

Identification and Antibacterial Activity of Volatile Flavor Components of *Cordyceps militaris*

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Abstract

Flavor characteristics of raw *Cordyceps militaris* significantly different from those of dried one. In the case of raw *Cordyceps militaris*, major flavor components were composed of 5 alcohols, 3 ketones, 4 phenols, 9 alkanes, and 3 alkenes. The major alcohol was 1-octen-3-ol (22.56%, 1147.3 ng/ml), which contributed to the characteristic green flavor. Ketones (3-ocatone, in particular) were present in the highest concentration in raw *Cordyceps militaris*. In contrast, major flavor components of dried *Cordyceps militaris* were composed of 4 alcohols, 4 ketones, 3 furans, 4 pyrazines, 2 dithiazines, 5 phenols, 8 alkenes, 17 alkanes, and 8 fatty acids. Dried *Cordyceps militaris* had unique sweet aroma of sesame as well as a milky flavor. Green or fruit flavor were rarely detected. In alkanes, 10 cosanes, component of wax were present. Typical flavor components of alkanes such as β -caryophyllen and Δ -cadinene were also detected. Fatty acids of dried *Cordyceps militaris* ranged from myristic acid (14:0) to linoleic acid (18:2). The sweet aroma of dried *Cordyceps militaris* was mostly due to pyrazines, dithiazines, and furans. Two dithiazines were identified and characteristics of these flavor components was a roasted bacon flavor. Strong antibacterial activity was observed toward *Vibrio* spp. such as *V. vulnificus*, *V. cholerae*, *V. parahaemolyticus*. Relatively high antibacterial activity was shown toward *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, and *Corynebacterium xerosis*.

Key words: *Cordyceps militaris*, antibacterial activity

INTRODUCTION

Cordyceps militaris is known to adhere to the surface of insect (lava, pupa, or adult) during the winter followed by penetration of its body to form a fruiting body and sporangium. These mushrooms have been used as medicine in China and Korea; 750 different species were reported worldwide and only 76 species were reported in Korea (1). These mushrooms were considered as one of the three most important medicines in China and believed to have positive effects on the kidney and immune system as well as in the detoxification of opium addicts. Furthermore, these mushrooms reportedly inhibited the growth of cancer cells from the esophagus, lung and prostate (2). These mushrooms are very difficult to collect because their sizes are very small and their growth were restricted to a specific area.

Recently artificial culturing process has been developed in laboratory scale. In the near future these mushrooms will be able to be produced on a large scale. In the meantime β -(1-3)-glucans separated from polysaccharides of these mushrooms have been extensively studied for their antibacterial and anti-cancer activities (3-7).

Our objectives are to elucidate changes in flavor profiles of *Cordyceps militaris* before and after drying. At the same time antibacterial activity of volatiles from dried *Cordyceps militaris* was evaluated for possible application to commercial tea products or health foods.

MATERIALS AND METHODS

Materials

Dried *Cordyceps militaris* was prepared by drying raw *Cordyceps militaris* at 40°C under vacuum for 18 hr just before the analysis of flavor components.

Collection of volatile flavor components and analysis

To extract and analyze volatile flavor components, internal standard, 4-decanol of 20 μ g/ml and sodium chloride solution was added to each homogenized sample. These mixtures were extracted by the method of Likens and Nickerson using SDE apparatus (8). Diethyl ether fraction was concentrated to 1 ml and analyzed by GC/MS (HP5890A series II GC with HP5989A mass selective detector). HP-5 crosslinked 5% ph silicon capillary column (30 m \times 0.32 mm id \times 1.05 μ m film thickness Hewlett Packard Co.) was used. Oven temperature was programmed from 60°C to 250°C at the rate of 4°C/min with initial and final hold time of 6 min and 5 min, respectively. Injector and interface temperature were kept at 250°C and helium was used as the carrier gas (1 ml/min). Split ratio was set to 1 : 15 and ionization voltage was 70 eV. Mass spectral data from each peak were identified by comparing with the MS library (Wiley/NBS data base) and percentage of the peak was calculated as a relative percentage. Each concentration of flavor components was based on the formulation of Choi et al. (9) and Kim et al. (10).

