaicinoids. The relatively low volatility of the capsaicinoids compared to that of the coextractives, as well as the use of a sensitive and selective detector, allows the extracts to be analyzed without any concentration or cleanup.

The GC injector liner tended to become contaminated by the uncleaned extracts and needed to be changed

Table 3. Results of Capsaicinoid Analysis for Pepper Varieties (Micrograms per Gram of Dry Weight) Grown in Prosser, WA[a]

pepper name, variety	ppm NDC	SHU NDC	ppm C	SHU C	ppm DC	SHU DC	total ppm	total SHV
C. frutescens								
Tabasco[b]	47	440	670	10800	370	5900	1100	17000
C. annuum								
Anaheim M[b]	21	190	89	1400	110	1700	220	3400
Big Jim	14	130	160	2500	100	1700	270	4300
Sandia Hot	21	200	140	2200	160	2600	320	5000
Espanola Improved	28	260	250	4000	190	3000	460	7200
Hot Cherry Pepper	120	1200	200	3300	260	4100	580	8500
Cayenne Short[b]	140	1300	310	5000	270	4300	720	11000
NewMex X Hot	54	500	640	10000	480	7700	1200	18000
Early Jalapeno	190	1700	770	12400	840	14000	1800	28000
Chile De Arbol	280	2600	1100	17000	620	10000	2000	30000
Small Serrano	440	4100	1100	18000	1100	18000	2700	40000
Cayenne Long Thick	430	4000	1200	19000	1100	18000	2800	41000
NewMex Centennial Chile[b]	190	1800	1400	23000	1100	18000	2800	43000
Serrano[b]	360	3400	1400	22000	1100	18000	2800	43000
Carolina Cayenne	380	3500	1900	31000	1100	17000	3400	52000
Jalapeno M	360	3300	1900	30000	1200	19000	3500	53000
Ring of Fire	210	2000	2600	43000	1200	20000	4100	64000
Ultra Cayenne (Slim)	760	7000	1500	25000	2100	33000	4400	65000
Bulgarian Carrot	630	5900	1500	23000	2300	37000	4400	67000
Yatsafusa	750	7000	2400	39000	1900	31000	5100	76000
C. chinense								
Chocolate Scotch Bonnet	20	190	7700	120000	1600	25000	9300	150000
Habanero	810	7600	12000	190000	6300	100000	19000	300000
Dom Habanero	250	2300	14000	230000	5200	83000	20000	320000

[a] The Scoville heat units (SHU) or values (SHV) are given for the individual capsaicinoids and the sum of the three, respectively. All peppers are of the genus *Capsicum* and are grouped by species. [b] Dry when prepared.

