ELSEVIER

Journal of Chromatography A, 740 (1996) 51–63

JOURNAL OF CHROMATOGRAPHY A

Characterization of powdered turmeric by liquid chromatography–mass spectrometry and gas chromatography–mass spectrometry

Richard Hiserodt[a,b,*], Thomas G. Hartman[b], Chi-Tang Ho[a], Robert T. Rosen[b]

[a]*Department of Food Science, Cook College, Rutgers University, New Brunswick, NJ 08903, USA*

[b]*Center for Advanced Food Technology, Cook College, Rutgers University, New Brunswick, NJ 08903, USA*

Received 1 November 1995; revised 26 January 1996; accepted 26 January 1996

Abstract

Five commercial powdered turmeric samples were analyzed to identify major and minor components. The developed HPLC method allows the separation of curcumin, demethoxycurcumin and bisdemethoxycurcumin, as well as three other major components and numerous minor components. The separation was accomplished on an octadecyl stationary phase using a mobile phase consisting of 50 m*M* ammonium acetate with 5% acetic acid and acetonitrile as the organic modifier. Thermospray mass spectra were obtained for all of the components. Particle beam EI-mass spectra were obtained for the curcuminoids, but could not be obtained for the other components due to the limitations of the particle beam interface when analyzing volatile and semi-volatile compounds. EI mass spectra for the volatile components were obtained by direct thermal desorption-gas chromatography–mass spectrometry (DTD-GC–MS).

Keywords: Turmeric; Mass spectrometry; Curcumin; Demethoxycurcumin; Bisdemethoxycurcumin

1. Introduction

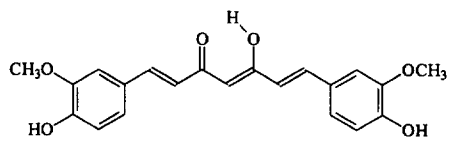
There is widespread interest in non-nutritive food chemicals or phytochemicals as antioxidants and anticancer agents; many of these are nonvolatile compounds. Curcumin, an important nonvolatile compound in turmeric, has been found to inhibit lipid-peroxide-induced DNA damage [1] and to inhibit the growth of tumors in model systems where tumors are induced by polynuclear aromatics [2].

Turmeric is a member of the family *Zingiberaceae* along with the other economically important members, ginger and cardamom. It is a member of the genus *Curcuma* which consists of hundreds of species of plants that grow from rhizomes, or underground roots. Turmeric generally refers to the economically most important species which is *domestica* [3]. It grows naturally or is cultivated in warm rainy regions of the world such as India, China, Indonesia, Haiti, Jamaica and Peru. Turmeric powder is used primarily today as a component in curry and as a food coloring in a wide range of products. The powder is produced by boiling the fresh rhizomes in water to gelatinize the starch and disperse the color. The rhizomes are sun dried for about 10 days which makes them brittle. They are then ground into a powder.

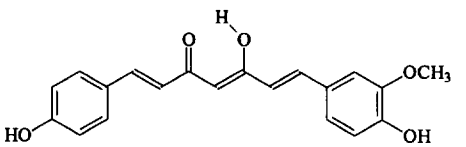
In addition to potential antioxidative and anticarcinogenic effects, curcumin, along with two other curcuminoids, is responsible for the distinctive yellow-orange color of turmeric (Fig. 1). Curcumin exists in a keto–enol tautomerism with the equilib-

*Corresponding author.

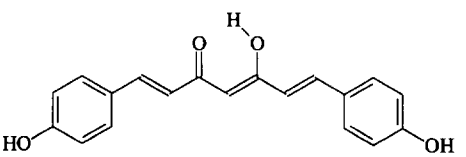
0021-9673/96/$15.00
PII S0021-9673(96)00103-3



curcumin
MW=368



demethoxycurcumin
MW=338



*bis*demethoxycurcumin
MW=308

Fig. 1. Structures of turmeric curcuminoids.

rium strongly favoring the enol form. The enol form owes its stability to resonance structures which give it pseudo-aromatic character. This enables curcumin to form inter and intramolecular hydrogen bonds and to complex with metals. The study of crystal structures has shown that the enol hydrogen can be on either oxygen and exchanges freely between the two [4]. These chemical characteristics have made separation of the curcuminoids in turmeric, by high-performance liquid chromatography (HPLC), somewhat of a challenge to chromatographers.

The first separation of the turmeric curcuminoids was reported by Tonnesen and Karlsen [5]. They used a mobile phase consisting of ethanol–water (94:4) with a Nucleosil amino stationary phase. They were able to obtain a separation of the three major curcuminoids but did not apply this method to the analysis of other components in turmeric. The concentration of the organic modifier was initially high and they reported that water content greater than 10% deactivated the column for the separation of the curcuminoids.

The chromatographic system developed by Cooper et al. [6] consists of a mobile phase with 1% citric acid with the pH adjusted to 3 with KOH and THF as the organic modifier. The researchers added citric acid to compete with active sites that cause tailing of the curcuminoids. This was an adaptation of work by Bailey et al. [7] who used citric acid in the mobile phase to quench secondary retention mechanisms caused by metals and active silanols on columns where the stationary phase was bonded to silica.

Other recent work on the analysis of turmeric has examined the total curcuminoid content only [4–6,8–11]. The curcuminoids were identified in samples of fresh or powdered turmeric based on retention time correlation with synthesized standards or characterization using UV-Vis spectra obtained from diode array detectors, or analysis by fluorescence or electrochemical detectors. Sanagi et al. [8] reported using a mobile phase consisting of an acetonitrile–acetate buffer for their analysis of curcuminoids extracted with supercritical CO_2. This mobile phase is well suited for interfacing liquid chromatography with mass spectrometry and was used for the work presented in this report (mobile phase 2).

