Leffingwell Reports, Vol. 5(1), 1-21, July 2013

Aroma Constituents of a Supercritical CO_2 Extract of Kentucky Dark Fire-Cured Tobacco.

by

John C. Leffingwell[1], E. D. Alford[2] and Diane Leffingwell[1]

[1]Leffingwell & Associates, Canton, GA 30115-8162, USA
[2]Alford Consulting, Inc., Louisville, KY 40207-1644, USA

SUMMARY

This report provides the results of a capillary gas chromatography-mass spectrometry (GC-MS) study and identification of the volatiles from a commercial, partially denicotinized, supercritical CO_2 Extract of Kentucky Dark Fire-Cured Tobacco. The results show that along with the expected tobacco constituents, among the major aroma volatiles, many are phenolics generated by pyrolysis of lignin from the hardwood smoke used in the curing process.

INTRODUCTION

In 2012, dark fire-cured Tobacco was the third largest tobacco crop of the United States at about 53.3 million lb. (trailing flue-cured at 494.6 million Lb. and Burley at 202.3 million lb.) (1).

Historically, the USA has produced slightly less than half of the world's production of dark fire-cured. Other producing areas include the African countries of Mozambique, Tanzania, Uganda, Malawi, Congo, Zambia and Kenya as well as Indonesia and Italy (2). It is employed in primarily in moist snuff (commonly referred to as "snus'), fine cut shag for roll-your own cigarettes and, to a lesser extent, chewing tobacco. In the Netherlands, where about 22% of cigarette smokers hand roll, 'halfzware' (half fire-cured, half flue-cured), is the most popular type of tobacco for hand-rolled cigarettes followed by 'zware' (all fire-cured) tobacco (3,4).

In recent years, the potential use of fire-cured and other tobacco extracts for use in smokeless tobacco products (e.g. "snus") and in cigarette type products that generate "vapors" (e.g. E-cigarettes) has become of increasing importance. Such extracts have the advantage that they can be tailored to be produced with very low levels of TSNA's (tobacco specific nitrosamines) and polynuclear hydrocarbons such as benzo[a]pyrene (e.g. Ref 5) as well as nicotine (9).

Other than our preliminary presentation in 1998 (6), only qualitative reports of the volatiles of fire-cured tobacco have been reported (7,8) . This paper provides additional details based on a more comprehensive data analysis of our prior work. In addition, this work provides the first report of the approximate relative amounts of the Fire-cured phenolics primarily derived from the hardwood smoke. As many of these phenolics that are responsible for the characteristic smoky aroma are GRAS (Generally Regarded As Safe) flavorants, this work may also have importance in the flavor formulation of smoke aromas for non-tobacco products as well as tobacco flavorings.

EXPERIMENTAL

Material

Commercial samples of a partially denicotinized, supercritical CO_2 Extract of Kentucky dark fire-cured tobacco were obtained from The Muller Extract Co., Coburg, Germany. These had been prepared as generally described in US Patent 4.506,682 (9) and were utilized for the GC-MS analysis.

Capillary gas chromatography analysis

Sample 1:
GC and GC/MS: The GC-MS was a Hewlett-Packard 5890/5970 High Performance combination. A DB-5 60m X 0.32mm I. D. fused silica column coated with a 0.25 micron film was employed. The column was held isothermally at 60°C for 2 minutes, and programmed from 60°C to 260°C at 1.2°C/min, with a final hold time of 30 minutes. The injection port was held at 260°C. Helium Carrier Gas was used with a column flow rate of 2.9 ml/min. The Mass spectrometer was scanned in the EI mode from 26m/z to 350m/z using 70eV ionizing voltage. Percentages are TIC% without correction for response factors. The injection amount was 0.5 µl (neat).

Sample 2:
GC and GC/MS: The GC-MS was a Hewlett-Packard G1530A-6890/5975 High Performance combination. A HP-5 60m X 0.32mm I. D. fused silica column coated with a 0.25 micron film was employed. The column was held isothermally at 30°C for 2 minutes, and programmed from 30°C to 260°C at 2°C/min, with a final hold time of 28 minutes. The injection port was held at 260°C. Helium Carrier Gas was used with a column flow rate of 2.9 ml/min. The Mass spectrometer was scanned in the EI mode from 26m/z to 350m/z using 70eV ionizing voltage. The injection amount was 0.35 µl (neat).
This sample was utilized primarily for the purpose of confirming component mass spectra and for determining the FID percentages of the important phenolics.

Component Identifications: Identifications were based on mass spectra from the Wiley 6 and NIST 05 MS libraries as well as from the authors MS library. Where available, retention time comparisons were used employing primarily the Retention Indices compilations of the NIST Mass Spec Data Center (10), as well as those of Boelens (11) and Adams (12). In addition, Relative Retention Times (RRT) for a number of components were used for confirmation where RI values were not available. Initial analysis was done on the Agilent MS Enhanced Chemstation program D.03.00.611 employing both normal and selective ion modes. The NIST AMDIS deconvolution program (Ver. 2.65) was also employed in both normal and high resolution modes using the MSP file format from data imported from the Wiley and NIST MS libraries, as well as the authors libraries. For purposes of comparison, RI values for a 5% diphenyl-95% methylpolysiloxane stationary phase (DB-5, HP-5, HP5-MS, VF-5MS, Ultra-2, etc.) were considered valid (and were comparable to our prior experience using values obtained using DB-5, HP-5 and VF-5MS columns).

