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Analysis of volatile compounds in fresh healthy and diseased peppers (*Capsicum annuum* L.) using solvent free solid injection coupled with gas chromatography-flame ionization detector and confirmation with mass spectrometry

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Abstract

The characteristic volatile flavor compounds in healthy peppers (*Capsicum annuum* L.) were evaluated using a solvent-free solid injector coupled with a-gas chromatography-flame ionization detector (SFSI-GC-FID) and the results of evaluation were confirmed using GC–mass spectrometry (GC–MS). These compounds were compared with those obtained from peppers that were naturally infected or artificially inoculated with *Colletotrichum* spp**.** Parameters influencing the vaporization efficiency, including the injector temperature, pre-heating time and holding time, were optimized to improve the analytical efficiency. A total of 96 compounds (excluding eight capillary compounds), 17 of which were identified in healthy peppers, 49 of which were found in naturally infected peppers, and 61 of which were identified in artificially inoculated peppers, were separated and identified under the optimal conditions of an injector temperature of 250 °C and 7-min preheating and holding times. Acetic acid and 2-furanmethanol were the major compounds detected in the volatiles of the healthy and diseased peppers. The major compound detected in both the healthy and naturally infected peppers was 3-hydroxypyridine, while hexadecanoic acid was the primary compound identified in the artificially inoculated peppers. Indole derivatives (1*H*-indole, 4-methylindole and 1-ethylindole) were suggested to be the key factors contributing to the pepper infection caused by *Colletotrichum* spp. We conclude that SFSI in combination with GC is a suitable approach for distinguishing between healthy and diseased peppers by the investigation of their volatile compounds. It does not require the use of solvents and complicated equipment.

Keywords: Pepper; Infection; SFSI; Volatile flavor compounds; Indole compounds

1. Introduction

The genus *Capsicum* includes many species widely cultivated in Asia, Africa, and countries along the Mediterranean. Peppers are native plants of America, and their fruits (pericarps) are consumed as vegetable foods, spices, and external medicines. They are also a source of vitamins A, C, and E. Being a member of the solanaceae family, the peppers are attacked by several diseases resulting in low production yields. The anthracnose disease caused by *Colletotrichum* spp. is the third most important disease after virus complex and phytophthora rot caused by *Phytophthora capsici* [1]. Anthracnose is an economically important disease affecting peppers. Pre- and post-harvest losses in peppers of up to 50% have been reported in certain conditions that are favorable for disease development [2]. *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* were the predominant fungal species causing anthracnose in peppers in the Republic of Korea [3,4].

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Numerous bacteria and fungi also have the ability to synthesize plant growth regulators such as indole-3-acetic acid (IAA) and other indole-related compounds [5–7]. Chung et al. [8] successfully identified several indole derivatives produced by the fungus *Colletotrichum acutatum*, which causes lime anthracnose and postbloom fruit drop in citrus. In most cases, the identification and quantitation of indole compounds were performed using high performance liquid chromatography as along with color reactions followed by fluorescence thin-layer chromatography [8]. Furthermore, Salkowski reagent was employed to specifically recognize the compounds via the formation of a pink color in solution, which can be further quantified using spectrophotometer [9]. The drawback of this test is the inability to visualize colors that are too faint in UV light [8]. The determination of the volatile flavor constituents of diseased peppers has not been investigated thus far.

Color and pungency are the main quality parameters for assessing Capsicum varieties [10,11]. However, the majority of research has been focused on using aroma as an important parameter for assessing the quality of fresh fruits and vegetables [12–14]. The volatile compound fractions of the pepper species have previously been isolated and more than 200 compounds were identified after hydrodistillation [15], and dynamic headspace sampling (purge and trap) [16] procedures. Nevertheless, none of the aforementioned methodologies directly studied the volatiles emitted from the pepper matrices in their natural state, without any pretreatment. It has been reported that thermal treatment and the presence of water promote many chemical or enzymatic reactions [17,18] that introduce major variations to the pepper aroma profile composition.

In a routine analytical laboratory, it is necessary to obtain analytical results from a large number of samples in a short period of time. Therefore, the introduction of direct sample injection using a conventional syringe-based sample injector by Amirav and Dagan [19], Jing and Amirav [20], and Morgan [21] has gained acceptance as a rapid, inexpensive and quantitative analytical technique to be used in the determination of a wide spectrum of analytes during recent years [22–26]. Shim et al. [22] originally modified the SFSI using a Keele injector for the gas chromatographic analysis of vinclozolin in lettuces. The technique is essentially solvent free, which is a highly desirable feature. To our knowledge, solvent free solid injector (SFSI) has not been applied to the analysis and identification of the volatile compounds in peppers. The present paper reports the identities of the volatile components of healthy peppers and compares them to those detected in diseased peppers. The identities of key odor compounds responsible for the characteristic flavor of the diseased peppers are suggested. The use of GC–MS determination in full scan mode allowed the volatiles found in the extract to be identified.

