

# **Grinding and Fractionation During Distillation Alter Hemp Essential Oil Profile and its Antimicrobial Activity**

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Supplemental Table S1. Industrial Hemp essential oil composition of control oil.

RT	RI	Name	Rep 1	Rep 2
			Control 0-180 min	Control 0-180 min
		EO content, %	0.5	0.43
8.49	899	heptanal	0.136	0.144
9.14	918	2-methyl-4-heptanone	0.152	0.161
9.38	924	$\alpha$ -thujene	0.320	0.338
9.65	932	$\alpha$ -pinene	5.728	6.053
10.11	946	camphene	0.217	0.229
10.92	969	sabinene	0.154	0.163
11.03	974	$\beta$ -pinene	3.075	3.250
11.52	988	myrcene	7.402	6.822
12.00	1002	$\alpha$ -phellandrene	0.078	0.082
12.10	1007	$\delta$ -3-carene	4.346	4.593
12.35	1014	$\alpha$ -terpinene	0.319	0.337
12.58	1020	<i>p</i> -cymene	0.144	0.152
12.78	1023	limonene	2.315	2.446
12.84	1026	eucalyptol	1.337	1.413
13.02	1032	$\beta$ -(Z)-ocimene	1.743	1.842
13.41	1044	$\beta$ -(E)-ocimene	8.345	8.819
13.72	1055	$\gamma$ -terpinene	0.456	0.482
14.07	1066	(Z)-sabinene hydrate	0.361	0.382
14.59	1087	terpinolene	4.428	4.179
14.71	1090	<i>p</i> -cymenene	0.132	0.139
15.02	1098	(E)-sabinene hydrate	0.674	0.712
15.59	1114	endo-fenchol	0.137	0.145
15.78	1117	exo-fenchol	0.125	0.132
15.91	1128	allo-ocimene	0.246	0.260
16.27	1135	(E)-pinocarveol	0.262	0.277
16.33	1140	(Z)-pinene hydrate	0.141	0.149
16.89	1159	pinocarvone	0.153	0.162
17.20	1166	borneol	0.159	0.168
17.41	1171	(Z)-pinocamphone	0.164	0.173
17.52	1175	m-Cymen-8-ol	0.195	0.206
17.62	1180	<i>p</i> -cymen-8-ol	0.108	0.114
17.81	1188	$\alpha$ -terpineol	0.363	0.384
18.24	1205	verbenone	0.206	0.218
18.69	1290	(E)-verbetyl acetate	0.071	0.075
22.77	1372	$\alpha$ -ylangene	0.065	0.069
22.94	1376	$\alpha$ -copaene	0.049	0.052
23.15	1388	$\beta$ -bourbonene	0.087	0.092
23.26	1393	7-epi-sesquithujene	0.102	0.108
23.71	1407	isocaryophyllene ( $\gamma$ -caryophyllene)	0.400	0.423
24.01	1411	$\alpha$ -(Z)-bergamotene	0.319	0.337
24.19	1418	$\beta$ -caryophyllene	9.850	8.409
24.32	1430	$\gamma$ -elemene	0.323	0.341
24.41	1432	$\alpha$ -(E)-bergamotene	2.216	2.342
24.60	1435	$\beta$ -gurjunene	0.160	0.169
24.89	1441	(Z)- $\beta$ -farnesene	2.335	2.468
25.05	1452	$\alpha$ -humulene ( $\alpha$ -caryophyllene)	4.698	4.165
25.13	1457	L-alloaromadendrene	0.393	0.415
25.47	1478	$\gamma$ -muurolene	0.155	0.164
25.52	1481	$\gamma$ -himachalene	0.162	0.171
25.60	1485	germacrene D	0.087	0.092