Retention Indices: Kovats RI Indices were derived using the classic (isothermal) formula of Kovats (13):

$$RIx = 100n + 100[\log(tx) - \log(tn)] / [\log(tn+1) - \log(tn)]$$

Additionally Linear Retention Indices (LRI), sometimes referred to as the Arithmetic Index (AI), based on n-alkanes using the methodology of Van den Dool and Kratz (14) were calculated using the formula:

$$LRIx = 100n + 100(tx-tn) / (tn+1 - tn)$$

where t_n and t_{n+1} are retention times of the reference n-alkane hydrocarbons eluting immediately before and after chemical compound "X"; t_x is the retention time of compound "X".

Lee retention indices were determined by analogy with the calculation of Kovats indices for the following reference compounds: benzene, naphthalene, phenanthrene and chrysene.

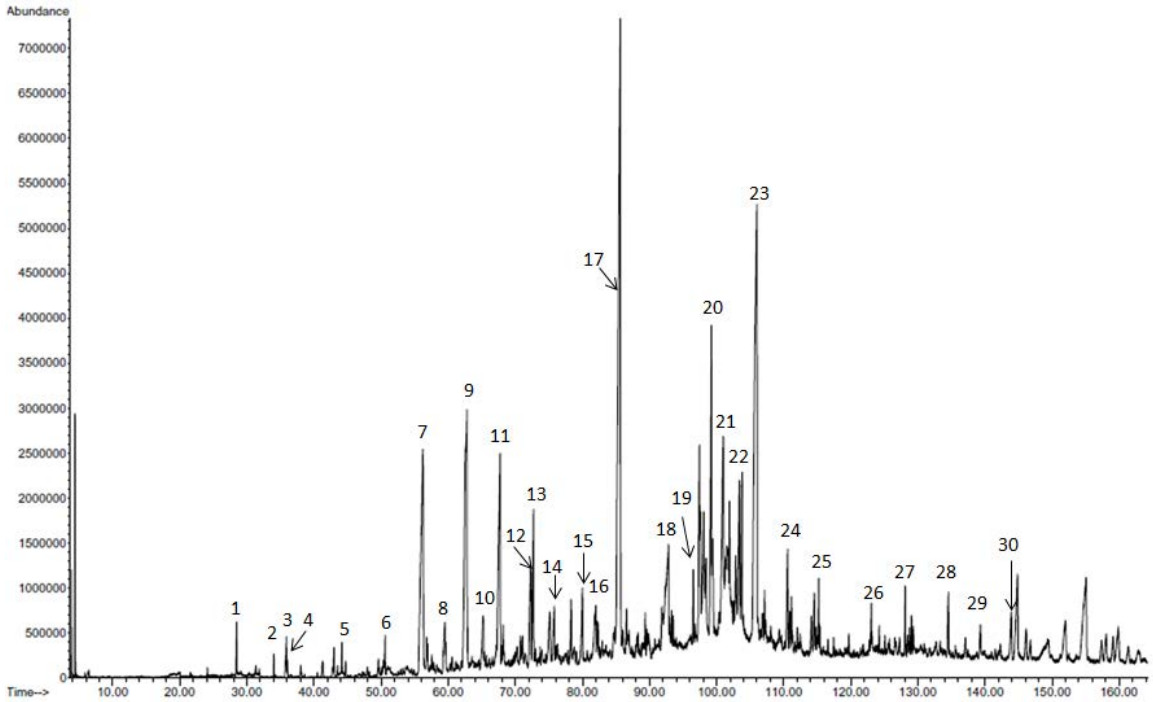
Both the calculated Kovats KI and the LRI retention indices are provided in our tables because some of the reported literature does not specify which RI calculations were actually employed. The NIST and Adams values used for comparison were both the van den Dool and Kratz temperature programmed LRI values and the Kovats (KI) values. The Boelens values in the ESO 2000 (Update 2006) database, that were derived from the literature, do not specify the method of RI calculation. In most cases the RI values by both methods are quite similar, but can vary by 1-9 units. The Lee retention index values were used as a further corroboration. As with all such reported retention index values, those below 500-700 KI/LRI must be considered as somewhat approximate as such values are subject to greater instrumental experimental fluctuations between laboratories.

RESULTS & DISCUSSION

The aroma of the CO_2 extract utilized had a distinct "smoky" aroma similar to Hickory smoked meats. This suggested that much of the aroma was due to the phenolic smoke constituents from the smoldering hardwoods used in the curing process. Bailey (15) has described the commercial fire curing process in detail.

The results of the GC-MS analysis confirmed the presence of many aromatic phenolic compounds not normally found in dark air-cured tobacco along with constituents previously identified in air cured tobaccos.