Particle beam EI mass spectra and thermospray mass spectra can provide molecular weight information in addition to characteristic fragmentation patterns that can be used to confirm the identity of known curcuminoids and provide important structural information to help identify unknown components. In this study, five commercial turmeric powders were analyzed for major and minor components by high-performance liquid chromatography (HPLC) using UV detection, HPLC interfaced to mass spectrometry using both particle beam and thermospray interfaces (HPLC–MS) and direct thermal desorption-gas chromatography–mass spectrometry (DTD-GC–MS).

2. Experimental

2.1. Apparatus and instrument conditions

HPLC–MS studies were conducted using a Varian 9012 pump (Sugar Land, TX, USA) interfaced to a Vestec 201 quadrupole mass spectrometer (Houston, TX, USA) equipped with Vestec thermospray and particle beam interfaces. Also used was a Varian 9050 UV detector. Mass spectral data analysis was

accomplished using a Technivent (St. Louis, MO, USA) Vector/One data system.

Samples analyzed using the Vestec thermospray interface were scanned from 150–550 amu at a rate of 3.2 s/scan. The source was operated in the discharge mode with the source block at 255°C. The control, tip and tip heater temperatures were 136, 185 and 283°C, respectively. The control temperature was held constant throughout the analysis.

Samples analyzed using the Vestec particle beam interface were scanned from 45–450 amu at 3.6 s/scan. The ion source was operated in the EI mode (70 eV and 200 μA). The source temperature was 270°C and the temperature of the momentum separator was 102°C. The control and tip temperatures were initially 140 and 137°C, respectively. The control temperature was continuously decreased to 115°C to compensate for the change in mobile phase composition due to the gradient.

The column used was a Supelcosil LC-18, d_p=5 μm, 250×4.6 mm I.D. (Supelco, Bellefonte, PA, USA). Two mobile phases were used. Mobile phase 1 consisted of 1% citric acid (pH adjusted to 3.0 with dilute NaOH) and acetonitrile as the organic modifier. A gradient was run from 50% acetonitrile, after an initial hold for 10 min, to 80% acetonitrile in 30 min at 1 ml/min. This method was used to determine the three major curcuminoids. Mobile phase 2 consisted of 50 m*M* NH_4OAc with 5% HOAc using acetonitrile as the organic modifier. The same gradient used for mobile phase 1 was used for mobile phase 2. This method was used when the HPLC was interfaced to the mass spectrometer to obtain identification of components other than the three main curcuminoids.

GC–MS studies were conducted using a Varian 3400 gas chromatograph interfaced to a Finnigan MAT 8230 magnetic sector mass spectrometer (San Jose, CA, USA) and a flame ionization detector. Mass spectral data analysis was accomplished using the Finnigan MAT SS300 data system. The column used for the GC analyses was a JW Scientific (Folsom, CA, USA) DB-1 capillary column, 60 m× 320 μm I.D., d_f=0.25 μm.

A DB-1 capillary column was used for both experiments and was temperature programmed from −20 to 150°C at 20°C/min and then 150–280°C at 5°C/min. Subambient conditions were obtained using solid CO_2. Injections were made for all GC analyses using a Short Path Thermal Desorption Model TD-1 (Scientific Instrument Services, Ringoes, NJ, USA). Samples were desorbed at 220°C for 5 min. For analyses with the flame ionization detector, the detector temperature was 325°C and the injector temperature was 220°C

The mass spectrometer was scanned from 35–350 amu at 1 s/decade in the EI mode (70 eV at 1 mA). The injector temperature was 220°C, the GC–MS interface line and MS inlet temperatures were 280 and 240°C, respectively, and the ion source temperature was 280°C.

2.2. Reagents

Water, methanol and acetonitrile were Optima-grade and were purchased from Fisher Scientific (Pittsburgh, PA, USA). Ammonium acetate, acetic acid, citric acid and ammonium hydroxide were A.C.S. reagent grade or better and were purchased from Aldrich (St. Louis, MO, USA). Chromosorb WHP, 80–100 mesh, was purchased from Supelco.

2.3. Internal standards

Naphthalene-d_8 (98+ atom %D) and 4-fluoro-4′-hydroxybenzophenone (97%) were purchased from Aldrich.

2.4. Standards

Curcumin, technical, 80%, was purchased from Aldrich.

2.5. Preparation of samples and standards

2.5.1. Samples

Samples for LC–MS analysis were prepared by vortexing 150.00 mg turmeric powder in 5 ml of water. The sample was centrifuged for 30 min at 2000 rpm and the water decanted. Methanol (10 ml) was added and the sample vortex-mixed. The sample was centrifuged for 30 min at 2000 rpm. A 1.0-ml aliquot of the extract was diluted quantitatively with 1.0 ml 4-fluoro-4′-hydroxybenzophenone solution (I.S.) [12,13]. The solutions were filtered through a 0.45-μm Nylon-66 filter disk.

Samples for the assay of turmeric curcuminoids by HPLC were prepared by sonicating 10.00 mg in 10.0 ml methanol for 10 min. The samples were filtered through a 0.45-μm Nylon-66 filter disk.

Samples for GC analysis were prepared by mixing 20 mg turmeric powder with 200 mg Chromosorb W HP (80–100 mesh) and mixing thoroughly. The Chromosorb was conditioned at 300°C for 2 h prior to use. A 15-mg amount of the turmeric powder–Chromosorb mixture was added to a 10.2 cm×4 mm I.D. glass-lined stainless steel tube. Silanized glass wool was added to both ends of the stainless steel tube to contain the sample. A 5-μl volume of d_8-naphthalene solution (I.S.) was added to each sample. This is equivalent to 3670 ppm, based on the turmeric in the turmeric powder–Chromosorb mixture. The methanol was removed by purging the sample tube with nitrogen at 80 ml/min for 30 min at room temperature.