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Table 1. Used strains for antibacterial activity test

Strain	Origin	Strain	Origin
<i>Bacillus cereus</i>	ATCC 11778	<i>Shigella dysenteriae</i>	ATCC9752
<i>Bacillus subtilis</i>	ATCC 6633	<i>Salmonella typhi</i>	Isolates
<i>Citrobacter freundii</i>		<i>Staphylococcus aureus</i>	ATCC 25923
<i>Corynebacterium xerosis</i>	ATCC 9755	<i>Vibrio cholerae O-1</i>	Isolates
<i>Enterobacter cloacae</i>	ATCC13047	<i>Vibrio parahaemolyticus</i>	ATCC 27519
<i>Escherichia coli O157</i>	E32511 ¹⁾	<i>Vibrio vulnificus</i>	Isolates
<i>Listeria monocytogenes</i>	ATCC15313	<i>Yersinia enterocolitica</i>	Isolates
<i>Micrococcus luteus</i>			

¹⁾Provided from College of Veterinary Medicine, Gyeong-Sang National University

$$\text{Concentration } (\mu\text{g/g}) = \frac{(\text{peak area of each flavor component}) (\text{i.s. weight}/\mu\text{g})}{(\text{i.s. peak area}) (\text{weight material/g})}$$

Screening for antibacterial activity

Six species of Gram positive bacteria and nine species of Gram negative bacteria obtained from the Pusan Institute of Health & Environment and standard bacteria from ATCC (American Type Culture Collection) were used for screening of antibacterial activity (Table 1). The medium for antibacterial activity test was TSB (Tryptic soy broth, Difco.). For *Vibrio spp.*, NaCl was added to the medium to make a final concentration of 3%. Bacterial suspension for the test was split in media and then cultured at 37°C. After 18hr, the media was adjusted to 10⁶ CFU/ml of each bacterial suspension with TSB media. 1,000 ppm of testing materials were added in 10⁶ CFU/ml of bacterial suspension and then cultured for 36 hr at 35°C. Turbidity was determined at 650 nm by using automatic bacterial growth analyzer (Labsystem, Bioscreen Co., USA) at intervals of 30 min. Diethyl ether was used as control.

RESULTS AND DISCUSSION

Flavor characteristics of raw *Cordyceps militaris* significantly differed from those of dried one. Dried *Cordyceps militaris* exhibited a strong sweet aroma, which is highly acceptable to consumers of health foods. We noted a possible application of *Cordyceps militaris* in health foods; therefore, flavor profiles of raw and dried *Cordyceps militaris* were compared by GC/MS (Fig. 1, 2).

Flavor components of raw *Cordyceps militaris*

In the case of raw *Cordyceps militaris*, major flavor components were composed of 5 alcohols, 3 ketones, 4 phenols, 9 alkanes, and 3 alkenes (Table 2). The formation of alcohol flavor components such as octanol and hexanol resulted from carbohydrates and fatty acids (for instance, glucose and linoleic acid, respectively) by way of hydroxy cleavage of unsaturated fatty acids and oxidation of those by lipoxygenase (11-13). Major alcohol was 1-octen-3-ol (22.56%, 1147.3 ng/ml), which contributed to the characteristic green flavor according to other researchers (9,10,14,15).

Ketones were present in highest concentrations in raw *Cor-*

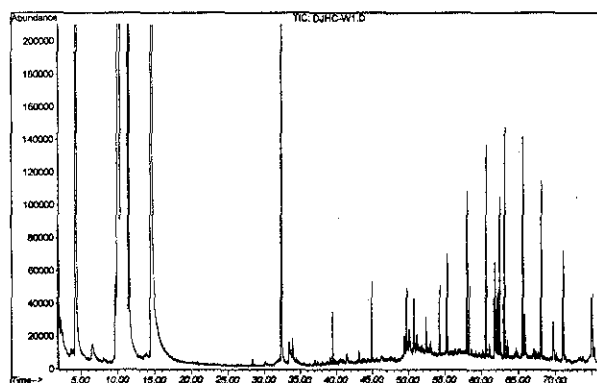


Fig. 1. GC/MS chromatogram of flavor components from raw *Cordyceps militaris*.