Figure 1 - The Kentucky Fire-Cured CO_2 Extract TIC Chromatogram (Sample 1)



(1) Phenol; (2) 2-Methylphenol; (3) 4-methylphenol + 3-Methylphenol; (4) Guaiacol; (5) 4-Methylguaiacol; (6) 4-Ethylguaiacol; (7) Syringol; (8) Vanillin; (9) 4-Methylsyringol; (10) Norsolanadione + Acetovanillone; (11) 4-Ethylsyringol; (12) 4-Allylsyringol; (13) 4-Propylsyringol; (14) Syringaldehyde; (15) Acetosyringone; (16) Syringic acid hydrazide; (17) Neophytadiene; (18) Palmitic acid; (19) Thunbergol; (20) Phytol; (21) A Duvatriendiol + Linoleic acid; (22) Duvatrienediols + Phytyl acetate; (23) α- and/or β-4,8,13-Duvatriene-1,3-diol; (24) Tricosane; (25) 1-Docosanol; (26) Heptacosane; (27) 10-Demethylsqualene; (28) Triacontane; (29) Hentriacontane; (30) Cholest-5-en-3β-ol

Among important tobacco derived compounds found were the diterpenoids (e.g. Neophytadiene, Phytol, Isophytol, Phytofuran), degraded carotenoids (e.g. E)-β-damascenone, E)-β-ionone, 4 isomers of Megastigma-4,6,8-trien-3-one, 3-Hydroxy-β-damascone, 3-Oxo-α-ionol, Dihydroactinidiolide), degraded acyclic isoprenoids (e.g. Farnesylacetone, 6,10,14-Trimethyl-2-pentadecanone), cembrenoids (e.g. Thunbergol, 4,8,13-Duvatriene-1,3-diol), degraded cembrenoids (e.g. Solanone, Norsolanadione) as well as fatty acids and esters (e.g. Dodecanoic acid, Myristic acid, Pentadecanoic acid, Palmitic acid, Stearic acid, (Z,Z)-Linoleic acid, Ethyl palmitate, Methyl stearate).

Approximately 25-28% of the extracts were composed of smoke constituents formed from the smoldering hardwoods used in the curing process. Hardwoods, such as Hickory and Oak, are generally higher in Syringyl type lignin than softwoods, such as pines, which contain predominately Guaiacyl type lignin. Thermal processes (i.e. burning) causes depolymerization of the respective lignins to produce syringol components or guaiacol components. Smoke from hardwoods produce the most desirable "smoke type" flavors having smoky, spicy, woody and sweet notes. The Syringyl/Guaiacyl lignin ratios are important in the selection of woods for smoke generation.

Table 1 provides the component identifications of over 200 constituents along with retention indices, CAS numbers and indicates materials previously found in Fire-cured tobacco as well as noting new materials not previously identified in tobacco.

Table 1. Identified Constituents of Fire-Cured Tobacco Supercritical CO_2 Extract (Sample 1)