2. Experimental

2.1. Samples and chemicals

Freshly harvested and naturally infected peppers were collected from an agricultural farm located in Sangmu-dong, Gwangju, Republic of Korea, and were immediately transported to our laboratory. The naturally infected fruits showed the typical symptoms of anthracnose including circular (or angular) sunken lesions, and pink to orange-colored masses of fungal spores arranged in concentric rings (Fig. 1), which were confirmed by a plant pathologist. The peppers to be used in the artificial inoculation were purchased from local market sales organic crops. All the samples were stored at −24 °C until analysis. The 3-hydroxypyridine, 1*H*-pyrrole and 1*H*-indole were purchased from Sigma (St. Louis, MO, USA). All other chemicals and solvents used in this study were of analytical grade, unless otherwise stated.

2.2. Fungal cultures and artificial inoculation

Monosporic isolates of *Colletotrichum gloeosporioides* and *C. acutatum* were kindly provided by the Laboratory of Environmental Microbiology, Chonnam National University, Gwangju, Republic of Korea, and cultured on potato dextrose agar (PDA, Difco Lab., USA) at 27 °C for 10 days. A conidial suspension (5×10^5 conidia/mL) of *C. gloeosporioides* or *C. acutatum* was prepared as follows: Fungal isolates were grown in Petri plates (90 mm diameter) containing 15 mL of PDA under constant fluorescent light. After 10 days of incubation at 27 °C, the conidial suspension were collected by scraping the colony surface with

Fig. 1. Field samples of (A) fresh healthy and (B) diseased peppers.

a sterile scalpel and 10 mL of sterile distilled water, then filtered through four layers of cheesecloth to remove any mycelial debris, and counted with a hemocytometer. The peppers were inoculated according to the method previously described by Oh et al. [27] with minor modifications. The surface of the peppers were sterilized with 70% ethanol for 1 min then washed three times with sterilized distilled water, and placed in containers (35 cm × 45 cm × 5 cm). Four layers of paper towels moistened with sterilized water were placed in the containers to maintain a 100% relative humidity. The peppers were then scratched and dot-marked around the equatorial region with a marking pen. Twenty microliters of the conidial suspension were then inoculated adjacent to the marks. The control peppers were inoculated with just 20 μL of sterilized water. The containers were then covered with a lid and maintained in darkness at 28 °C.

2.3. Solvent-free solid injector (SFSI) extraction procedure

An SFSI manufactured by Han Jin Precision Co. (Gwangju, Republic of Korea) was used for the injection to a gas chromatograph in our laboratory. A 1-mg sample weight was dropped into a soft glass capillary tube (1.2 mm i.d. × 30 mm in length); both ends of the tube were sealed briefly in a flame, and the tube was then placed in a solvent-free solid injector (SFSI). The tube was crushed by lowering the injector plunger to carry the sample analytes onto a GC column by a carrier gas in a normal manner. The SFSI was held at the injection port during the pre-heating phase until the injector plunger reached the top of the injector septum, which allowed a constant pressure of carrier gas to be maintained during analysis.

The optimal SFSI conditions were attained by sequentially varying one experimental parameter while all of the other parameters remained fixed. The parameters were varied in the order of the injector temperature, pre-heating time, and holding time. The results of the current test were used to determine the next extraction parameter changes to be used for the optimization. The various extraction conditions were injector temperatures (200, 250, and 300 °C), pre-heating times (3, 5, 7, and 10 min), and holding times of 0, 3, 5, 7, and 10 min.

2.4. GC and GC/MS analyses

An HP 4890 gas chromatograph (Hewlett-Packard, Pale Alto, CA, USA) equipped with a 30 m × 0.25 mm × 0.25 μm HP-5 fused silica capillary column and a flame ionization detector (FID) was used. The injector and detector temperatures were 250 and 300 °C, respectively. The oven temperature was held at 50 °C for 5 min and then increased to 280 °C at a rate of 5 °C/min and was then held constant for 10 min. The carrier gas (nitrogen) flow rate was 1 mL/min. Injection was made using split mode of 20:1. The linear retention indices were calculated against those of *n*-paraffins.