2.5.2. Standards

A curcumin stock solution was prepared by dissolving 36.1 mg curcumin in 100.0 ml methanol. The standard for HPLC analysis was prepared by taking 70 ml of this dilution and diluting it to 100.0 ml with methanol for a final concentration of 25.2 μg/ml. Standards were prepared in low actinic glass to protect against photo-decomposition.

2.5.3. Internal standards

4-Fluoro-4′-hydroxybenzophenone (146.0 mg) was dissolved in 100 ml methanol and diluted to 250.0 ml with water for a final concentration of 0.58 mg/ml.

Naphthalene-d_8 (10.0 mg) was diluted in 10.0 ml methanol for a final concentration of 1.0 mg/ml.

3. Results and discussion

3.1. HPLC analysis

Five commercially available turmeric powders were analyzed using mobile phase 1 to determine the amounts of the three main curcuminoids present.

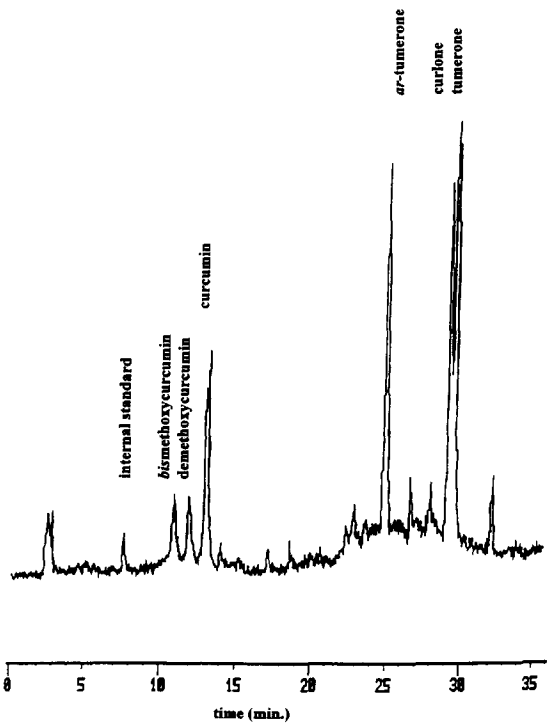


Fig. 2. Thermospray LC–MS chromatogram of turmeric. Column, Supelcosil LC-18, d_p 5 μm, 250×0.46 mm I.D.; mobile phase A, 50 m*M* NH_4OAc–5% HOAc; mobile phase B, acetonitrile; gradient, initial 50% B (hold 10 min), 80% B at 30 min (hold 10 min); masses scanned, 150–550 amu at 3.2 s/scan; source temperature, 255°C; control temperature, 136°C.

Table 1
Quantitative results for curcuminoids in turmeric powder by HPLC

Curcuminoid	Powder 1 (wt%)	Powder 2 (wt%)	Powder 3 (wt%)	Powder 4 (wt%)	Powder 5 (wt%)
Bisdemethoxycurcumin	0.49	0.60	0.55	0.50	0.90
Demethoxycurcumin	0.60	0.72	0.66	0.58	1.10
Curcumin	1.68	1.70	1.94	1.63	3.18

Column, Supelcosil LC-18, *dp*=5 μm, 250 mm×0.46 mm I.D.; mobile phase A, 1% citric acid (pH 3.0); mobile phase B, acetonitrile; gradient, initial 50% B (hold 10 min), 80% B at 30 min (hold 10 min).

Gradient elution also enabled detection of three major late eluting components and numerous minor components.

Values for curcumin, demethoxycurcumin and bisdemethoxycurcumin are listed in Table 1. These values were determined using an external standard method and by assuming the Vis responses for these components at 423 nm are equal [6].

The linearity of response versus pg (injected on column) for curcumin was evaluated in the range of 186 to 929 pg. Regression analysis generated the equation $y=726x-63\,809$ with $r^2=0.9989$. The linearity of response versus pg (injected on column) for demethoxycurcumin was evaluated in the range of 52 to 258 pg. Regression analysis generated the equation $y=699x-14\,598$ with $r^2=0.9996$. The linearity of response versus pg (injected on column) for bisdemethoxycurcumin was evaluated in the range of 14 to 71 pg. Regression analysis generated the equation $y=695x-3890$ with $r^2=0.9999$.

The precision for this assay was determined by analyzing five weighings of powder 1. The standard deviation was found to be 0.0367 at the 1.68% level for curcumin, 0.0137 at the 0.60% level for de-