			DB-5	DB-5				
CAS #	R.T. (Min.)	Lee RI	Kovats RI	LRI	Compounds	TIC %	Ident.	Lit. KI-LRI
64-17-5	4.384		430	427	Ethanol	0.41	MS,RI	427-472
67-64-1	4.632		499	499	2-Propanone (Acetone)	0.01	MS,RI	477-503
78-93-3	5.853		600	600	2-Butanone	0.01	MS,RI	600-622
64-19-7	6.434		625	621	Acetic acid[a]	0.05	MS,RI	600-663
79-09-4	9.500	106.5	724	720	Propanoic acid[a]	<0.01	MS,RI	702-748
108-88-5	11.915	113.1	777	773	Toluene (Methylbenzene)	<0.01	MS,RI	754-784
107-92-6	14.323	119.8	818	815	Butanoic acid	<0.01	MS,RI	790-841
98-00-0	17.917	129.8	868	862	Furfuryl alcohol[a] [(Furan-2-yl)methanol]	<0.01	MS,RI	852-875
95-47-6	18.682	131.9	877	872	o-Xylene (1,2-Dimethylbenzene)	<0.01	MS,RI	870-900
503-74-2	18.909	132.5	879	875	Isovaleric acid (3-Methylbutanoic acid)	0.10	MS,RI	833-875
116-53-0	19.816	135.0	890	887	2-Methylbutanoic acid	0.09	MS,RI	851-894
*96-48-0; **109-52-4	21.568	139.9	911	910	Butyrolactone*[b] [Dihydrofuran-2(3H)-one] + Pentanoic acid**	0.02	MS,RI	*908-918; **908-933
541-85-5	24.132	147.0	945	941	5-Methyl-3-heptanone[c]	0.03	MS,RI	939-952
100-52-7	25.441	150.6	961	957	Benzaldehyde[a,b]	<0.01	MS,RI	958-965
105-43-1	26.150	152.6	969	966	3-Methylpentanoic acid	<0.01	MS,RI	946-971
110-93-0	27.767	157.1	988	986	6-Methyl-5-hepten-2-one[a]	0.01	MS,RI	985-990
108-95-2	28.469	159.0	995	994	Phenol[a]	0.17	MS,RI	980-996
142-62-1	29.115	160.8	1002	1002	Hexanoic acid	0.03	MS,RI	990-1037
1003-29-8	30.354	164.2	1019	1017	2-Formylpyrrole[a] (1H-Pyrrole-2-carbaldehyde)	0.01	MS,RI	1013-1030
138-86-3	31.201	166.6	1030	1027	Limonene [1-Methyl-4-(prop-1-en-2-yl)cyclohexene]	0.02	MS,RI	1027-1035
80-71-7	31.347	167.0	1032	1029	2-Hydroxy-3-methylcyclopent-2-en-1-one (3-Methyl-1,2-cyclopentanedione) [Cyclotene]	0.04	MS,RI	1027-1036; Lee 166.7
100-51-6	31.844	168.3	1038	1035	Benzyl alcohol[b] (Phenylmethanol)	0.04	MS,RI	1029-1037
13494-07-0	33.647	173.3	1060	1057	2-Hydroxy-3,5-dimethylcyclopent-2-en-1-one (3,5-Dimethyl-1,2-cyclopentanedione)	<0.01	MS	1057
95-48-7	34.023	174.4	1065	1062	2-Methylphenol[a]	0.07	MS,RI	1054-1079
1072-83-9	34.288	175.1	1068	1065	2-Acetylpyrrole [1-(1H-Pyrrol-2-yl)ethanone]	<0.01	MS,RI	1060-1067
13494-06-9	35.009	177.1	1076	1074	2-Hydroxy-3,4-dimethylcyclopent-2-en-1-one (3,4-Dimethyl-1,2-cyclopentanedione)	<0.01	MS	1075
106-44-5	35.723	179.1	1084	1082	4-Methylphenol[a]	0.08	MS,RI	1075-1093
108-39-4	35.886	179.5	1086	1084	3-Methylphenol[a]	0.15	MS,RI	1077-1105
90-05-1	36.040	180.0	1088	1086	Guaiacol[a] (2-Methoxyphenol)	0.06	MS,RI	1087-1102
111-14-8	36.545	181.4	1093	1092	Heptanoic acid	<0.01	MS,RI	1078-1097
16647-04-4	37.417	183.8	1103	1103	(E)-6-Methyl-3,5-heptadien-2-one[a,b]	<0.01	MS,RI	1097-1107
350-03-8	37.528	184.1	1105	1105	3-Acetylpyridine [1-(3-Pyridinyl)ethanone]	<0.01	MS,RI	1109-1117
576-26-1	37.648	184.4	1107	1106	2,6-Dimethylphenol	<0.01	MS,RI	1105-1117
118-71-8	38.032	185.5	1112	1111	Maltol (5-Hydroxy-6-methyl-4H-pyran-4-one)	0.05	MS,RI	1110-1112
60-12-8	38.161	185.8	1114	1113	2-Phenethyl alcohol[a,b] (2-Phenylethanol)	0.02	MS,RI	1113-1130
21835-01-8	38.597	187.0	1120	1119	3-Ethyl-2-Hydroxycyclopent-2-en-1-one (3-Ethyl-1,2-cyclopentanedione) [Ethylcyclopentenolone]	0.01	MS,RI	1091-1140
1125-21-9	40.219	191.5	1143	1140	4-Ketoisophorone[b] (2,6,6-Trimethyl-2-cyclohexene-1,4-dione)	<0.01	MS,RI	1140-1144
90-00-6	40.501	192.3	1146	1144	2-Ethylphenol	0.01	MS,RI	1138-1169

Table 1. Identified Constituents of Fire-Cured Tobacco Supercritical CO_2 Extract (Sample 1)