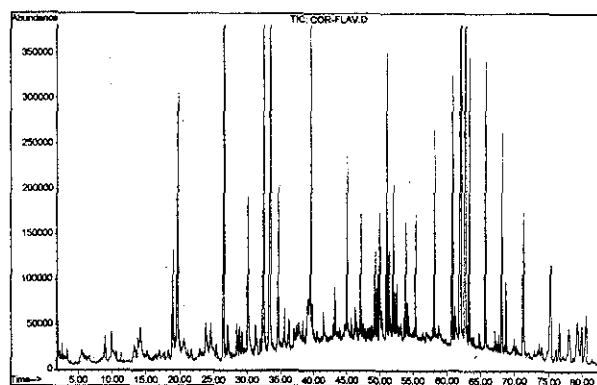


Fig. 2. GC/MS chromatogram of flavor components from dried *Cordyceps militaris*.

dyceps militaris. In particular, 3-ocatone, major ketone in French Lavenda oil consisted of 50.11% of flavor components. Its fruit flavor, in part, contributes to the composite flavor of Lavenda or Fougne class (16).

Flavor components of dried *Cordyceps militaris*

In contrast, the major flavor components of dried *Cordyceps militaris* were composed of 4 alcohols, 4 ketones, 3 furans, 4 pyridines, 2 dithiazines, 5 phenols, 8 alkenes, 17 alkanes, and 8 fatty acids. Dried *Cordyceps militaris* had a unique sweet aroma of sesame as well as a milky flavor. The green or fruit flavor was rarely detected in dried *Cordyceps militaris* which

Table 2. Concentration of volatile compounds in *Cordyceps militaris*

Compounds	Dry		Compounds	Dry	
	Before Concentration (ng/ml)	After		Before Concentration (ng/ml)	After
Alcohols			Triacotane		8.5
2-Methyl 3-penten-1-ol	3.6		Octacosane	18.6	104.6
Sec-butyl carbinol		7.1	Nonacosane	10.2	99.7
2-Furan carbinol		2.4	Hexatriacosane		54.6
1-Hexen-3-ol	0.2		Alkenes		
1-Octen-3-ol (Vinylamyl cabinol)	1147.3		5-Methyl-2-hexene		4.5
2-Nonen-1-ol	1.4		2,5-Dimethyl-2-hexene		12.0
3-Octanol	37.2		Cycloocten	256.7	
2-Butyl-2-octanol		26.4	β -Caryophyllene		9.0
d-Nerolidol		91.4	t-Ocinene	1.2	
Ketons			4-Methyl-2,4,-diphenyl-pent- 1-ene		11.4
5-Methyl 2-cyclohexen-1-one		9.1	Δ -Cadinene		6.1
Cycloheptanone	807.0		2,4-Diphenyl-2(E)-pentene		35.8
1-Octen-3-one	25.3		1,4 α - β -Dimethyl-7-isopropyl -4-octahydrophenanthrene		6.8
3-Octanone	2548.6		2-Bis(2-methyl benzocyclo butenylidene	7.2	
2-Undecanone		31.8	1,6-Diphenyl-1,5,-hexadiene		20.6
2,6-Di(t-butyl)-4-hydroxy-4- methyl-5-cyclo-hexadiene-1-one		21.3	Fatty acids		
Cis-9,10-epoxy heptadec-b-one		9.2	Ethyl myristate		11.7
Phenols			Methyl palmitate	8.6	52.3
Bis(1,1dimethylethyl)phenol	2.3		Ethyl palmitate	2.9	38.3
2-Methyl-4-(1,1,3,3,tetramethyl) phenol		10.2	Methyl linolelitate	16.3	76.6
2-t-Buthyl-4-(dimethyl benzyl) phenol		102.5	Methyl oleate	6.6	21.4
2,6-Bis(t-buthyl)-4-(dimethyl benzyl) phenol	10.5		Methyl stearate		10.0
2,4,6-Tri(dimethyl benzyl)phenol		30.7	Ethyl linoleate	6.2	50.4
2,4-Bis(dimethyl benzyl)phenol	31.8	361.4	Methyl 6-octadecanoate		14.9
2,4-Bis(dimethyl benzyl) -b-t-butyl phenol	21.3	299.8	Pyrazines		
Alkans			2-Ethyl-3,5-dimethylpyrazine		14.8
Tetradecane	2.1	12.1	2-Butyl-3-methylpyrazine		5.8
Pentadecane		7.0	2,5-Dimethyl-3-n-butyl pyrazine		9.1
Hexadecane	4.8	13.5	3-Isoamyl-2,5,-dimethyl pyrazine		39.9
Heptadecane	11.8	18.1	Dithiazines		
Octadecane	19.5	52.3	5,6-Dihydro-2,4,6,-trimethyl- 4H-1,3,5-dithiazine		16.2
2-Phenyltridecane		22.5	5,6-Dihydro-4-butyl-2,6- dimethyl-4H-1,3,5-dithiazine		67.6
7,9-Dimethyl hexadecane		11.2	Furans		
Nonadecane	24.1		2,3-Dihydroxy-4- (1-methylpropyl) furan		12.8
Docosane	27.8		2-Amylfuran		9.9
Tricosane	2.6	93.2	3-t-Butyl-2,3-dihydrofuran		3.1
Tetracosan	28.2	146.2			
Pentacosan		159.3			
9-Octyl heptadecane		7.2			
Hexacosane	20.2	156.6			
Heptacosane	14.5	136.2			