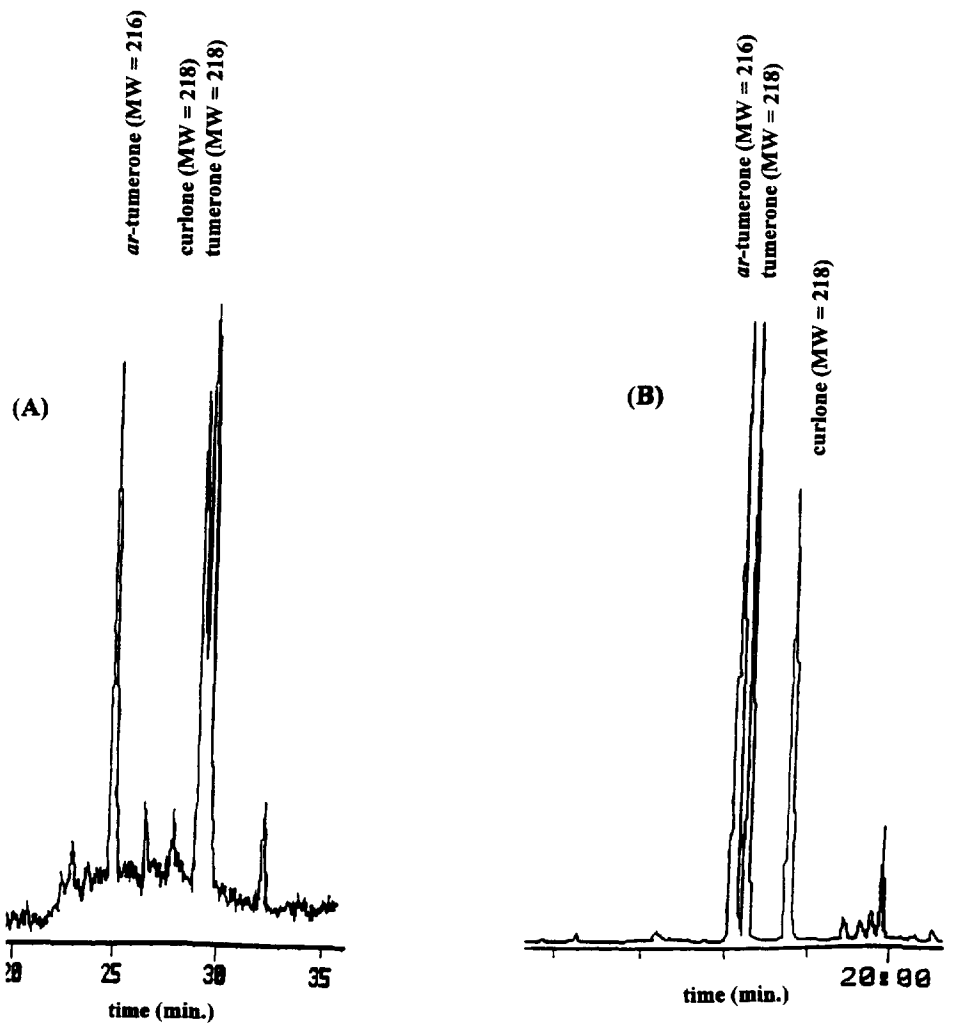


Fig. 3. Comparison of late eluting components in thermospray LC–MS (A) and GC–MS (B) partial chromatograms.

Table 2
Semi-quantitative results for some major and minor components in turmeric powders

Retention time (min)	Kovat index[a]	Assignment	MW	Powder 1 (ppm)	Powder 2 (ppm)	Powder 3 (ppm)	Powder 4 (ppm)	Powder 5 (ppm)
15.71	747.85	3,4,5-Trimethyl-2-cyclo-pentene-1-one	140	1000	674	519	478	866
17.28	779.74	I.S. (internal standard)	136	4000	4660	4760	3290	2580
18.04	794.44	Coumaran	120	532	327	129	98	231
18.96	809.00	2-Hydroxy-5-methyl-acetophenone	150	2590	1150	1183	848	1650
20.23	826.54	Vanillin[b]	152	411	148	229	272	245
22.25	852.29	Curcumene	204	544	601	895	370	347
22.55	855.92	Compound 1[c]	204	537	295	292	315	296
22.83	859.26	Zingiberene	204	107	137	173	98	108
23.17	863.26	β-Bisabolene	204	632	495	679	435	549
24.00	872.78	Dehydrocurcumene (proposed)	200	1710	647	690	707	1180
24.46	877.92	Compound 2	216	1440	611	754	924	1240
25.05	884.37	*ar*-Tumerol	218	1510	327	822	533	1340
25.28	886.84	Compound 3	204	3490	1340	1230	1590	2430
25.68	891.09	*ar*-Tumerone	216	59 500	25 700	25 450	29 200	33 200
25.89	893.29	Tumerone	218	45 700	13 900	6420	20 500	25 600
26.59	900.64	Curlone	218	48 000	15 900	13 100	23 800	26 900
27.47	911.74	Compound 4	216	3470	906	1250	1480	2300
27.72	914.83	Compound 5	216	5950	1620	963	1200	ND
28.05	918.86	Compound 6	218	11 400	3620	3510	4250	7960
28.40	923.09	Dehydrozingerone	192	10 700	2640	3890	3930	5830
28.94	929.51	Compound 7	234	3320	632	467	1370	2460
29.95	941.20	Compound 8	232	1470	432	301	978	939
30.447	946.73	Compound 9	216	2370	780	1740	946	2300
30.98	952.72	Compound 10	234	2310	464	878	1480	2060

Column, DB-1 capillary, 60 m×320 μm I.D., d_f=0.25 μm; temperature program, -20–150°C at 20°C/min; 150–280°C at 5°C/min; carrier, He at 1 ml/min; injection temperature, 220°C; FID temperature, 325°C.

[a] See Majlat et al. [20].

[b] Possible decomposition product [21].

[c] See Table 3.

methoxycurcumin and 0.0128 at the 0.49% level for bisdemethoxycurcumin.

This extraction procedure was determined to be quantitative by extracting 1.05 g of turmeric powder 1 with 750 ml of methanol for 1.25 h using a Soxhlet extractor. The extract was quantitatively transferred to a 1000-ml volumetric flask and diluted to volume with methanol. This sample was analyzed using the same instrument conditions as the samples described above. The curcumin level was determined to be 1.75%, the demethoxycurcumin level was determined to be 0.64% and the bisdemethoxycurcumin level was determined to be 0.57%. This compares favorably to the values obtained for this sample from the precision study (Table 1).

Although resolution and column performance were very good using this system, citric acid and NaOH cannot be used in the mobile phase when interfaced with a mass spectrometer because of the requirement for volatile mobile phase additives such as the acetonitrile–ammonium acetate buffer used for this work. The reason for this is that nonvolatile components in the mobile phase will clog the mass spectrometer interface leading to high back pressures; they will also contaminate the mass spectrometer ion source.