			DB-5	DB-5				
CAS #	R.T. (Min.)	Lee RI	Kovats RI	LRI	Compounds	TIC %	Ident.	Lit. KI-LRI
105-67-9	41.236	194.4	1156	1154	2,4-dimethylphenol	0.06	MS,RI	1150-1160
95-87-4	41.340	194.6	1158	1155	2,5-dimethylphenol[a]	0.05	MS,RI	1151-1167
123-07-9	42.750	198.6	1176	1174	4-Ethylphenol	0.04	MS,RI	1167-1192
*620-17-7; **108-68-9	42.999	199.2	1179	1177	3-Ethylphenol* + 3,5-Dimethylphenol**[a]	0.15	MS,RI	*1171-1184; **1169-1187
91-20-3	43.273	200.0	1182	1181	Naphthalene[a]	<0.01	MS,RI	1182-1190
122-00-9	43.410	200.4	1184	1183	4-Methylacetophenone	<0.01	MS,RI	1178-1189
526-75-0	43.496	200.6	1185	1184	2,3-dimethylphenol	0.05	MS,RI	1181-1190
1195-09-1	43.590	200.8	1186	1185	3-Methylguaiacol[tent] (3-Methyl-2-Methoxyphenol)	<0.01	MS	
124-07-2	43.924	201.7	1190	1190	Octanoic acid	0.02	MS,RI	1180-1202
93-51-6	44.181	202.3	1194	1193	4-Methylguaiacol (4-Methyl-2-methoxyphenol)	0.16	MS,RI	1190-1207
65-85-0	44.344	202.7	1196	1195	Benzoic acid	0.02	MS,RI	1170-1210
95-65-8	44.713	203.7	1200	1200	3,4-Dimethylphenol[a]	0.05	MS,RI	1190-1200
527-60-6	45.115	204.7	1206	1206	2,4,6-Trimethylphenol	<0.01	MS,RI	1202-1215
120-80-9	46.619	208.6	1228	1227	1,2-Benzenediol[tent] (Pyrocatechol)	0.12	MS	~1197-1210
644-35-9	46.748	208.9	1230	1229	2-Propylphenol	<0.01	MS,RI	1222-1229
1687-64-5	47.193	210.0	1237	1235	2-Ethyl-6-methylphenol	0.02	MS,RI	1232[(16)]-1236
3855-26-3	47.493	210.8	1241	1239	2-Ethyl-4-Methylphenol	0.02	MS	
150-76-5	47.700	211.3	1241	1239	4-Methoxyphenol	<0.01	MS,RI	1235-1240
1687-61-2	47.929	211.9	1247	1245	2-Ethyl-5-methylphenol	0.04	MS	1242[(16)]
698-71-5	48.255	212.7	1252	1250	3-Ethyl-5-Methylphenol[a]	0.01	MS	1247[(16)]
645-56-7	49.487	215.9	1268	1267	4-Propylphenol[a]	0.01	MS,RI	1260-1262
621-27-2	49.573	216.1	1270	1268	3-Propylphenol	0.01	MS,RI	~1265-1270
1123-94-0	49.607	216.2	1270	1269	4-Ethyl-3-Methylphenol	0.04	MS	
934-00-9	49.693	216.4	1271	1270	3-Methoxy-1,2-benzenediol (3-Methoxycatechol)	0.02	MS, RI	1268-1272
2416-94-6	50.028	217.3	1276	1274	2,3,6-Trimethylphenol	0.03	MS,RRT	RRT[(17)]
97678-77-8	50.320	218.0	1280	1279	3-Ethylguaiacol[tent] (3-Ethyl-2-Methoxyphenol)	0.04	MS	
697-82-5	50.328	218.1	1280	1279	2,3,5-Trimethylphenol	0.04	MS,RI	1276-1280
2785-89-9	50.581	218.7	1283	1282	4-Ethylguaiacol (4-Ethyl-2-Methoxyphenol)	0.18	MS,RI	1281-1288
89-83-8	50.604	218.8	1284	1282	Thymol [5-methyl-2-(1-methylethyl)phenol]	0.01	MS,RI	1282-1295
2785-88-8	50.700	219.0	1285	1284	5-Ethylguaiacol[tent] (5-Ethyl-2-Methoxyphenol) (Locustol)	<0.01	MS	
488-17-5	50.931	219.6	1288	1287	3-Methyl-1,2-benzenediol[tent] (3-Methylcatechol) + unknown	0.06	MS	1263
112-05-0	51.215	220.3	1292	1291	Nonanoic acid + unknown	0.01	MS,RI	1275-1308
103-82-2	51.361	220.7	1294	1293	Benzeneacetic acid (Phenylacetic acid)	0.06	MS,RI	1262-1279
91-57-6	51.550	221.2	1296	1296	2-Methylnaphthalene	<0.01	MS,RI	1296-1299
499-75-2	51.653	221.4	1297	1297	Carvacrol[a] [2-Methyl-5-(1-methylethyl)phenol]	0.01	MS,RI	1296-1325
496-78-6	52.511	223.6	1310	1310	2,4,5-trimethylphenol[a]	0.01	MS,RRT	RRT[(17)]
90-12-0	52.751	224.3	1314	1313	1-Methylnaphthalene[a]	<0.01	MS,RI	1307-1325
634-36-6	52.888	224.6	1316	1315	1,2,3-Trimethoxybenzene	0.02	MS,RI	1309-1317
7786-61-0	53.034	225.0	1318	1317	4-Vinylguaiacol (4-Vinyl-2-Methoxyphenol) + unknown	0.01	MS,RI	1312-1325

Table 1. Identified Constituents of Fire-Cured Tobacco Supercritical CO_2 Extract (Sample 1)