25.70	1489	$\beta$ -selinene	0.580	0.613
25.84	1492	$\delta$ -selinene	1.309	1.383
25.90	1497	$\alpha$ -selinene	0.324	0.342
26.01	1501	$\beta$ -himachalene	0.945	0.999
26.16	1513	$\gamma$ -cadinene	0.522	0.552
26.25	1522	$\delta$ -cadinene	0.641	0.677
26.44	1536	$\alpha$ -cadinene	0.336	0.355
26.57	1544	selina-3,7(11)-diene	0.377	0.398
26.68	1548	elemol	0.468	0.495
26.89	1566	maaliol	0.913	0.965
27.02	1570	caryophyllenyl alcohol	2.074	2.192
27.11	1572	germacrene D-4-ol	1.589	1.679
27.55	1576	spathulenol	2.312	2.443
27.68	1579	himachalene epoxide	0.115	0.122
28.12	1584	caryophyllene oxide	3.691	3.901
28.39	1590	globulol	0.313	0.331
28.45	1601	ledol	0.186	0.197
28.72	1608	humulene epoxide 2	1.557	1.645
28.83	1615	$\beta$ -himachalene oxide	0.395	0.417
29.01	1626	selina-6-en-4-ol	1.092	1.154
29.25	1637	caryophylla-4(12),8(13)-dien-5 $\alpha$ -ol	0.574	0.607
29.33	1639	caryophylla-4(12),8(13)-dien-5 $\beta$ -ol	0.728	0.769
29.53	1663	14-hydroxy-(Z)-caryophyllene	0.319	0.337
29.74	1668	14-hydroxy-9-epi-(E)-caryophyllene	0.901	0.952
29.95	1671	bulnesol	0.423	0.447
30.07	1675	$\beta$ -bisabolol	1.047	1.106
30.40	1686	$\alpha$ -bisabolol	1.290	1.363
30.69	1694	eudesm-7(11)-en-4-ol	0.458	0.484
33.68	1822	(E)-nerolidyl isobutyrate	0.129	0.136
38.83	1943	phytol	0.136	0.144
40.64	2095	methyl linoleate	0.182	0.192
41.82	2132	linoleic acid	0.054	0.057
42.34	2283	$\delta$ 9-tetrahydrocannabivarin	0.105	0.111
43.30	2306	cannabicyclol	0.091	0.096
44.11	2381	CBD	5.923	5.426
44.28	2399	cannabichromene	0.327	0.346
45.05	2462	$\delta$ 8-tetrahydrocannabinol	0.126	0.133
45.65	2484	$\delta$ 9-tetrahydrocannabinol (dronabinol)	0.138	0.146

Monoterpenes	43.904	44.897264
Sesquiterpenes	47.009	46.878594
Cannabinoids	6.71	6.2576291
Others(acid esters, ketone, alcohols)	0.66	0.697