Curcumin, demethoxycurcumin and bisdemethoxycurcumin were identified in the methanol extracts of commercial powdered turmeric based on molecular mass data obtained from thermospray HPLC–MS (Fig. 2) and molecular mass and fragmentation data obtained from particle beam EI-mass spectra. The 70 eV fragmentation patterns are consistent with the structures of the curcuminoids [14].

None of the numerous late eluting components detected by thermospray HPLC–MS were detected when the samples were analyzed by particle beam HPLC–MS. One disadvantage of the particular particle beam interface used is that volatile and some semi-volatile compounds are lost in the membrane separator used to remove solvents [12,13]. The powdered turmeric was subsequently analyzed by GC–MS. The components detected by thermospray LC–MS and the semi-volatile components detected by GC–MS were correlated from molecular mass information and relative response. (Fig. 3).

The samples were analyzed by direct thermal desorption gas chromatography (DTD-GC) using both a flame ionization detector and a mass spectrometer. Direct thermal desorption is a technique where dry samples are lightly packed into short glass-lined stainless steel tubes (10 cm×4 mm). A mixture with a diatomaceous support, such as Chromosorb W, can be made so small samples can be more easily weighed into the column. The volatile components are swept directly onto the GC capillary column by purging the heated sample column with helium. The GC capillary column is maintained at subambient conditions while the volatiles are purged to minimize band broadening. The samples must be <5–10% water to keep the GC capillary column from plugging with ice. An overview of DTD-GC–MS has been published by Hartman et al. [15].

The data obtained by GC using a flame ionization detector were used to obtain semi-quantitative results for the volatile components in five turmeric powders. Calculations were based on comparing the responses of each component to the response of d_8-naphthalene used as an internal standard. These results are listed in Table 2.

EI-mass spectra were obtained for each component. Fig. 4, Fig. 5 and Fig. 6 show the EI-mass spectra for the three major components detected by DTD-GC–MS. Table 3 contains the eight most abundant ions and relative intensities for the minor components in turmeric powder. Table 4 contains a list of structures for the components detected. Some of these identifications are based on correlation with mass spectra in the NIST/EPA/NIH Mass Spectral Library. However, the mass spectra for most of the components could not be found in the library and many of the structures listed in Table 4 are proposed structures. For proposed, structures the identifications are based on similarities with components for which there was a good library match and by evaluation of the fragmentation patterns.

Fig. 4 shows the EI-mass spectrum of *ar*-tumerone [16,17]. The spectrum shows the molecular ion at *m/z* 216, ions for loss of methyl (*m/z* 201), α-cleavage to the aromatic ring (*m/z* 119) and α-cleavage to the carbonyl (*m/z* 83). There are also two odd electron ions at *m/z* 132 and *m/z* 98 that result from McLafferty rearrangements. The ion at *m/z* 132 is formed by transfer of the hydrogen, *ortho* to the 2-methyl-2-heptene-4-one moiety, to the carbonyl group followed by inductive cleavage (charge

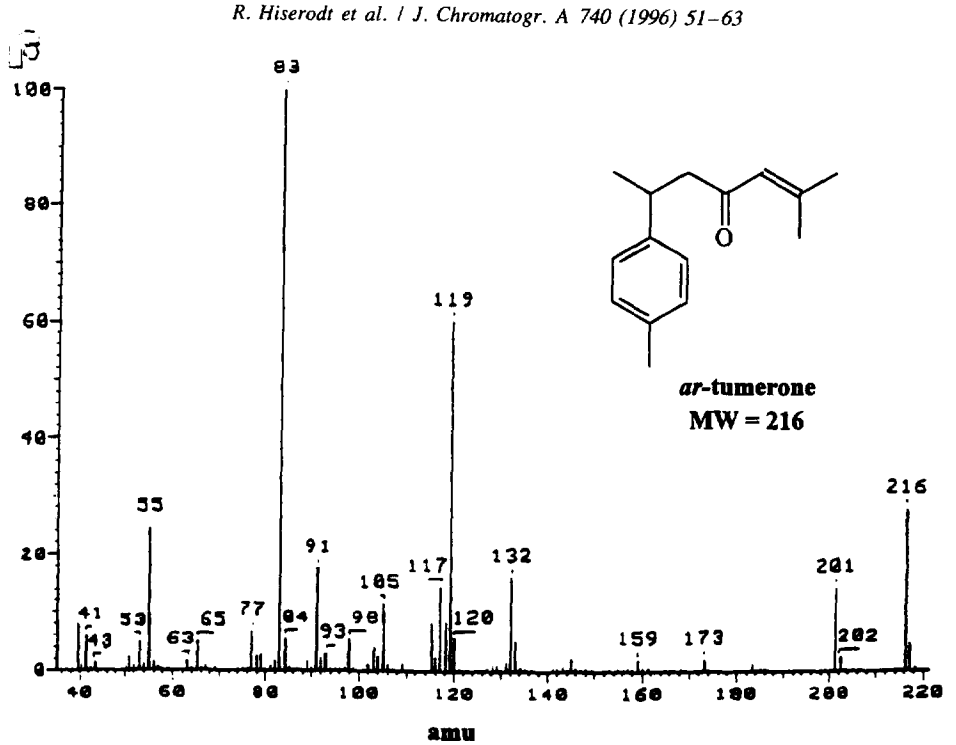


Fig. 4. Mass spectrum of *ar*-tumerone. GC–MS interface line, 280°C; MS inlet temperature, 240°C; ion source, 280°C; masses scanned, 35–350 amu at 1 s/decade, 70 eV.