CAS #	R.T. (Min.)	Lee RI	DB-5 Kovats RI	DB-5 LRI	Compounds	TIC %	Ident.	Lit. KI-LRI
452-86-8	53.258	225.6	1321	1320	4-Methyl-1,2-benzenediol[tent] (4-Methylcatechol)	0.03	MS	1293-1295
527-54-8	53.721	226.7	1328	1327	3,4,5-trimethylphenol	0.01	MS,RI	1320-1331
3209-13-0	54.340	228.3	1338	1336	3-Methoxy-5-methylphenol[c] (Orcinol methyl ether)	0.01	MS,RRT[18,19]	1317[20]~1337
91-10-1	56.140	232.9	1364	1363	Syringol (2,6-Dimethoxyphenol)	3.24	MS,RI	1353-1367
97-53-0	56.288	233.3	1366	1365	Eugenol [2-Methoxy-4-(2-propenyl)phenol]	0.07	MS,RI	1356-1370
54-11-5	56.331	233.4	1367	1365	Nicotine[a,b] [(S)-3-(1-Methyl-2-pyrrolidinyl)pyridine)	0.09	MS,RI	1355-1375
2033-89-8	56.608	234.1	1371	1369	3,4-Dimethoxyphenol	0.01	MS,RRT	RRT[17,21]
54868-48-3	56.815	234.7	1374	1372	Solanone[a,b] [(6E)-5-Isopropyl-8-methyl-6,8-nonadien-2-one)	0.22	MS,RI	1374
2785-87-7	56.936	235.0	1375	1374	4-Propylguaiacol (2-Methoxy-4-propylphenol)	0.15	MS,RI	1357-1382
135-77-3	57.575	236.6	1384	1384	1,2,4-trimethoxybenzene	0.06	MS,RI	1374-1378
58539-27-8	57.635	236.8	1385	1384	5-Propylguaiacol[tent] (2-Methoxy-5-propylphenol)	0.03	MS	
334-48-5	57.851	237.3	1388	1388	Decanoic acid	0.08	MS,RI	1380-1402
23726-93-4	58.051	237.8	1391	1391	(E)-β-damascenone[a,b] [(2E)-1-(2,6,6-Trimethyl-1,3-cyclohexadien-1-yl)-2-buten-1-one)	<0.01	MS,RI	1386-1394
629-59-4	58.700	239.5	1400	1400	Tetradecane	0.01	MS,RI	Lee 239.4
6443-69-2	59.273	240.9	1409	1409	5-Methyl-1,2,3-trimethoxybenzene[c] (3,4,5-Trimethoxytoluene)	0.07	MS,RI	1400-1419
121-33-5	59.506	241.5	1413	1412	Vanillin (4-Hydroxy-3-methoxybenzaldehyde)	0.44	MS,RI	1400-1415
5912-86-7	59.654	241.9	1415	1414	(Z)-Isoeugenol [(Z)-2-Methoxy-4-(prop-1-enyl)phenol]	0.12	MS,RI	1407-1423
99-93-4	60.292	243.6	1425	1424	4-Hydroxyacetophenone	0.06	MS	1442-1447
532-12-7	61.802	247.4	1448	1446	Myosmine [3-(3,4-Dihydro-2H-pyrrol-5-yl)pyridine]	<0.01	MS,RI	1427~1456
99-76-3	61.983	247.9	1450	1449	Methyl 4-hydroxybenzoate	0.09	MS	1459-1465
5932-68-3	62.520	249.3	1458	1457	(E)-isoeugenol[a] [(E)-2-Methoxy-4-(prop-1-enyl)phenol]	0.81	MS,RI	1451-1460
6638-05-7	62.799	250.0	1463	1461	4-methylsyringol (4-Methyl-2,6-dimethoxyphenol)	3.25	MS,RI	1149-1461
20194-67-6	63.059	250.6	1466	1465	(5S*,9S*)-3,4-dihydro-3-oxoedulan[b]; [(1S*3S*)-1,3,7,7-Tetramethyl-2-oxabicyclo[4.4.0]dec-5-en-9-one; (2S*,8aS*)-2,3,5,5,8,8a-Hexahydro-2,5,5,8a-tetramethyl-7H-1-benzopyran-7-one; (Z)-9-oxo-Dihydroedulan I (trivial name)]	<0.01	MS,RI	1464[22]
112-37-8	64.150	253.4	1482	1481	Undecanoic acid	0.01	MS,RI	1468-1490
20194-67-6	64.600	254.6	1489	1488	(5S*,9R*)-3,4-dihydro-3-oxoedulan[b]; [(1S*,3R*)-1,3,7,7-Tetramethyl-2-oxabicyclo[4.4.0]dec-5-en-9-one; (2R*,8aS*)-2,3,5,5,8,8a-Hexahydro-2,5,5,8a-tetramethyl-7H-1-benzopyran-7-one; (E)-9-oxo-Dihydroedulan II (trivial name)]	0.01	MS,RI	1488[22]
79-77-6	64.859	255.2	1492	1492	(E)-β-Ionone[b] [(3E)-4-(2,6,6-trimethylcyclohex-1-enyl)but-3-en-2-one]	0.03	MS,RI	1475-1498
55023-57-9	65.145	256.0	1497	1496	Norsolanadione[a] [(E)-5-isopropylnon-3-ene-2,8-dione]	0.23	MS, RRT	RRT[23,24]
498-02-2	65.200	256.1	1497	1497	Acetovanillone [1-(4-hydroxy-3-methoxyphenyl)ethanone]	0.35	MS,RI	1480-1496
629-62-9	65.389	256.6	1500	1500	Pentadecane + unknowns	0.09	MS,RI	Lee 256.1
132-64-9	66.610	259.7	1521	1520	Dibenzofuran[a]	0.08	MS,RI	1520-1522

Table 1. Identified Constituents of Fire-Cured Tobacco Supercritical CO_2 Extract (Sample 1)