The GC/MS analyses were performed on an Agilent model 6890N GC equipped with a 5973 mass-selective detector (Agilent Technologies, USA). It was fitted with an HP-5MS fused silica column (30 m × 0.25 mm × 0.25 μm). The chromatographic conditions were the same as those described for the GC (FID) with the exception of a split mode of 10:1. High-purity helium (99.9999%) at a constant flow rate of 1 mL/min was used as the carrier gas. An electron impact mass spectral (EI-MS) analysis was carried out at an ionization energy of 70 eV at 250 °C. The detection was performed in the scan mode between 10 and 400 amu at 3.71 scans/s.

2.5. Compound identification

The tentative identification of components was based on a comparison of the retention indices (RI) and mass spectra. The RI was calculated using a standard alkane–alkene mixture (prepared in hexane, 100 ng/μL) as the external references [28]. The preliminary experiments were initially conducted by syringe injection in the split mode of a solution of hydrocarbons in order to find the optimal conditions for the separation of the high molecular mass alkanes and alkenes. One-microliter aliquots of the standard hydrocarbon solution were then placed in the soft glass capillaries, most of the hexane was allowed to evaporate, and they were then sealed, heated and crushed in the gas chromatograph using the solid sampler [29]. The temperature of the injector and the time of heating were varied until good peak shapes were obtained for all, including the last eluted peaks, and until their peak–area ratios corresponded to those from the syringe injection. Tentative identifications were based on matching the mass spectra of unknowns with those in the Wiley 7N mass spectral database [30]. The relative peak areas obtained from the GC–MS total ion chromatogram were used to calculate the percentages of each compound.

3. Results and discussions

Achieving maximum efficiency is the greatest concern when using the SFSI method. A general discussion of these parameters is presented since there are a number of parameters that influence the extraction efficiency.

3.1. Injector temperatures

Injector temperatures between 200 and 300 °C with a 45 min extraction period were assayed to determine the extraction efficiency of SFSI. The results showed that temperatures above 250 °C reduced the proportion of 1*H*-pyrrole and 3-hydroxypyridine; however, the proportion of 1*H*-indole increased with increasing the temperature (Fig. 2). The injection temperature in the GC injector must be high enough to volatize the compounds, but it is necessary to account the decomposition of some compounds when the injection temperature is too high. Therefore, a relatively low injection temperature was selected (250 °C) as the sufficient temperature for obtaining an adequate extraction and better resolution.

3.2. Pre-heating times

Pre-heating times of 3, 5, 7, and 10 min were tested at 250 °C. In the case of 3-hydroxypyridine, an increase in the pre-heating

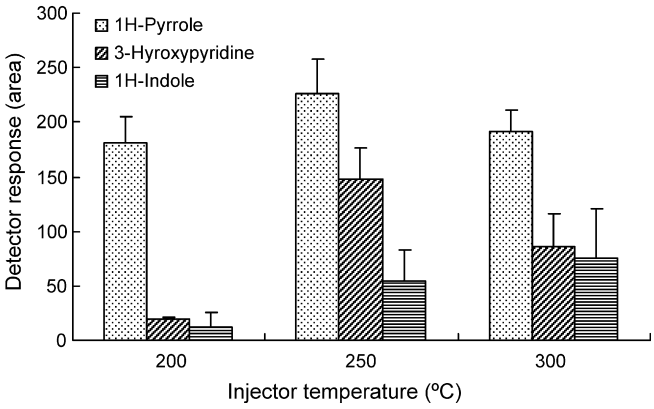

Fig. 2. Effect of injector temperatures on the proportion of volatile flavor components in fresh healthy and diseased peppers.

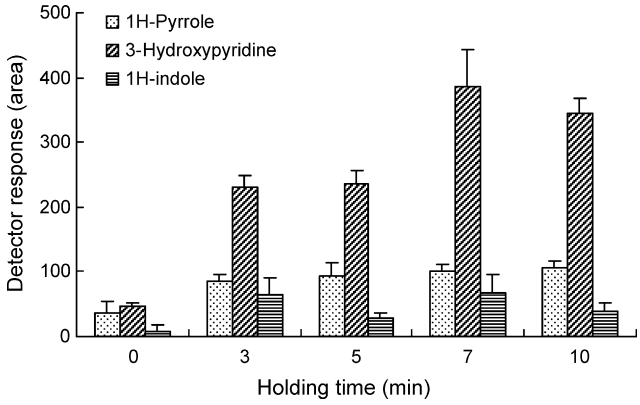

Fig. 4. Effect of holding time on the proportion of volatile flavor components in fresh healthy and diseased peppers.

time up to 7 min produced an improvement in the extraction efficiency, which decreased thereafter. On the other hand, the extraction efficiency of 1*H*-pyrrole and 1*H*-indole were not affected by the pre-heating time up to 7 min (Fig. 3). These results indicated that a pre-heating time of 7 min was a convenient compromise for the extraction of volatiles in all subsequent studies of the pepper samples.