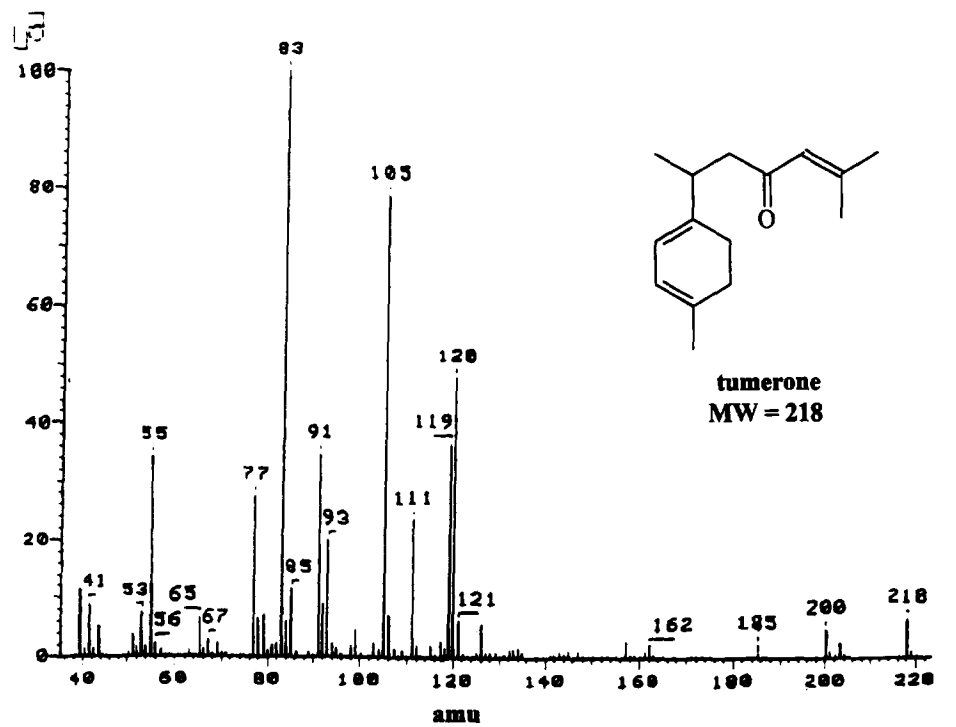


Fig. 5. Mass spectrum of tumerone. Conditions as given in the legend to Fig. 4.

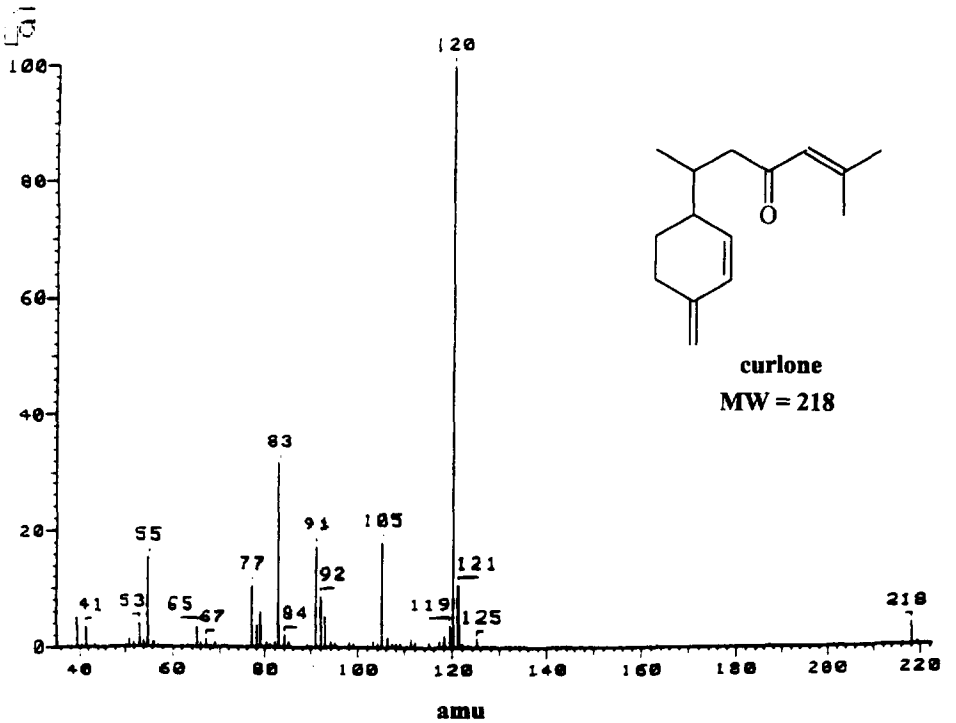
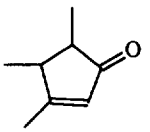
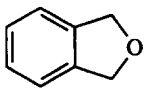
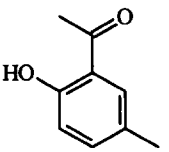
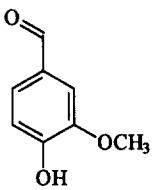
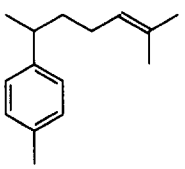
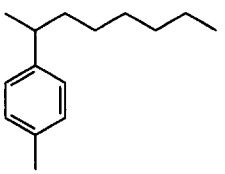
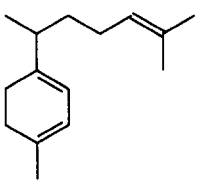
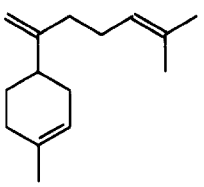
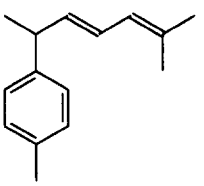
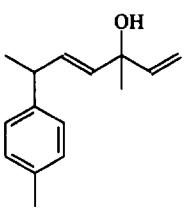
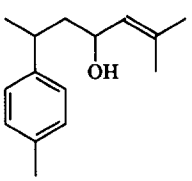
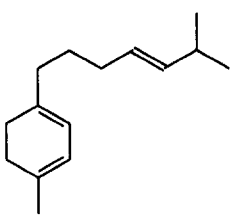


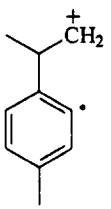
Fig. 6. Mass spectrum of curlone. Conditions as given in the legend to Fig. 4.