CAS #	R.T. (Min.)	Lee RI	DB-5 Kovats RI	DB-5 LRI	Compounds	TIC %	Ident.	Lit. KI-LRI
3943-74-6	67.147	261.1	1530	1529	Methyl vanillate (Methyl 4-hydroxy-3-methoxybenzoate)	0.17	MS,RI	1516-1526
14059-92-8	67.739	262.6	1539	1538	4-Ethylsyringol (4-Ethyl-2,6-dimethoxyphenol)	2.59	MS,RRT	1528
2503-46-0	67.973	263.2	1543	1542	2-Propiovanillone [1-(4-Hydroxy-3-methoxyphenyl)propan-2-one]	0.09	MS,RI	1536-1541
581-50-0	68.199	263.8	1547	1546	2,3'-Dipyridyl[b] [2-(Pyridin-3-yl)pyridine]	0.18	MS,RI	1536-1576
17092-92-1	68.277	264.0	1548	1547	Dihydroactinidiolide[a,b] [5,6,7,7a-Tetrahydro-4,4-7a-trimethyl-2-(4H)-benzofuranone]	<0.01	MS,RI	1522-1548
5492-79-5	69.725	267.7	1572	1571	(6Z,8E)-Megastigma-4,6,8-trien-3-one[a,b] (Isomer I); [(Z,E)-3,5,5-trimethyl-4-(2-butenylidene)-cyclohexen-1-one]	0.04	MS,RI	1568[22]
1131-62-0	69.777	267.8	1572	1571	Acetoveratrone [1-(3,4-dimethoxyphenyl)ethanone]	0.06	MS,RI	1573
143-07-7	70.299	269.2	1581	1580	Dodecanoic acid	0.24	MS,RI	1567-1589
15964-80-4	70.690	270.2	1587	1586	Methyl homovanillate[c] [Methyl 2-(4-hydroxy-3-methoxyphenyl)acetate]	<0.01	MS	
*5164-79-4 **86-73-7	70.786	270.4	1588	1588	(6Z,8Z)-Megastigma-4,6,8-trien-3-one*[a,b] (Isomer II); [(Z,Z)-3,5,5-trimethyl-4-(2-butenylidene)-cyclohexen-1-one] + 9H-Fluorene[a]**	0.17	MS,RI	*1586[22]; **1580-1595
1835-14-9	71.081	271.2	1593	1593	1-Propiovanillone[c] [1-(4-hydroxy-3-methoxyphenyl)propan-1-one]	0.25	MS,RI,RRT	1582-1588
544-76-3	71.542	272.3	1600	1600	Hexadecane	0.07	MS,RI	Lee 272.0
6627-88-9	72.309	274.3	1614	1613	4-allylsyringol [2,6-Dimethoxy-4-(2-propenyl)phenol]	0.96	MS,RI	1602-1615
6766-82-1	72.719	275.4	1621	1620	4-Propylsyringol[c] (4-Propyl-2,6-dimethoxyphenol)	1.07	MS,RI,RRT[17]	1616
5164-78-3	72.877	275.8	1624	1623	(6E,8E)-Megastigma-4,6,8-trien-3-one[a,b] (Isomer III); [(E,E)-3,5,5-trimethyl-4-(2-butenylidene)-cyclohexen-1-one]	0.04	MS,RI	1621[22]
102488-09-5	73.025	276.1	1626	1625	3-Hydroxy-β-damascone[b] [1-(3-Hydroxy-2,6,6-trimethyl-1-cyclohexen-1-yl)-2-buten-1-one]	0.11	MS,RI	1617-1640
5298-13-5	73.696	277.9	1638	1637	(6E,8Z)-Megastigma-4,6,8-trien-3-one[a,b] (Isomer IV); [(E,Z)-3,5,5-trimethyl-4-(2-butenylidene)-cyclohexen-1-one]	0.09	MS,RI	1633[22]
92-83-1	74.551	280.0	1652	1651	9H-Xanthene[a]	0.08	MS,RI	Lee 280.6 (Avg of 4)
34318-21-3	75.161	281.6	1663	1662	3-Oxo-α-ionol[b] [4-(3-Hydroxy-1-butenyl)-3,5,5-trimethyl-2-cyclohexen-1-one]	0.71	MS,RI	1665-1682
134-96-3	75.800	283.2	1674	1673	Syringaldehyde (3,5-Dimethoxy-4-hydroxybenzaldehyde)	0.52	MS,RI	1658-1670
26624-13-5	76.045	283.9	1678	1677	(Z)-4-(1-propenyl)syringol (2,6-dimethoxy-4-[(1Z)-prop-1-en-1-yl]phenol)	0.10	MS,RI,RRT	1659; RRT[28,29]
1136-86-3	76.683	285.5	1688	1688	1-(3,4,5-trimethoxyphenyl)ethanone[c]	0.09	MS,RRT	RRT[17]
629-78-7	77.389	287.3	1700	1700	Heptadecane	0.05	MS,RI	Lee 287.2
*2523-39-9; **1556-99-6	77.730	288.2	1706	1706	3*- and 4-Methyl-9H-fluorene**	<0.01	MS,RI	Lee 286.9-288.2
20675-95-0	77.887	288.6	1709	1709	(E)-4-(1-propenyl)syringol (2,6-dimethoxy-4-[(1E)-prop-1-en-1-yl]phenol)	0.07	MS,RI	1709
1430-97-3	78.105	289.1	1713	1713	2-Methyl-9H-fluorene	<0.01	MS,RI	Lee 288.5-289.5
2215-80-7	78.210	289.4	1715	1715	1-(4-hydroxy-2,6-dimethoxyphenyl)propan-2-one[tent] (or isomer) + unknown	0.13	MS	

Table 1. Identified Constituents of Fire-Cured Tobacco Supercritical CO_2 Extract (Sample 1)