was confirmed by the absence of 1-octen-3-ol and 3-octanone. These green or fruit flavor components presumably evaporated during drying.

In the case of phenols in dried *Cordyceps militaris*, 2,4-bis (dimethyl benzyl) phenol was detected in highest concentrations followed by 1,4-bis (dimethyl benzyl)-6-butylphenol in dried *Cordyceps militaris*. These components were frequently detected in other flavor researches; however, in-depth study

was rarely made. Therefore, further study on these components should be carried out.

In alkanes, 10 cosanes and component of wax were present. Typical flavor components of alkanes such as β -caryophyllen and Δ -cadinene were also detected. These sesquiterpene compounds were synthesized by forming a ring structure from 3 isoprene units of farnesyl pyrophosphate upon enzymatic reaction (17). Caryophyllen is a major component of clove oil; in

Table 3. Inhibitory effect of the extract of dried *Cordyceps militaris* against pathogenic bacteria by incubation time

Incubation time (hour)		Absorbance at 650 nm														
		a ¹⁾	b	c	d	e	f	g	h	i	j	k	l	m	n	o
0	Control	0.758	0.760	0.770	0.760	0.768	0.779	0.752	0.761	0.303	0.303	0.309	0.732	0.772	0.771	0.771
	Sample	0.727	0.677	0.667	0.663	0.679	0.680	0.673	0.745	0.306	0.335	0.305	0.673	0.681	0.683	0.662
2	Control	0.814	0.784	0.812	0.815	0.810	0.823	0.793	0.812	0.335	0.345	0.313	0.769	0.829	0.796	0.824
	Sample	0.747	0.687	0.679	0.684	0.703	0.690	0.682	0.750	0.294	0.321	0.298	0.677	0.698	0.694	0.668
4	Control	0.959	0.905	0.979	1.093	0.989	1.132	0.873	0.973	0.433	0.488	0.316	0.942	0.896	0.811	1.055
	Sample	0.774	0.727	0.702	0.810	0.719	0.686	0.700	0.770	0.287	0.314	0.294	0.744	0.728	0.704	0.729
6	Control	1.196	1.192	1.264	1.379	1.449	1.715	1.091	1.570	0.846	0.654	0.471	1.358	0.997	0.838	1.582
	Sample	0.803	0.873	0.767	1.159	0.735	0.701	0.781	0.984	0.286	0.313	0.294	1.089	0.757	0.699	1.120
8	Control	1.359	1.378	1.444	1.590	1.731	1.884	1.301	1.866	0.940	0.712	0.880	1.796	1.161	0.897	1.666
	Sample	0.865	1.056	0.974	1.293	0.693	0.726	0.879	1.547	0.284	0.311	0.294	1.415	0.813	0.701	1.319
10	Control	1.420	1.427	1.503	1.692	1.835	1.958	1.450	1.964	1.024	0.750	0.951	2.261	1.356	0.980	1.775
	Sample	1.023	1.200	1.163	1.402	0.725	0.772	1.055	1.827	0.281	0.307	0.293	1.849	0.889	0.705	1.354
12	Control	1.473	1.561	1.573	1.767	1.893	1.981	1.531	2.038	1.035	0.775	1.111	2.390	1.526	1.072	1.876