Table 3
Eight most abundant ions and intensities for the minor components in turmeric powder

Component	*m/z* (intensity)							
Compound 1 MW 204 (2%)	41 (100%)	119 (75%)	93 (38%)	91 (38%)	69 (36%)	77 (34%)	39 (32%)	43 (26%)
Dehydrocurcumene MW 200 (4%)	119 (100%)	91 (22%)	120 (14%)	105 (12%)	41 (11%)	27 (10%)	43 (8%)	83 (7%)
Compound 2 MW 216 (4%)	119 (100%)	71 (38%)	43 (31%)	41 (29%)	91 (20%)	39 (18%)	132 (16%)	117 (12%)
ar-tumerol MW 218 (4%)	119 (100%)	85 (32%)	120 (16%)	117 (14%)	91 (12%)	157 (10%)	200 (8%)	185 (6%)
Compound 3 MW 204 (8%)	120 (100%)	41 (52%)	69 (50%)	91 (48%)	55 (47%)	93 (46%)	119 (44%)	105 (30%)
Compound 4 MW 220 (6%)	41 (100%)	137 (62%)	95 (46%)	110 (43%)	135 (41%)	55 (38%)	109 (34%)	67 (32%)
Compound 5 MW 216 (1%)	119 (100%)	83 (76%)	55 (27%)	91 (21%)	43 (16%)	39 (14%)	41 (13%)	120 (12%)
Compound 6 MW 218 (26%)	83 (100%)	135 (67%)	55 (53%)	123 (50%)	41 (40%)	107 (34%)	67 (32%)	91 (28%)
Compound 7 MW 234 (3%)	83 (100%)	55 (50%)	93 (40%)	114 (38%)	121 (33%)	91 (32%)	77 (30%)	41 (26%)
Compound 8 MW 232 (14%)	83 (100%)	199 (52%)	135 (46%)	55 (32%)	91 (21%)	39 (20%)	41 (15%)	43 (15%)
Compound 9 MW 216 (3%)	118 (100%)	83 (64%)	55 (30%)	117 (21%)	91 (18%)	136 (15%)	119 (14%)	39 (13%)
Compound 10 MW 234 (3%)	83 (100%)	55 (65%)	137 (55%)	110 (50%)	39 (38%)	41 (35%)	43 (28%)	95 (25%)

Table 4
Known and proposed structures for components in turmeric powders

		
3,4,5-trimethyl-2-cyclopentene-1-one (MW=124)	coumaran (MW=120)	2-hydroxy-5-methyl acetophenone (MW=150)
		
vanillin (MW=152)	curcumene (MW=202)	compound 1 (proposed) (MW=204)
		
zingiberene (MW=204)	β-bisabolene (MW=204)	dehydrocurcumene (proposed) (MW=200)
		
compound 2 (proposed) (MW=216)	*ar*-tumerol (MW=218)	compound 3 (proposed) (MW=204)

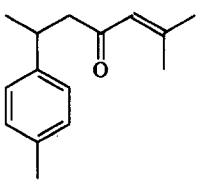


m/z = 132

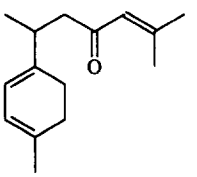
transfer) to form the odd electron ion shown below. This proceeds by way of a seven membered ring intermediate [18].

The odd electron at m/z 98 is formed by transfer of the γ-hydrogen from C7 of the 2-methyl-2-heptene-4-one moiety to the carbonyl carbon followed by β-cleavage to the carbonyl carbon to form the odd electron ion shown below.

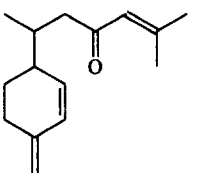
Table 4. (Continued)



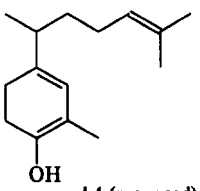
ar-tumerone
(MW=216)



tumerone
(MW=218)



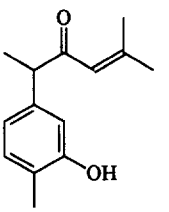
curlone
(MW=218)



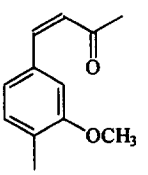
compound 4 (proposed)
(MW=220)

Unidentified Component

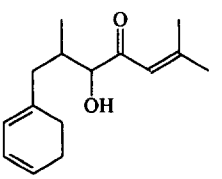
compound 5
(MW=216)



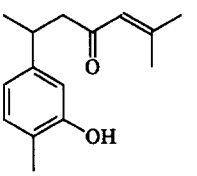
compound 6 (proposed)
(MW=218)



dehydrozingerone
(MW=190)



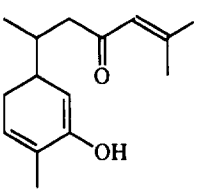
compound 7 (proposed)
(MW=234)



compound 8 (proposed)
(MW=232)

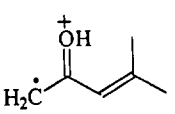
Unidentified Component

compound 9
(MW=216)



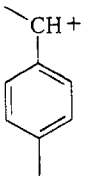
compound 10 (proposed)
(MW=234)

Column, DB-1 capillary, 60 m×320 μm I.D., d_f=0.25 μm; temperature program, −20–150°C at 20°C/min, 150–280°C at 5°C/min; carrier, He at 1 ml/min; injection temperature, 220°C; GC–MS interface line, 280°C; MS inlet temperature, 240°C; ion source, 280°C; masses scanned, 35–350 amu at 1 s/decade, 70 eV.