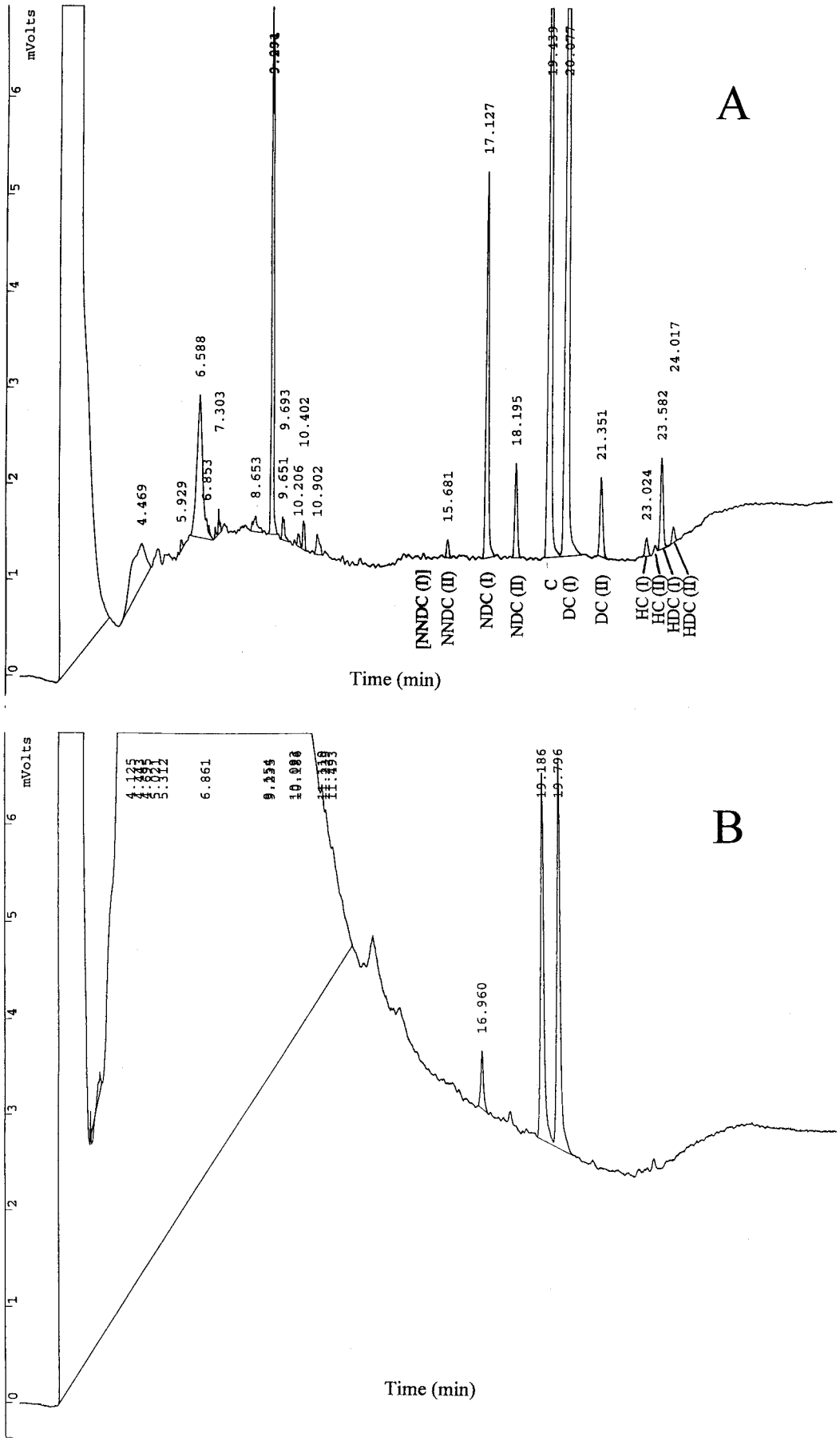

Figure 2. Chromatograms from GC analyses of capsaicinoids in pepper extracts: (A) 1:5 dilution of Bulgarian Carrot extract; (B) undiluted Sandia Hot extract.

after every 25−30 injections to maintain peak resolution. The use of a different GC injector configuration, such as a split/splitless injector and one of the array of liners that can be used with it, might extend liner performance. We also experienced a slight quenching of the detector response over the course of a long analysis set. To correct for this, the injections of the standards used for the standard curve were interspersed with sample injections throughout the analyses.

Acetone was chosen as the extraction solvent because it is fairly nontoxic and has been shown to give one of the most complete extractions of the capsaicinoids from pepper tissue (Sankarikutty et al., 1978; Weaver et al., 1984; Weaver and Awde, 1986; Attuquayefio and Buckle, 1987; Hawer et al., 1994). Methanol, 95% ethanol, and especially acetonitrile give almost as good extraction efficiencies and reduce the amounts of pigments and oils extracted with the capsaicinoids (Sticher et al., 1978; Woodbury, 1980; Hoffman et al., 1983; Chiang, 1986; Attuquayefio and Buckle, 1987; Collins et al., 1995). Due to chromatographic separation of the coextractives from the capsaicinoids by capillary GC, maximum extraction efficiency during a short time was the primary concern. A different solvent, however, might reduce some of the coextractives and thereby reduce the injector liner fouling by the pigments and oils which are visibly present in the acetone extract.

Capillary GC was able to effectively separate the capsaicinoids from one another (Figure 2), facilitating quantitation of the individual compounds. Quantitation was performed only for NDC, C, and DC as they were the only compounds available as reference standards. All six identified capsaicinoids would be quantitatable by this method if external standards were available or if relative response to an external or internal standard was used. The three major capsaicinoids were quantitated in 23 varieties of peppers (Table 3).