3.3. Holding time

The effect of varying holding times, ranging from 0 to 10 min at 250 °C (optimal injector temperature) and 7 min (optimal pre-heating time) were also investigated. The peak areas of *1H*-indole and 3-hydroxypridine increased for up to 7 min and decreased thereafter. The effects of holding times between 3 and 10 min on 3-hydroxypyridine were very similar (Fig. 4). The volatile components were completely swept onto the GC/MS column by the carrier gas after 7 min, which was then selected as the optimal holding time.

No further improvement was observed in the majority of the compounds when the extraction time was increased from 45 to 60 min (data not shown). Therefore, an extraction time of 45 min was selected.

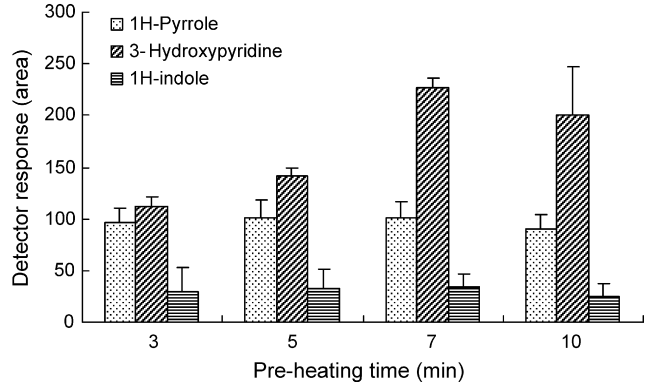

Fig. 3. Effect of preheating time on the proportion of volatile flavor components in fresh healthy and diseased peppers.

3.4. Volatile compounds emitted from peppers

A detailed identification of the volatile flavor compounds confirmed on the HP-5ms column via GC–MS coupled SFSI and their relative percentages of peak area, retention time (RT) and the respective retention indices calculated according to the Kovats index [28] are reported in Table 1 and Fig. 5. A total of 104 compounds were extracted and identified, including acids, alcohols, aldehydes, amides, amines, benzene, esters, hydrocarbons, ketones, phenol, pyrazines, pyridines, pyrroles, and other miscellaneous compounds (Table 2). Most of the acids found in peppers primarily originated from the degradation of lipids [31] and these compounds could significantly contribute to the odor [31]. Wu and Liou [32] indicated that tissue disruption increased the amount of volatile unsaturated C6 aldehydes and alcohols. These aldehydes and alcohols might play an important role in determining the green bell and hot pepper flavors.

Eight of the volatile compounds that were detected, including decanal, tetradecanoic acid, tetradecanoic acid 1-methylethyl ester, 9-octadecanol, 1-octadecanol, dibuthyl phthalate, tritetracontane, and bis-phthalate belonged to the capillary tube (referred as blank) (Fig. 5A). All of these compounds were excluded from the total volatiles. Therefore, a total of 96 compounds were emitted from healthy and both naturally infected and artificially inoculated peppers. The composition of volatile compounds of peppers clearly differs between healthy and diseased peppers. Table 1 shows the majority of volatile compounds from both healthy and diseased peppers. A comparison of Fig. 5B–D illustrates that the patterns of healthy and diseased pepper fruits differ greatly. In general, the intensity of the MS response of the diseased peppers was higher than that of the healthy peppers, which indicates that the number and the area percents of some volatile compounds in the diseased peppers are higher than healthy peppers. Seventeen volatile compounds were identified in the healthy peppers by SFSI coupled with GC–MS (Fig. 5). The primary compounds (concentration >3.0%, calculated as % peak area of GC–MS) detected in the SFSI samples of healthy peppers were: 3-hydroxypyridine (26.15%), acetic acid (25.02%), 2-furanmethanol (7.15%), butyrolactone (3.36%), and 2-propanone (3.24%). A strong almond note was exhib-

Table 1
Identified compounds and their average relative GC–MS peak areas in healthy and diseased peppers