	Sample	1.094	1.305	1.197	1.540	0.751	0.834	1.231	1.926	0.277	0.303	0.292	2.174	0.980	0.709	1.461
14	Control	1.453	1.536	1.594	1.804	1.927	1.983	1.565	2.108	1.044	0.817	1.286	2.496	1.704	1.217	1.968
	Sample	1.193	1.391	1.300	1.711	0.848	0.772	1.337	2.019	0.307	0.337	0.312	2.264	1.130	0.764	1.541
16	Control	1.456	1.551	1.571	1.804	1.948	1.963	1.586	2.120	0.982	0.749	1.314	2.455	1.685	1.230	1.926
	Sample	1.142	1.324	1.288	1.678	0.795	0.882	1.366	2.048	0.282	0.304	0.293	2.179	1.147	0.709	1.505
18	Control	1.479	1.648	1.593	1.855	1.983	1.957	1.598	2.177	1.014	0.748	1.417	2.440	1.771	1.428	2.018
	Sample	1.195	1.305	1.298	1.734	0.823	0.979	1.340	2.055	0.244	0.268	0.262	2.211	1.235	0.780	1.594
20	Control	1.585	1.753	1.592	1.900	2.044	1.987	1.699	2.240	1.061	0.771	1.504	2.502	1.826	1.697	2.097
	Sample	1.300	1.410	1.287	1.788	1.125	0.986	1.423	2.180	0.308	0.329	0.320	2.247	1.312	0.905	1.653
22	Control	1.606	1.795	1.572	1.906	2.004	1.966	1.674	2.240	1.076	0.721	1.610	2.497	1.845	1.823	2.195
	Sample	1.337	1.460	1.253	1.841	1.126	1.114	1.448	2.149	0.248	0.276	0.279	2.278	1.358	0.945	1.808
24	Control	1.632	1.862	1.592	1.966	2.016	1.989	1.715	2.303	1.107	0.730	1.732	2.511	1.870	1.967	2.311
	Sample	1.417	1.615	1.270	1.892	1.224	1.127	1.520	2.233	0.283	0.304	0.301	2.354	1.444	1.046	1.926
26	Control	1.714	1.938	1.605	1.997	2.028	2.018	1.753	2.336	1.116	0.756	1.817	2.539	1.884	2.069	2.366
	Sample	1.460	1.782	1.230	1.896	1.137	1.051	1.590	2.269	0.276	0.296	0.296	2.347	1.468	1.076	2.005
28	Control	1.727	1.943	1.597	2.016	2.021	2.008	1.742	2.352	1.106	0.759	1.859	2.522	1.877	2.147	2.451
	Sample	1.502	1.903	1.192	1.911	1.122	1.014	1.597	2.288	0.275	0.292	0.293	2.369	1.476	1.113	2.040
30	Control	1.739	1.944	2.019	2.025	2.101	1.999	1.734	2.372	1.092	0.809	1.877	2.578	1.889	2.174	2.436
	Sample	1.529	1.942	1.152	1.919	1.227	1.027	1.617	2.299	0.276	0.292	0.295	2.377	1.480	1.138	2.097
32	Control	1.786	2.019	1.914	2.038	2.142	2.024	1.760	2.400	1.120	0.917	1.910	2.563	1.881	2.213	2.519
	Sample	1.564	1.985	1.091	1.928	1.327	1.014	1.614	2.315	0.275	0.291	0.292	2.387	1.489	1.201	2.118
34	Control	1.817	2.077	2.036	2.049	2.143	2.038	1.751	2.412	1.169	1.017	1.900	2.525	1.899	2.315	2.494
	Sample	1.584	2.017	1.102	1.922	1.253	0.984	1.609	2.345	0.275	0.289	0.291	2.380	1.498	1.306	2.119
36	Control	1.883	2.146	2.142	2.057	2.128	2.049	1.764	2.471	1.214	1.142	1.879	2.517	1.905	2.307	2.549
	Sample	1.594	2.085	1.325	1.936	1.262	1.234	1.571	2.365	0.275	0.288	0.295	2.358	1.508	1.402	2.125