In addition to quantitation in parts per million, the pungencies of the peppers were also expressed in Scoville heat units (SHU; Table 3). This relates the analytically determined capsaicinoid concentrations to how "hot" the pepper would taste. The SHU is derived from the dilution of the pure capsaicinoid homologue needed in an aqueous sugar solution to reach the taste threshold. The reciprocal of this dilution is the SHU for that capsaicinoid (Scoville, 1912; Govindarajan et al., 1977; Todd et al., 1977). Values were taken from Todd et al. (1977): $C = 16.1 \times 10^6$; $DC = 16.1 \times 10^6$; $NDC = 9.3 \times 10^6$; [$HC = 6.9 \times 10^6$; $HDC = 8.1 \times 10^6$]. The SHU is multiplied by the absolute amount of capsaicinoid contained in the pepper, such as grams of capsaicinoid per gram of pepper, to give the heat intensity of the pepper due to that capsaicinoid (DiCecco, 1978). Pepper capsaicinoid concentrations expressed in parts per million allow elimination of the 10^6 factor from the SHU value. In this study, the SHU coefficient was multiplied by the parts per million of capsaicinoid in the pepper to give the SHU pungency of the pepper contributed by that capsaicinoid. This value was obtained for each quantitated capsaicinoid, and the individual capsaicinoid SHUs summed to provide the total Scoville heat value (SHV) for the pepper.

Among the peppers, the three tested members of the *C. chinense* species contained the greatest capsaicinoid concentrations, from 3 to 5 times the total SHV of the highest *C. annuum* tested, the Yatsafusa (Table 3). Tabasco, the single *C. frutescens* tested, had a total SHV similar to the moderate pungency of the *C. annuum*. The majority of peppers grown in the United States are *C. annuum*, with *C. chinense* species added more recently to produce hotter peppers than had been available (Bosland, 1992). Since few *C. chinense* varieties were available for testing, it is unknown if other cultivars include examples with low to no pungency as is seen in *C. annuum*.

For the major capsaicinoids capsaicin (C), dihydrocapsaicin (DC), and nordihydrocapsaicin (NDC) (Hoffman et al., 1983; Cooper et al., 1991), peak identification in the extracts was made by comparison of retention times with those of characterized external standards. Capillary GC/MS was used on several extracts to confirm peak identities and to identify the other peaks as the minor capsaicinoids homocapsaicin (HC), homodihydrocapsaicin (HDC), and nornordihydrocapsaicin (NNDC). NNDC identification was based upon the fragmentation pattern of the postulated NNDC molecule, which was identical to that of NDC except that the molecular ion was 14 mass units less ($-CH_2$) (Figure 3A−C). This 14 amu difference was the same pattern seen when the mass spectra of the other capsaicinoid homologues in both the saturated and unsaturated series were compared. The two other minor capsaicinoids, norcapsaicin (NC) and nornorcapsaicin (NNC), were not positively confirmed in this study, although they have been previously reported (Gannett et al., 1988). The mass spectra generated during peak confirmation and identification generally agree with the literature (Masada et al., 1971; Hoffman et al., 1983). The capsaicinoids all had similar fragmentation patterns, as the most stable portions of the molecules are identical. Fragments are tentatively identified in Figure 4.

During GC/MS analyses, two peaks (I, II) were identified for each of NNDC, NDC, DC, HC, and HDC (Figure 2) that exhibited identical mass spectral patterns (see NDC spectra in Figure 3A,B as an example). Peaks with retention times corresponding to these pairs were present in nearly all of the varieties we analyzed via GC/TSD. These isomers have been seen before via HPLC for HC and HDC (Heresch and Jurenitsch, 1979; Jurenitsch et al., 1979a,b; Cooper et al., 1991), and a postulation of the existence of an isomer of DC was made by Collins et al. (1995). Hawer et al. (1994) also saw peaks by GC corresponding to what we have called NDC I and DC II. They use mass spectral data to identify NDC I as pelargonic acid vanillylamide (PAV) and DC II as capric acid vanillylamide (CAV). These are reasonable assignments since, for example, PAV and NDC both have the same molecular formula and, except for the terminal *n*-propyl versus isopropyl structures, are identical. Because the most stable parts of the molecules and the molecular weights are the same, the mass spectra probably look very similar (they were not presented in the paper). What we see for the mass spectrum for our NDC I, however (Figure 3A), does not support the structure of NDC I as PAV, due to the presence of a fragment of *m*/*z* 43. This is often indicative of a terminal isopropyl group. This fragment is seen at approximately the same intensity in the mass spectra of our NDC I and II (Figure 3A,B), and the peak in our chromatogram for NDC II is also referred to by Hawer et al. as NDC. This would indicate that the terminal structures of the NDC I/PAV and NDC II are identical and that PAV is probably not the true identity