No.	Compounds	RT	RI	Area (%)			
				Blank	Fresh healthy pepper	Naturally infected pepper	Artificially inoculated pepper
1	3-Methylbutanal	2.07	<800	–	2.10	4.55	–
2	Acetic acid	2.37	<800	–	25.02	21.39	22.01
3	2-Propanone	2.42	<800	–	3.24	3.79	–
4	Pyrazine	2.84	<800	–	–	4.98	0.36
5	*N*,*N*-dimethylaminoethanol	2.91	<800	–	–	–	0.61
6	1H-pyrrole	3.16	<800	–	–	2.63	–
7	1,2-Ethanediamine	3.70	<800	–	–	2.05	0.13
8	Ethanamine, *N*-methyl-	3.73	<800	–	–	–	0.97
9	2-Amino-4-hydroxypteridine-6-carboxylic acid	3.89	801.4	–	0.54	–	–
10	1,4-Dideuterio-2-methylbutane	4.02	806.9	–	–	0.91	–
11	Trimethylurea	4.19	814.2	–	0.63	3.04	–
12	2[3H]-Furanone	4.37	821.1	–	–	0.89	–
13	Topotecan	4.43	823.6	–	–	0.83	–
14	2-Methyl-pyrazine	4.63	831.2	–	–	3.39	4.86
15	2-Furanmethanol	5.95	874.0	–	7.15	10.26	5.64
16	2-ethylpyrazine	7.31	912.8	–	–	–	0.23
17	2,6-Dimethylpyrazin	7.55	920.6	–	–	1.53	2.07
18	Ethylpyrazine	7.65	923.8	–	–	0.99	0.27
19	Butyrolactone	7.84	929.6	–	3.36	2.37	–
20	Gamma valerolactone	8.78	956.7	–	–	–	0.21
21	2-Furancarboxaldehyde	9.43	973.7	–	1.81	–	–
22	Phenol	10.07	989.5	–	–	–	0.21
23	2-Ethyl-6-methyl-pyrazine	10.62	1003.5	–	–	0.51	0.82
24	2-Ethyl-5-methyl-pyrazine	10.73	1006.9	–	–	0.35	0.46
25	2,3,5-Trimethylpyrazine	10.80	1009.5	–	–	1.38	0.06
26	4(H)-Pyridine	11.08	1018.6	–	–	–	0.25
27	Endo-2-methyltricyclo [4,10]decane	11.42	1029.5	–	–	0.60	–
28	2-Cyclopenten-1-one	11.83	1041.9	–	1.45	0.85	1.84
29	Benzeneacetaldehyde	12.15	1051.6	–	–	0.46	–
30	1-[1H-pyrrol-2-yl]-Ethanone	13.08	1078.0	–	–	0.68	1.56
31	Hydroxy dimethyl furanone	13.19	1080.9	–	–	–	0.80
32	2,5-Dimethyl-4-hydroxy-3[2H]-furanone	13.32	1084.5	–	2.84	1.19	0.04
33	Phenol, 2-methoxy-	13.4	1086.6	–	-	–	1.29
34	2-Butanamine, hydrochloride	13.72	1095.1	–	-	–	0.17
35	Cyclobutanol	13.98	1102.6	–	2.65	2.63	–
36	1,2-Propanediol, 3-chloro-	14.02	1103.9	–	-	–	0.19
37	1-Propanol	14.23	1111.3	–	-	0.42	–
38	1-Guanidinosuccinimide	14.31	1114.0	–	-	0.73	–
39	4H-Pyran-4-one	14.61	1124.4	–	0.77	–	–
40	3-Ethyl-2-hydroxy-2-cyclopenten-1-one\	14.79	1130.5	–	–	0.27	–
41	Erythro-1,2,4-trimethylpnet-4-en-1-ol	15.27	1146.5	–	–	0.46	–
42	β-D_2-γ-picoline	15.36	1149.3	–	–	0.68	–
43	4-Pyridinol	15.56	1155.8	–	–	2.20	0.15
44	3-Hydroxypyridine	15.90	1166.5	–	26.15	7.02	–
45	4-Hydroxypyridine	16.35	1180.3	–	–	–	1.61
46	Decanal	16.55	1186.4	0.74	0.37	0.36	0.38
47	6-Methyl-3-pyridinol	17.01	1200.0	–	–	1.00	0.04
48	5-Ethyldihydro-2[3H]-furanone	17.26	1209.3	–	–	1.00	–
49	Dianhydromannitol	17.57	1220.9	–	–	–	0.02
50	4-Pyridinamine	19.63	1292.4	–	–	0.68	–
51	1H-Indole	19.95	1303.4	–	–	0.43	1.01
52	2-Methoxy-5-vinylphenol	20.11	1309.9	–	–	–	0.51
53	Phenol, 2,6-dimethoxy-	21.15	1350.1	–	–	–	0.25
54	1,3-Benzenediamine	21.86	1376.2	–	–	0.63	–
55	4-Methylindole	22.39	1395.4	–	–	0.43	2.18
56	1-Ethylindole	24.79	1491.6	–	–	1.12	–
57	1,4,8-Dodecatriene, (*E*,*E*,*E*)-	24.89	1495.4	–	–	–	0.30
58	9-Octadecenamide, (*Z*)-	25.22	1507.9	–	–	–	3.19
59	Acetamide	25.59	1525.5	–	–	0.40	–
60	Tetradecanamide	25.60	1525.9	–	–	–	0.55