¹⁾a, *Shigella dysenteriae*; b, *Yersinia enterocolitica*; c, *Bacillus subtilis*; d, *Salmonella typhi*; e, *Bacillus cereus*; f, *Staphylococcus aureus*; g, *Listeria monocytogenes*; h, *E. coli* O157; i, *Vibrio vulnificus*; j, *Vibrio cholerae*; k, *Vibrio parahaemolyticus*; l, *Enterobacter cloacae*; m, *Micrococcus luteus*; n, *Corynebacterium xerosis*; o, *Citrobacter freundii*

addition, it showed activity of repellent and antifeedent as well as relation to allelopathy (18,19).

Fatty acids of dried *Cordyceps militaris* ranged from myristic acid (14 : 0) to linoleic acid (18 : 2). All the fatty acids were present in the form of either a methyl or ethyl ester, indicating that they were thermal artifact compounds formed during the drying of raw *Cordyceps militaris* or in the distillation and concentration of the volatile flavor components (9,15).

The sweet aroma of dried *Cordyceps militaris* was mostly due to pyrazines, dithiazines, and furans. In spite of very

low concentrations of these components (3.1~67.6 ng/g), the thresholds of these flavor components were also very low. Pyrazines, major flavor components of heat treated foods, were reportedly formed by the Maillard reaction or pyrolysis during heating (20). Typical sensory expressions of pyrazines were the roasted and sweet aromas.

Two dithiazines were identified, both in the form of methyl and hydroxy group was bound to a dithiazine ring. The characteristics of these flavor components were a roasted bacon flavor. *Cordyceps militaris* also appeared to have the same flavor, in-

dicating dithazines contributed to the flavor of dried *Cordyceps militaris*.

Furans represents a sweet flavor and is formed by autooxidation of unsaturated fatty acids in fats and oils. Grosch (21) claimed that among volatiles formed by autooxidation of fatty acids, R' structure of alkoxy radical varied depending upon the location of break and the furan was formed only when two double bonds were present in R'. Sulfur-containing components such as furan do not play an important role in terms of flavor intensity; however, they can act synergistically with other components.

Antibacterial activity of volatiles from *Cordyceps militaris*

Antibacterial effect of volatile from *Cordyceps militaris* was varied depending upon the strain of bacteria tested (Table 3). Strong antibacterial activity was observed toward *Vibrio* spp. such as *V. vulnificus*, *V. cholerae*, *V. parahaemolyticus*; conversely, no or insignificant antibacterial activity was exhibited toward most Gram negative bacteria such as *Shigella dysenteriae*, *Yersinia enterocolitica*, *Salmonella typhi*, *Escherichia coli* O157:H7, *Enterobacter cloacae*, and *Citrobacter freundii*. Relatively high antibacterial activity was shown toward *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, and *Corynebacterium xerosis*.

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