## Supplemental Table S2. Essential oil constituents from nonground hemp material cv. Novosadská.



28.12	1584	caryophyllene oxide	3.691	3.901	1.101	1.053	3.735	3.254	5.133	5.591	6.576	6.146	7.447	7.870	6.985	7.468	6.606
28.39	1590	globulol	0.313	0.331	nd												
28.45	1601	ledol	0.186	0.197	nd	nd	nd	nd	0.158	0.151	0.248	0.262	0.242	0.256	nd	nd	nd
28.72	1608	humulene epoxide 2	1.557	1.645	0.259	0.248	0.919	0.971	1.535	1.469	2.078	2.407	2.544	2.169	2.432	2.327	2.422
28.83	1615	$\beta$ -himachalene oxide	0.395	0.417	nd	nd	nd	nd	0.298	0.285	0.486	0.514	0.516	0.545	nd	nd	nd
29.01	1626	selina-6-en-4-ol	1.092	1.154	nd	nd	0.307	0.324	0.687	0.657	1.062	1.122	1.377	1.455	1.444	1.382	nd
29.25	1637	caryophylla-4(12),8(13)-dien-5 $\alpha$ -ol	0.574	0.607	nd	nd	nd	nd	0.481	0.460	0.659	0.696	0.967	1.022	0.800	0.765	nd
29.33	1639	caryophylla-4(12),8(13)-dien-5 $\beta$ -ol	0.728	0.769	nd	nd	0.298	0.315	0.381	0.365	1.244	1.315	0.827	0.874	0.862	0.825	nd
29.53	1663	14-hydroxy-(Z)-caryophyllene	0.319	0.337	nd	nd	nd	nd	0.202	0.193	0.374	0.395	0.753	0.796	0.900	0.861	nd
29.74	1668	14-hydroxy-9-epi-(E)-caryophyllene	0.901	0.952	nd	nd	0.150	0.159	0.436	0.417	0.782	0.826	0.926	0.979	0.936	0.896	nd
29.95	1671	bulnesol	0.423	0.447	nd	nd	nd	nd	nd	nd	0.195	0.206	0.228	0.241	nd	nd	nd
30.07	1675	$\beta$ -bisabolol	1.047	1.106	nd	nd	0.215	0.227	0.475	0.454	1.014	1.072	1.365	1.443	1.527	1.461	2.116
30.40	1686	$\alpha$ -bisabolol	1.290	1.363	nd	nd	0.318	0.336	0.863	0.826	1.751	1.585	2.308	2.044	2.603	2.491	2.502
30.69	1694	eudesm-7(11)-en-4-ol	0.458	0.484	nd	nd	nd	nd	0.164	0.157	0.364	0.385	0.460	0.486	0.539	0.516	nd
33.68	1822	(E)-nerolidyl isobutyrate	0.129	0.136	nd	0.133	0.141	nd									
38.83	1943	phytol	0.136	0.144	nd	0.115	0.108	nd									
40.64	2095	methyl linoleate	0.182	0.192	nd	0.165	0.152	nd									
41.82	2132	linoleic acid	0.054	0.057	nd	0.043	0.047	nd									
42.34	2283	$\delta$ 9-tetrahydrocannabivarin	0.105	0.111	nd	0.089	0.093	nd									
43.30	2306	cannabicyclol	0.091	0.096	nd	0.077	0.072	nd									
44.11	2381	CBD	5.923	5.426	nd	nd	nd	nd	0.345	0.318	1.675	1.844	3.351	3.541	5.137	5.271	4.696
44.28	2399	Cannabichromene	0.327	0.346	nd	0.312	0.305	nd									
45.05	2462	$\delta$ 8-tetrahydrocannabinol	0.126	0.133	nd	0.116	0.122	nd									
45.65	2484	$\delta$ 9-tetrahydrocannabinol (Dronabinol)	0.138	0.146	nd	0.102	0.111	nd									

Monoterpenes	43.904	44.89726	76.933	76.6027	68.157	67.63604	60.4888	59.69709	45.844	45.14743	29.991	31.01546	20.454	22.19476	13.658
Sesquiterpenes	47.009	46.87859	21.079	21.50766	29.92	30.31141	37.45	38.34458	50.8802	51.50048	65.563	63.96761	72.141	70.34695	80.1942
Cannabinoids	6.71	6.257629	0	0	0	0	0.345	0.318	1.6745	1.844097	3.351	3.5413	5.83318	5.97392	4.696
Others(acid esters, ketone, alcohol)	0.66	0.697	0.574	0.549197	0.208	0.219812	0.167	0.159784	0.103	0.108849	0	0	0.323	0.307	0

Supplemental Table S3. Essential oil constituents from ground hemp material cv. Novosadska.