Table 1 (*Continued*)

No.	Compounds	RT	RI	Area (%)			
				Blank	Fresh healthy pepper	Naturally infected pepper	Artificially inoculated pepper
61	Benzeneacetic acid, 4-hydroxy-3-mehoxy-	25.73	1531.6	–	–	–	0.53
62	(-)-(1R,5S)-exo-2(R)-Methylbicyclo [3.2.1.]octan-3-one	26.30	1556.0	–	–	–	1.92
63	3-Pyrrolidin-2-yl-propionic acid	30.10	1724.8	–	–	–	1.25
64	Tetradecanoic acid	30.80	1758.2	1.58	–	–	0.91
65	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	31.26	1779.7	–	–	–	0.15
66	2-Decene, 3-methyl-	32.09	1819.8	–	–	–	1.54
67	Neophytadiene	32.22	1826.3	–	–	–	1.29
68	Cyclododecane	32.36	1833.4	–	–	–	0.13
69	Tetradecanoic acid, 1-methylethyl ester	32.55	1842.6	0.56	0.92	0.61	0.70
70	9-Octadecanol	32.81	1855.5	0.30	0.23	0.38	0.97
71	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	33.41	1885.1	–	–	0.27	-
72	2-Octylfuran	33.56	1892.3	–	–	–	2.32
73	Cyclohexanol, 1-ethynyl-	33.77	1902.7	–	–	–	3.74
74	Hexadecanoic acid, methyl ester	33.98	1913.8	–	–	–	2.47
75	4a[2H]Naphthalenemethanol	34.08	1919.2	–	–	0.21	–
76	Oxacycloheptadecan-2-one	34.16	1923.3	–	–	–	0.44
77	Benzene	34.51	1941.8	–	0.61	0.40	–
78	Pyrrolo[1,2,*a*]pyrazine-1,4-dione	34.52	1941.9	–	0.98	–	–
79	1-Octadecanol	34.66	1949.3	0.47	0.38	0.16	1.27
80	Butanoic acid	34.73	1953.1	–	–	0.29	–
81	Hexadecanoic acid	34.93	1963.3	–	–	–	6.53
82	Dibuthyl phthalate	35.08	1971.2	14.98	6.36	2.37	1.01
83	d-Nerolidol	35.97	2017.8	–	–	–	0.15
84	6-C14H26	36.73	2059.3	–	–	–	0.72
85	(1S, 15S)-Bicyclo[13.1.0]hexadecan-2-one	36.84	2065.2	–	–	–	0.40
86	1-Octadecene	36.93	2070.1	–	–	–	0.30
87	9-Octadecenoic acid (*Z*)-, methyl ester	37.25	2087.2	–	–	–	1.56
88	9,12-Octadecadienoic acid	37.47	2098.9	–	–	0.23	2.26
89	Octadecanoic acid, methyl ester	37.71	2112.8	–	–	–	0.25
90	Phytol	37.81	2118.5	–	–	0.34	0.55
91	9,12-Octadecadienoic acid (*Z*,*Z*)-	37.99	2128.9	–	–	–	1.12
92	9-Octadecanoic acid, (*E*)-	38.08	2134.0	–	–	–	1.75
93	1-Epoxy-2-methyl-3-isobutenyl-1,4-pentadiene	38.74	2171.4	–	–	0.39	–
94	Dodecanamide	38.76	2172.6	–	–	–	1.12
95	α-Farnesene	38.82	2175.9	–	–	0.29	–
96	β-Myrcene	38.90	2180.3	–	–	0.95	–
97	2-Cyclohexenecarboxanilide	39.83	2234.6	–	–	–	1.06
98	(*E*,*Z*)-alpha-Farnesene	40.01	2245.2	–	–	–	0.17
99	Camphene	40.17	2254.9	–	1.10	–	0.06
100	7-Propylidene-bicyclo-[4,1,0]heptane	40.64	2281.8	–	1.22	–	–
101	Bicyclo[10.1.0]tridec-1-ene	41.68	2345.5	–	–	–	0.32
102	3-Methyl-thiophene	41.77	2351.3	–	–	0.43	–
103	Tritetracontane	43.85	2481.8	1.64	–	–	–
104	Bis-phthalate	45.00	2557.6	79.73	10.75	3.34	5.79