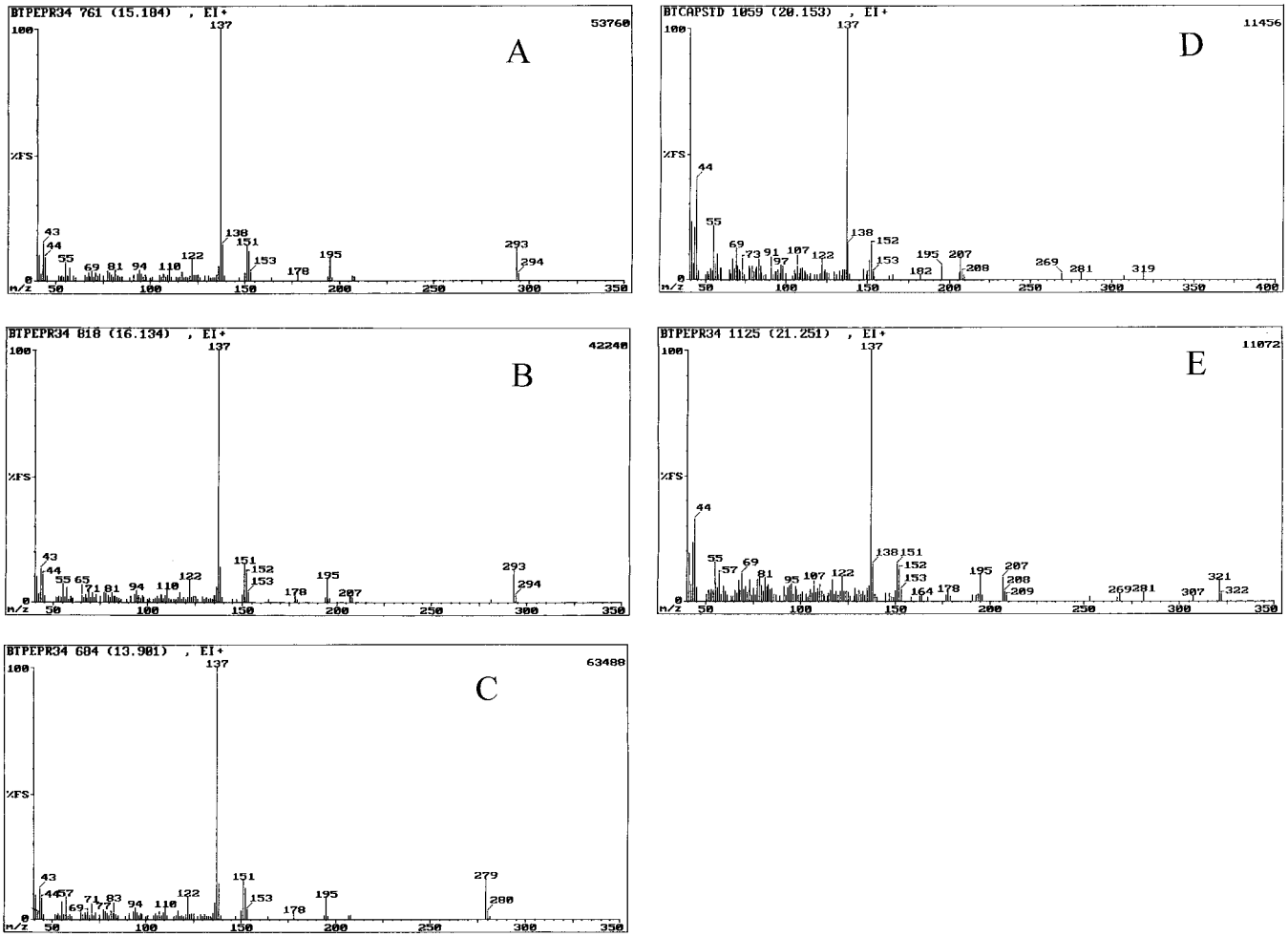

Figure 3. EI^+ mass spectra of selected capsaicinoids from Dom Habanero extract: (A) NDC I; (B) NDC II; (C) NNDC II; (D) HC II; (E) HDC II.