m/z = 98

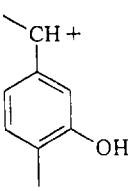
All of the proposed structures have the curcumene or *ar*-tumerone backbone. The most common ion was detected at *m/z* 119 as a moderate (37% base peak) to base peak in half of the proposed structures. This is attributed to the even electron ion shown below.



m/z = 119

The ion at m/z 119 can also be attributed to the aromatization of the cyclohexadienyl moiety at m/z 121 at the elevated temperatures in the GC–MS transfer line and the MS inlet temperature [16]. Both ions are present when the ring is not aromatic. This is seen in zingiberene and compound 3 (Table 4), where the 119/121 ratio is approximately 2:1. This ratio varies from 6:1 in tumerone (Fig. 5) to 1:6 in compound 7, both of which are non-aromatic compounds (Table 4). Mass spectral data for tumerone was reported by Su et al. [16]. They reported a base peak at m/z 121 with a probe temperature equal to 70°C. They also reported the appearance of an ion at m/z 119 when the probe temperature was increased to 150°C. The difference between their data and the data reported here may due to the high GC–MS temperatures stated above.

The aromatization of cyclohexadiene systems is also seen in compound 4 (Table 4) where the cation is detected at m/z 137 from α-cleavage to the cyclohexadiene ring; and the aromatized form, shown below, is detected at m/z 135. The 137/135 ion ratio in compound 4 is 1.5:1.



m/z = 135

α-Cleavage to the double bond in side chains of components that do not have a carbonyl group yields ions at m/z 41, 55 and 69 in addition to loss of a propyl radical from the molecular ion as in the spectra of curcumene and zingiberene. Loss of the propyl radical from the molecular ion was used as an indication that there was no carbonyl group in the side chain. This reasoning was used to postulate structures for compound 3 and compound 4 (Table 4) both of which have ions at m/z 69 due to α-cleavage at the double bond and loss of a propyl radical from the molecular ion.

Curlone (Fig. 6) shows a greater tendency to form a rearrangement product at m/z 120 (base peak) than tumerone (48%) or *ar*-tumerone (5%). The data presented in this report agrees with the data for curlone reported by Kiso et al. [19].

4. Conclusion

Extraction of natural products yields complex mixtures of volatile, semi-volatile, and nonvolatile components and no one technique for obtaining mass spectral data is generally applicable. Many of these components were determined by thermospray LC–MS. Thermospray LC–MS provided molecular weight information for the components but with limited fragmentation. Particle beam LC–MS was used to obtain EI–mass spectra for the nonvolatile components but because of the limitations of the particle beam interface, EI-mass spectra could not be obtained for the volatile and semi-volatile components. Volatile and semi-volatile components were determined by DTD-GC–MS.

Acknowledgments

We acknowledge the Center for Advanced Food Technology (CAFT) mass spectrometry facility for providing instrumentation support. CAFT is an initiative of the New Jersey Commission on Science and Technology. This is New Jersey Agricultural Experiment Station (NJAES) publication No. 10569-1-95.

References

[1] S. Toda, T. Miyase, H. Arichi, H. Tanizawa and Y. Takino, Chem. Pharm. Bull., 33 (1985) 1725.

[2] M.A. Azuine and S.V. Bhide, Nutr. Cancer, 17 (1992) 77.

[3] V.S. Govindarajan, Crit. Rev. Food Sci. Nutr., 12 (1980) 199.

[4] H.H. Tonnesen, ACS Symp. Ser., 506 (1992) 143.
[5] H.H. Tonnesen and J. Karlsen, J. Chromatogr., 259 (1983) 367.
[6] T.H. Cooper, J.G. Clark and J.A. Guzinski, ACS Symp. Ser., 547 (1992) 231.
[7] R.G. Bailey, H.E. Nursten and I. McDowell, J. Chromatogr., 542 (1991) 115.
[8] M.M. Sanagi, U.K. Ahmad and R.M. Smith, J. Chromatogr. Sci., 31 (1993) 20.
[9] S.J. Taylor and I.J. McDowell, Chromatographia, 34 (1992) 73.
[10] R.L. Rouseff, J. Food Sci., 53 (1988) 1823.
[11] R.M. Smith and B.A. Witowska, Analyst, 109 (1984) 259.
[12] L.B. Clark, R.T. Rosen, T.G. Hartman, J.B. Louis and J.D. Rosen, Int. J. Environ. Anal. Chem., 45 (1991) 169.
[13] L.B. Clark, R.T. Rosen, T.G. Hartman, J.B. Louis, I.H. Suffet, R.L. Lippincott and J.D. Rosen, Int. J. Environ. Anal. Chem., 47 (1992) 167.
[14] H.H. Tonnesen, Chemistry, Stability and Analysis of Curcumin i – A Naturally Occurring Drug Molecule, Institute of Pharmacy, University of Oslo, Oslo, 1986.
[15] T.G. Hartman, S. Overton, J. Manura, C.W. Baker and J.N. Manos, Food Technol., 45 (1991) 104.
[16] H.C.F. Su, R. Horvat and G. Jilani, J. Agric. Food Chem., 30 (1982) 290.
[17] A.S. Rao, B. Rajanikanth and R. Seshadri, J. Agric. Food Chem., 37 (1989) 740.
[18] D.G.I. Kingston, J.T. Bursey and M.M. Bursey, Chem. Rev., 74 (1974) 215.
[19] Y. Kiso, Y. Suzuki, Y. Oshima and H. Hikino, Phytochemistry, 22 (1983) 596.
[20] P. Majlat, Z. Erdos, J. Takacs, J. Chromatogr., 91 (1974) 89.
[21] A. Khurana and C.-T. Ho, J. Liq. Chromatogr., 11 (1988) 2295.