ited by 2-furancarboxaldehyde (1.81%), which was found in the healthy peppers as reported by Luning et al. [14] in a red pepper (Spanish type). On the other hand, 49 and 61 compounds were extracted and identified in the naturally infected and artificially inoculated peppers, respectively. Acetic acid (21.39%), 2-furanmethanol (10.26%), 3-hydroxypyridine (7.02%), pyrazine (4.98%), 3-methylbutanal (4.55%), 2-propanone (3.79%), 2-methyl-pyrazine (3.39%), and trimethylurea (3.04%) were the main compounds found in the naturally infected peppers. Acetic acid (22.01%), hexadecanoic acid (6.53%), 2-furanmethanol (5.64%) and 2-methyl-pyrazine (4.86%) were the primary compounds found in the artificially inoculated peppers. Notably, 4-hydroxypyridine (1.61%) rather than 3-hydroxypyridine was found among the volatiles of the artificially inoculated peppers.

All of the studies reported in the literature have been limited to the identification of the most abundant volatile components in plants. In many cases, the most abundant components make little contribution to the flavor. It is often the less abundant aroma compounds that primarily contribute to the flavor, and they may contribute to the aroma through low sensory thresh-

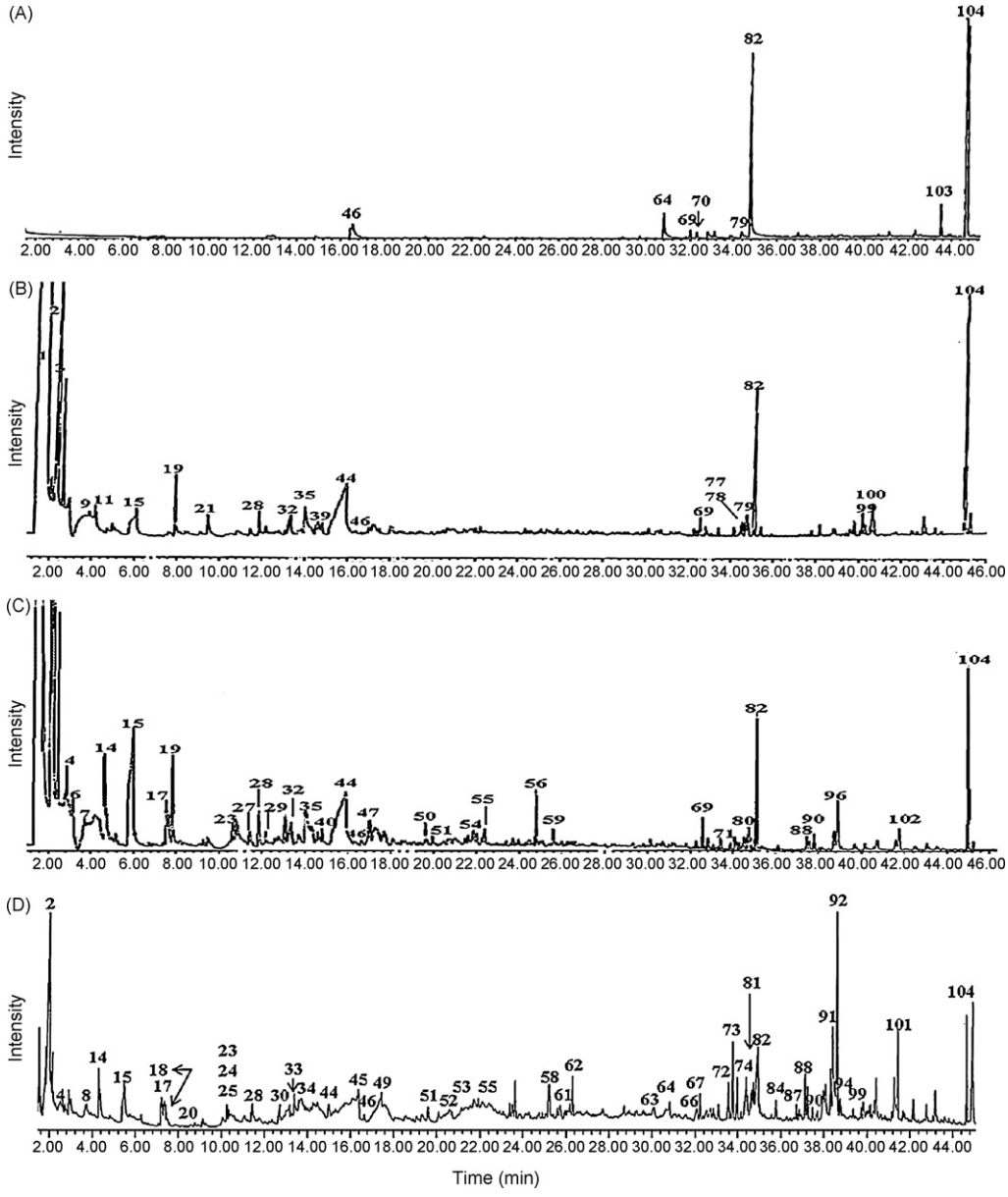

Fig. 5. Typical GC–MS chromatograms of volatile flavor components extracted by SFSI from (A) blank, (B) fresh healthy, (C) naturally infected, and (D) artificially inoculated peppers. (See Table 1 for peaks identification).

olds. In the present study, minor constituents such as 1*H*-indole, 4-methylindole and 1-ethylindole were present in small quantities in both the naturally infected and the artificially inoculated peppers. We suggested that these compounds might be the key factors responsible for the anthracnose caused by *Colletotrichum* spp. in peppers and that they could contribute to the flavor of the pepper samples [8].

Our present experiment was performed at an injector temperature of 250 °C using solvent free solid injection. This might explain why volatile compounds with higher KI values were released compared to the results of the experiment by Luning et al. [14], who used a dynamic headspace method at room temperature to isolate volatile compounds from Dutch bell peppers. Buttery et al. [33] identified about 60 volatile compounds in green bell peppers (*C. annuum* var. *grossum*, Sendt), one of which was