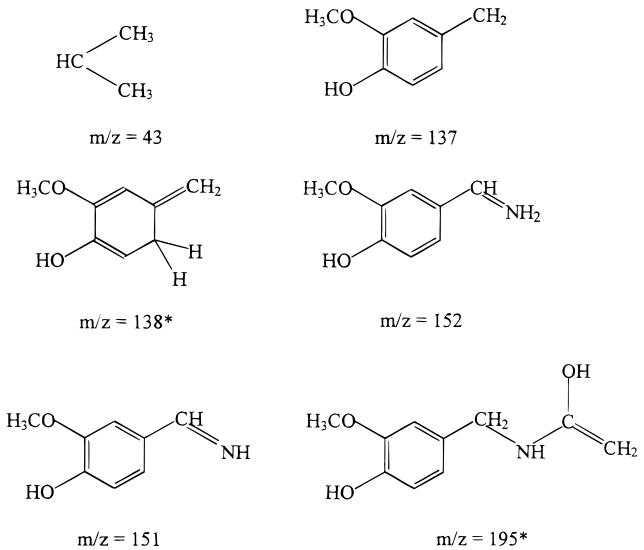

Figure 4. Proposed structures for fragments seen in mass spectra. *McLafferty rearrangement (Pavia et al., 1979).

of NDC I. The same situation exists and conclusions are made for DC II/CAV.

We are unsure of the exact structures of these isomers. A cis isomer of HC could be postulated for HC II. However, the cis isomer of C is reportedly not found in natural products (Kosuge and Furuta, 1970), and the same may also be true for the C homologues. Heresch and Jurenitsch (1979) and Jurenitsch et al. (1979b) propose structures for HC I and II that are not consistent with the C homologous series presented in most of the recent literature (the commonly accepted structures in Figure 1). Heresch and Jurenitsch (1979) and Jurenitsch et al. (1979b) position the double bond in the alkyl side chain one bond closer to the ring than the commonly accepted position. They also have the branch methyl two positions back from the alkyl chain terminus (in a non-isopropyl configuration) for HC II and HDC II. Our spectra for HC II and HDC II (Figure 3D,E) reveal the fragment of *m/z* 43 for I and II for both homologues as seen for NDC I and II. This again suggests the presence of a terminal isopropyl group in both isomers, in agreement with accepted structures. An *E/Z* configuration difference between I and II in the DC homologous series is not possible since, unlike the C series, there is no unsaturated bond in the alkyl side chain. The presence of identical mass spectra and two very different but consistent retention times may indicate a difference in the positioning of the methoxy or the hydroxyl group on the vanillyl portion of the capsaicinoid. A preparative scale fractionation of the isomers of HC and HDC (I and II) by HPLC (Cooper et al., 1991) and possibly DC I and II (Collins et al., 1995) may provide pure compounds for further characterization by a technique such as NMR. The apparent existence of an isomeric pair for DC may be reason for

Table 4. Differences in Capsaicinoid Profiles in Peppers of the Same Variety Grown at Different Locations

pepper	location	ppm NDC	SHU NDC	ppm C	SHU C	ppm DC	SHU DC	total ppm	total SHV
Serrano[a]	Prosser, WA	360	3400	1400	22000	1100	18000	2800	43000
Serrano	Othello, WA	200	1900	690	11000	700	11000	1600	24000
Yatsafusa	Prosser, WA	750	7000	2400	39000	1900	31000	5100	76000
Yatsafusa	Othello, WA	260	2500	580	9400	900	15000	1800	26000
Carolina Cayenne	Prosser, WA	380	3500	1900	31000	1100	17000	3400	52000
Carolina Cayenne	Othello, WA	180	1700	900	14000	940	15000	2000	31000
Habanero	Prosser, WA	810	7600	12000	190000	6300	100000	19000	300000
Habanero	Othello, WA	580	5400	9700	160000	4700	76000	15000	240000

[a] Dry when prepared.

Table 5. Ratios of Each of the Isomers Relative to Capsaicin (Set to 1000) for Selected Peppers Grown in Prosser, WA[a]

pepper	NNDC I	NNDC II	NDC I	NDC II	C	DC I	DC II	HC I	HC II	HDC I	HDC II	total SHV
Carolina Cayenne	6	6	180	16	1000	920	11	24	6	39	16	31000
Jalapeno M	4	4	150	22	1000	540	5	5	4	28	4	53000
Bulgarian Carrot	7	16	400	95	1000	1400	91	21	9	100	17	67000
Yatsafusa	4	11	280	36	1000	800	27	11	8	47	3	76000
Habanero	1	5	58	26	1000	470	21	8	4	12	4	310000
Habanero	2	10	13	13	1000	300	25	11	1	3	4	320000

[a] Ratios are based upon GC peak area. (The two Habanero samples are from different plants.)

reconsideration of the *E*/*Z* configuration difference as the source for detection of other isomeric pairs.