RT	RI	Name	840	841	842	843	844	845	846	847	848	849	850	851
			0-5min	0-5min	5-10min	5-10 min	10-20min	10-20min	20-80min	20-80min	80-120min	80-120 min	120-180min	120-180 min
8.49	899	heptanal	0.242	0.232	0.191	0.202	0.177	0.169	nd	nd	nd	nd	nd	nd
9.14	918	2-methyl-4-heptanone	0.336	0.321	0.304	0.321	0.278	0.266	0.225	0.238	0.126	0.121	0.104	0.109
9.38	924	$\alpha$ -thujene	0.466	0.446	0.410	0.433	0.418	0.400	0.390	0.412	0.242	0.232	0.286	0.299
9.65	932	$\alpha$ -pinene	15.850	15.317	17.806	16.817	16.695	16.974	12.818	13.546	11.189	11.457	14.830	14.495
10.11	946	camphene	0.405	0.387	0.378	0.399	0.352	0.337	0.276	0.292	0.153	0.146	0.177	0.185
10.92	969	sabinene	0.183	0.175	0.196	0.207	0.222	0.212	0.228	0.241	0.150	0.144	0.138	0.144
11.03	974	$\beta$ -pinene	7.728	7.394	7.835	8.280	7.078	6.772	5.511	5.824	3.721	3.956	4.166	4.354
11.52	988	myrcene	19.077	18.525	21.078	20.275	18.296	18.505	14.232	13.704	12.559	12.890	12.980	12.521
12.00	1002	$\alpha$ -phellandrene	0.246	0.235	nd	nd	0.063	0.060	0.173	0.183	0.106	0.101	0.090	0.094
12.10	1007	$\delta$ -3-carene	5.502	5.826	5.173	5.467	4.821	5.361	4.449	4.702	3.367	3.722	5.558	5.809
12.35	1014	$\alpha$ -terpinene	0.225	0.215	nd	nd	0.213	0.204	0.216	0.228	0.129	0.123	nd	nd
12.58	1020	p-cymene	0.284	0.272	0.293	0.310	0.220	0.210	0.121	0.128	0.098	0.094	0.103	0.108
12.78	1023	limonene	3.107	2.973	2.652	2.280	2.527	2.842	2.591	2.738	1.896	1.814	2.403	2.118
12.84	1026	eucalyptol	5.470	5.823	4.076	4.307	2.382	2.628	0.814	0.860	0.230	0.243	0.204	0.213
13.02	1032	$\beta$ -(Z)-ocimene	2.234	2.137	1.996	2.109	2.168	2.074	4.138	3.737	1.276	1.221	0.962	1.005
13.41	1044	$\beta$ -(E)-ocimene	15.639	16.319	17.004	17.603	15.775	16.105	13.451	12.921	12.329	12.580	9.982	10.433
13.72	1055	$\gamma$ -terpinene	0.398	0.381	0.270	0.285	0.321	0.307	0.382	0.404	0.232	0.222	0.244	0.255
14.07	1066	(Z)-sabinene hydrate	0.634	0.607	0.494	0.522	0.378	0.362	0.280	0.296	nd	nd	nd	nd
14.59	1087	terpinolene	5.125	4.904	4.533	4.790	4.610	4.411	4.449	3.702	3.687	3.853	2.843	2.536
14.71	1090	p-cymenene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15.02	1098	(E)-sabinene hydrate	0.755	0.722	0.625	0.660	0.576	0.551	0.481	0.508	nd	nd	nd	nd
15.59	1114	endo-fenchol	0.199	0.190	nd	nd	0.120	0.115	0.089	0.094	nd	nd	nd	nd
15.78	1117	exo-fenchol	nd	nd	nd	nd	0.112	0.107	0.092	0.097	nd	nd	nd	nd
15.91	1128	allo-ocimene	nd	nd	nd	nd	nd	nd	0.116	0.123	nd	nd	nd	nd
16.27	1135	(E)-pinocarveol	0.156	0.149	nd	nd	nd	nd	0.054	0.057	nd	nd	nd	nd
16.33	1140	(Z)-pinene hydrate	nd	nd	nd	nd	nd	nd	0.213	0.225	nd	nd	nd	nd
16.89	1159	pinocarvone	nd	nd	nd	nd	nd	nd	0.097	0.103	nd	nd	nd	nd
17.20	1166	borneol	nd	nd	nd	nd	0.145	0.139	0.155	0.164	nd	nd	nd	nd
17.41	1171	(Z)-pinocamphone	0.162	0.155	nd	nd	0.161	0.154	0.141	0.149	nd	nd	nd	nd