an alkyl-methoxy-pyrazine. This pyrazine and other alkyl-methoxypyrazines are the character impact compounds of the genus *Capsicum* [34]. Moreover, 102 volatile compounds were identified in Yucatan Habanero chile pepper (*Capsicum chinense* Jack. cv. Habanero) at two ripening stages (green and orange) using simultaneous steam distillation-solvent extraction apparatuses coupled with GC and GC/MS. The major compounds identified in Habanero chile pepper were (*E*)-2-hexenal, hexyl-3-methylbutanoate, (*Z*)-3-hexenyl 3-methylbutanoate, hexyl pentanoate, 3,3-dimethylcyclohexanol, and hexadecanoic acid [15]. Keller et al. [35] reported that volatiles of fresh red Jalapeňo pepper extracts had a pleasant floral aroma derived from 3-carene.

Table 2
Relative content of functional groups in identified volatiles emitted from healthy and diseased peppers

Functional group	Blank		Fresh healthy pepper		Naturally infected pepper		Artificially inoculated pepper	
	No.	Relative area (%)	No.	Relative area (%)	No.	Relative area (%)	No.	Relative area (%)
Acid	2	2.14	2	25.56	3	21.91	8	36.36
Alcohol	2	0.77	4	10.41	11	18.33	13	14.83
Aldehyde	1	0.74	3	4.28	4	5.97	1	0.38
Amide	–	–	1	0.63	2	3.44	3	4.86
Amine	–	–	–	–	3	3.36	3	1.27
Benzene	–	–	1	0.61	1	0.4	–	–
Ester	2	94.71	3	18.02	3	6.32	6	11.78
Hydrocarbon	1	1.64	2	2.32	5	2.97	9	4.83
Ketone	–	–	6	12.64	8	11.04	9	7.36
Phenol	–	–	–	–	–	–	2	0.76
Pyrazine	–	–	–	–	7	13.13	8	9.13
Pyridine	–	–	1	26.15	1	7.02	2	1.86
Pyrrole	–	–	–	–	4	4.61	2	3.19
Miscellaneous	–	–	–	–	3	2.24	2	3.38

No., number of recorded compounds in each functional group.

4. Conclusion

The indole-related compounds present in symptomatic and asymptomatic tissues after infection may be partially produced by the fungal species *Colletotrichum.* SFSI is a solvent-free, rapid, and simple sample preparation technique based on direct vaporization. It may contribute to the early diagnosis of the disease based on the presence of indole-related compounds. There is no dilution or contamination with solvent or its impurities and no loss of quickly eluted components in the solvent peak. The only contaminants or artifacts observed over some time have been squalene, from the hands of manipulator, and isopropyl myristate [36], from hand creams. Squalene peaks, if they interfere, can be avoided by handling the glass capillaries with rubber gloves.

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