Isomers can be expected to have differences in characteristics such as pungency, as is displayed by the different capsaicinoid homologues. Capsaicinoid profile differences within a given variety are well established and can be caused by variations in growing conditions or maturity (Todd et al., 1977; Huffman et al., 1978; Govindarajan and Sathyanarayana, 1991; Cordell and Araujo, 1993). Differences in capsaicinoid profiles between peppers of the same variety grown in different locations for this study are presented in Table 4. Even peppers from the same plant can vary in their capsaicinoid profiles due merely to differences in postharvest ripening conditions (Iwai et al., 1977). Characterization of the capsaicinoid profile for a given pepper or extract is necessary to analytically determine its pungency, and this should include the characterization of the isomers as well. Since the properties of each isomer relative to the other are not yet known, and I isomers were usually greater in concentration than II isomers, we quantitated NDC and DC on isomer I only. [Note: references to the capsacinoids in the paper without the isomer delineation refer to isomer I.] Quantitation of the capsacinoids on isomer I only probably causes an underestimation of the heat value of the pepper, the extent of the error dependent upon the ratio of I to II. This approach was considered to be more valid than the summation of I and II. If ring position of the methoxy or hydroxy substituents distinguishes the isomers, large differences in biological activity (such as heat receptor stimulation) will probably exist. The activity of a capsaicinoid generally decreases if ring position is different from that in Figure 1 (Walpole et al., 1993a).

The ratio of the various capsaicinoid isomers of tested *C. chinense* varieties is similar to those of the *C. annuum* varieties (Table 5). The increased pungency of the Habanero is due to increased concentrations of all isomers rather than to particularly high concentrations of capsaicin or another isomer. The Habanero was the only pepper variety tested at both growing sites as well as from two different plants at the same location (Tables 4 and 5). The samples from the two plants at the same location showed nearly identical results, with a difference in total SHV within the variability of the analysis method. The sample from the plant grown in Othello, WA, however, contained a total SHV ≈80% of that found in the Prosser, WA, samples. The lower total SHV for peppers grown at the Othello site was typical of all peppers grown at both locations. Total SHVs in peppers from the Othello site were 30, 50, and 60% of the total SHVs for the Prosser peppers for the Yatsafusa, Serrano, and Carolina Cayenne varieties, respectively (Table 4).

Various biological activities of the capsaicinoids have recently been investigated (Kaga et al., 1989), such as their potential mutagenicity (Gannett et al., 1988), mammalian repellency (Mason et al., 1991; Rouhi, 1996), metabolic stimulatory effects (Watanabe et al., 1993), analgenicity (Szolcsányi and Jancsó-Gábor, 1975, 1976; Hayes et al., 1984; Walpole et al., 1993a–c; Surh et al., 1995), ulcerogenicity and other effects [see review by Govindarajan and Sathyanarayana (1991)]. Analgenicity of C and related synthetic structures has been examined most intensively, and C itself has proven to be a fairly potent pain reliever. In a different application, use of the capsaicinoids as synergists with organophosphate insecticides has also recently been investigated by Nishimura and Schreiber (Nishimura, 1996). In this study the organophosphate diazinon was found to be more effective against Colorado potato beetle when applied with *Capsicum* oleoresin than when applied alone. For most pharmacological studies, C has been the only natural individual homologue studied. However, natural oleoresins have been used for various purposes and have exhibited significant variability in efficacy (Cordell and Araujo, 1993), possibly due to differences in the ratios of the constituents making up the oleoresins prepared from different sources. To assess the potency of an extract for a given application, it is probably necessary to characterize the extract to determine not only concentrations of each homologue but also relative ratios of the isomers represented. As is revealed in Table 3, different varieties of peppers can differ substantially in their capsaicinoid profiles. This is more pronounced when the minor capsaicinoids are taken into account and even more so when the ratios of the isomers of each capsaicinoid are examined (Table 5). Characterization would be of most practical utility after studies have been undertaken to determine the activity of each of the capsaicinoids and isomers associated with them.

CONCLUSIONS

Acetone extraction followed by GC quantitation of capsaicinoids is an accurate, rapid, simple, and relatively inexpensive technique that does not involve specialized equipment or unusually hazardous chemicals. It should allow quantitation of the eight capsaicinoids without need for derivatization if analytical standards could be obtained. The high resolution provided by capillary GC has revealed the presence of possible isomers for all four DC homologues. Further studies into the structure and activity of these compounds would likely prove valuable.

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