17.52	1175	<i>m</i> -cymen-8-ol	nd	nd	nd	nd	0.145	0.152	0.202	0.213	nd	nd	nd	nd
17.62	1180	<i>p</i> -cymen-8-ol	nd	nd	nd	nd	0.111	0.120	0.089	0.095	nd	nd	nd	nd
17.81	1188	$\alpha$ -terpineol	0.147	0.141	nd	nd	0.170	0.163	0.375	0.396	nd	nd	nd	nd
18.24	1205	verbenone	nd	nd	nd	nd	nd	nd	0.193	0.204	nd	nd	nd	nd
18.69	1290	(E)-verbenyl acetate	nd	nd	nd	nd	nd	nd	0.128	0.135	nd	nd	nd	nd
22.77	1372	$\alpha$ -ylangene	nd	nd	nd	nd	nd	nd	0.061	0.067	nd	nd	nd	nd
22.94	1376	$\alpha$ -copaene	nd	nd	nd	nd	nd	nd	0.055	0.059	nd	nd	nd	nd
23.15	1388	$\beta$ -bourbonene	nd	nd	nd	nd	nd	nd	0.086	0.090	nd	nd	nd	nd
23.26	1393	7-epi-sesquithujene	nd	nd	nd	nd	nd	nd	0.074	0.079	nd	nd	nd	nd
23.71	1407	isocaryophyllene ( $\gamma$ -caryophyllene)	nd	nd	nd	nd	0.082	0.078	0.120	0.127	0.191	0.183	0.169	0.177
24.01	1411	$\alpha$ -(Z)-bergamotene	nd	nd	nd	nd	nd	nd	0.071	0.075	0.117	0.112	nd	nd
24.19	1418	$\beta$ -caryophyllene	6.175	6.454	5.872	5.421	6.467	6.188	7.694	6.913	10.850	10.295	12.836	12.371
24.32	1430	$\gamma$ -elemene	nd	nd	nd	nd	0.142	0.136	0.201	0.212	0.285	0.273	0.178	0.186
24.41	1432	$\alpha$ -(E)-bergamotene	0.699	0.669	0.496	0.524	0.525	0.502	0.690	0.729	1.142	1.093	0.920	0.962
24.60	1435	$\beta$ -gurjunene	nd	nd	nd	nd	nd	nd	0.073	0.077	0.095	0.091	nd	nd
24.89	1441	(Z)- $\beta$ -farnesene	0.579	0.554	0.281	0.297	0.438	0.419	0.676	0.714	1.037	0.992	0.635	0.664
25.05	1452	$\alpha$ -humulene ( $\alpha$ -caryophyllene)	2.014	2.493	1.873	1.979	2.469	2.171	2.871	3.034	4.266	4.082	3.957	4.136
25.13	1457	<i>L</i> -alloaromadendrene	0.141	0.135	nd	nd	0.170	0.163	0.223	0.236	0.339	0.324	0.295	0.308
25.47	1478	$\gamma$ -muurolene	nd	nd	nd	nd	nd	nd	0.108	0.114	0.071	0.068	nd	nd
25.52	1481	$\gamma$ -himachalene	nd	0.066	0.063	nd	nd							
25.60	1485	germacrene D	nd	0.085	0.092	nd	nd							
25.70	1489	$\beta$ -selinene	0.088	0.084	nd	nd	0.136	0.130	0.217	0.229	0.296	0.290	0.189	0.198
25.84	1492	$\delta$ -selinene	0.304	0.291	0.260	0.275	0.402	0.385	0.572	0.604	0.844	0.808	0.616	0.644
25.90	1497	$\alpha$ -selinene	nd	nd	nd	nd	0.080	0.077	0.122	0.129	0.167	0.160	nd	nd
26.01	1501	$\beta$ -himachalene	0.246	0.235	nd	nd	0.312	0.299	0.443	0.468	0.655	0.627	0.453	0.473
26.16	1513	$\gamma$ -cadinene	nd	nd	nd	nd	0.083	0.079	0.155	0.164	0.201	0.192	nd	nd
26.25	1522	$\delta$ -cadinene	nd	nd	nd	nd	0.140	0.134	0.229	0.242	0.321	0.307	nd	nd
26.44	1536	$\alpha$ -cadinene	nd	nd	nd	nd	0.097	0.093	0.139	0.147	0.194	0.186	nd	nd
26.57	1544	selina-3,7(11)-diene	nd	nd	nd	nd	0.106	0.101	0.152	0.161	0.221	0.211	0.189	0.198
26.68	1548	elemol	nd	nd	nd	nd	0.114	0.109	0.201	0.212	0.242	0.232	0.166	0.173
26.89	1566	maaliol	0.202	0.193	nd	nd	0.236	0.226	0.370	0.391	0.498	0.476	0.265	0.277
27.02	1570	caryophyllenyl alcohol	0.566	0.542	0.535	0.565	0.651	0.623	1.015	1.073	1.522	1.456	0.924	0.966
27.11	1572	germacrene D-4-ol	0.573	0.548	0.607	0.641	0.697	0.667	0.915	0.967	1.379	1.319	0.899	0.940
27.55	1576	spathulenol	0.655	0.627	0.787	0.832	0.976	0.934	1.614	1.706	2.127	2.350	1.272	1.329
27.68	1579	himachalene epoxide	nd	0.135	0.129	0.140	0.142							
28.12	1584	caryophyllene oxide	1.248	1.494	nd	nd	2.881	2.757	4.068	4.383	5.529	5.829	5.281	5.052

28.39	1590	globulol	nd	nd	2.013	2.127	0.105	0.100	0.227	0.240	0.257	0.246	nd	nd
28.45	1601	ledol	nd	nd	nd	0.094	0.090	0.166	0.175	0.191	0.183	0.165	0.172	
28.72	1608	humulene epoxide 2	0.258	0.247	0.447	0.472	0.761	0.728	1.222	1.291	1.673	1.601	1.700	1.777
28.83	1615	$\beta$ -himachalene oxide	nd	nd	nd	nd	0.140	0.134	0.289	0.305	0.364	0.348	0.333	0.348
29.01	1626	selina-6-en-4-ol	nd	nd	nd	nd	0.413	0.395	0.780	0.824	1.093	1.046	0.897	0.937
29.25	1637	caryophylla-4(12),8(13)-dien-5 $\alpha$ -ol	nd	nd	nd	nd	0.214	0.205	0.404	0.427	0.602	0.576	0.543	0.568
29.33	1639	caryophylla-4(12),8(13)-dien-5 $\beta$ -ol	nd	nd	nd	nd	0.121	0.116	0.525	0.555	0.879	0.841	1.098	1.148
29.53	1663	14-hydroxy-(Z)-caryophyllene	nd	nd	nd	nd	0.147	0.141	0.216	0.228	0.267	0.255	0.232	0.242
29.74	1668	14-hydroxy-9-epi-(E)-caryophyllene	nd	nd	nd	nd	0.146	0.140	0.556	0.519	0.814	0.779	0.587	0.613
29.95	1671	bulnesol	nd	nd	nd	nd	0.066	0.063	0.255	0.269	0.336	0.321	0.213	0.223
30.07	1675	$\beta$ -bisabolol	nd	nd	nd	nd	0.094	0.090	0.528	0.558	0.845	0.808	1.043	1.090
30.40	1686	$\alpha$ -bisabolol	nd	nd	nd	nd	0.492	0.471	1.197	1.265	1.683	1.610	1.044	1.091
30.69	1694	eudesm-7(11)-en-4-ol	nd	nd	nd	nd	0.101	0.097	0.264	0.279	0.386	0.369	0.263	0.275
33.68	1822	(E)-nerolidyl isobutyrate	nd	0.108	0.116	nd	nd							
38.83	1943	phytol	nd	0.122	0.131	nd	nd							
40.64	2095	methyl linoleate	nd	0.111	0.106	nd	nd							
41.82	2132	linoleic acid	nd	0.049	0.056	nd	nd							
42.34	2283	$\delta$ 9-tetrahydrocannabivarin	nd	0.075	0.068	nd	nd							
43.30	2306	cannabicyclol	nd	0.070	0.061	nd	nd							
44.11	2381	CBD	nd	nd	nd	nd	nd	nd	1.692	1.788	3.495	3.148	5.859	6.124
44.28	2399	cannabichromene	nd	0.244	0.235	nd	nd							
45.05	2462	$\delta$ 8-Tetrahydrocannabinol	nd	0.088	0.790	nd	nd							
45.65	2484	$\delta$ 9-Tetrahydrocannabinol (Dronabinol)	nd	0.101	0.089	nd	nd							

Monoterpenes	83.992	83.2945062	84.819	84.746445	78.079	79.2652561	66.944	66.4813541	51.3643796	52.7977541	54.9647728	54.5686
Sesquiterpenes	13.7476	14.5659859	13.171	13.1340494	20.098	19.0381875	29.844	30.3394514	42.373	41.3394554	37.5027659	37.6799
Cannabinoids	0	0	0	0	0	0	1.692	1.78808699	4.073	4.39128675	5.85937584	6.124
Others(acid esters, ketone, alcohol)	0.578	0.55302404	0.495	0.52311056	0.455	0.435339	0.225	0.23777753	0.408	0.414	0.10429	0.109