CAS #	R.T. (Min.)	Lee RI	DB-5 Kovats RI	DB-5 LRI	Compounds	TIC %	Ident.	Lit. KI-LRI
*486-56-6; **1730-37-6	78.779	290.9	1726	1725	Cotinine*[b] [1-Methyl-5-(3-pyridinyl)-2-pyrrolidinone] + 1-Methyl-9H-fluorene**	0.12	MS,RI	*1730-1740; **1719; Lee 289.5-290.5
1916-07-0	78.910	291.2	1728	1727	Methyl 3,4,5-trimethoxybenzoate[c]	0.06	MS,RRT	RRT[(26)]
2140-82-1	79.138	291.8	1732	1731	1-Pristene = 1-Norphytene (2,6,10,14-tetramethylpentadec-1-ene)	0.06	MS,RI	1731[(27)]
2478-38-8	80.012	294.0	1748	1747	Acetosyringone [1-(4-Hydroxy-3,5-dimethoxyphenyl)ethanone]	0.60	MS,RI	1741-1744
544-63-8	81.673	298.6	1778	1777	Myristic acid (Tetradecanoic acid)	0.39	MS,RI	1765-1778
1443-76-1	81.832	299.0	1781	1780	Syringic acid hydrazide[c]	0.24	MS,RI	1777
19037-58-2	82.025	299.2	1784	1783	2-Propiosyringone (Syringylacetone) [1-(4-hydroxy-3,5-dimethoxyphenyl)propan-2-one]	0.11	MS,RI	1781-1785
85-01-8	82.350	300.0	1790	1789	Phenanthrene	0.23	MS,RI	1776-1794
112-88-9	82.499	300.5	1792	1792	1-Octadecene	0.04	MS,RI	1792-1795
120-12-7	82.814	301.4	1797	1797	Anthracene[a]	0.06	MS,RI	1786-1806
593-45-3	82.963	301.9	1800	1800	Octadecane	0.18	MS,RI	Lee 301.7
5650-43-1	85.139	308.6	1842	1841	1-Propiosyringone[c] [1-(4-hydroxy-3,5-dimethoxyphenyl)propan-1-one]	0.50	MS,RRT	RRT[(28,29)]
504-96-1	85.410	309.4	1847	1846	Neophytadiene[a,b] [3-methylene-7,11,15-trimethylhexadec-1-ene]	8.57	MS,RI	1830-1844
*502-69-2; **56554-34-8	85.729	310.4	1853	1852	6,10,14-Trimethyl-2-pentadecanone*[a,b] + 2-Phytene** (2,6,10,14-Tetramethyl-2-hexadecene)	0.26	MS,RI	*1842-1855; **1849-1859
1002-84-2	86.951	314.2	1876	1875	Pentadecanoic acid	0.30	MS,RI	1856-1883
18435-45-5	87.951	317.3	1894	1894	1-Nonadecene	0.07	MS,RI	1890-1899
629-92-5	88.276	318.3	1900	1900	Nonadecane	0.11	MS,RI	Lee 318.1
832-71-3	88.610	319.3	1907	1907	3-Methylphenanthrene	0.08	MS,RI	Lee 318.6-319.5
2531-84-2	88.917	320.3	1913	1913	2-Methylphenanthrene	0.09	MS,RI	Lee 319.8-320.3
203-64-5	89.330	321.5	1921	1921	4H-Cyclopenta[def]phenanthrene (4,5-Methylenephenanthrene)	0.07	MS,RI	Lee 320.0-322.2
1117-52-8	89.374	321.7	1922	1922	(E,E)-Farnesylacetone[b] [(5E,9E)-6,10,14-trimethylpentadeca-5,9,13-trien-2-one]	0.27	MS,RI	1915-1927
112-39-0	89.620	322.4	1927	1927	Methyl palmitate[a,b] (Methyl hexadecanoate) + unknown	0.14	MS,RI	1915-1930
832-64-4 and or 883-20-5	89.752	322.8	1930	1929	4-(and/or 9)Methylphenanthrene + unknown	0.08	MS,RI	1923-1927; Lee 322.9-323.1
832-69-9	89.884	323.3	1933	1932	1-Methylphenanthrene	0.07	MS,RI	1931-1932; Lee 323.5-324
505-32-8	90.806	326.1	1951	1950	Isophytol (3,7,11,15-Tetramethyl-1-hexadecen-3-ol)	0.08	MS,RI	1949-1954
54869-11-3	91.853	329.3	1972	1971	Phytofuran; 3-(4,8,12-Trimethyltridecyl)furan	0.30	MS,RI	1971[(25)]
57-10-3	92.249	330.6	1979	1979	Palmitic acid [Hexadecanoic acid]	2.29	MS,RI	1963-2010
612-94-2	92.355	330.9	1981	1981	2-Phenylnaphthalene	0.10	MS,RI	1987; Lee 330.5-331.2
3452-07-1	92.999	332.9	1994	1994	1-Eicosene	0.25	MS,RI	1990-1995
628-97-7	93.040	333.0	1995	1995	Ethyl palmitate (Ethyl hexadecanoate)	0.04	MS,RI	1990-1997
112-95-8	93.308	333.8	2000	2000	Eicosane	0.25	MS,RI	Lee 333.6
1576-67-6	94.592	337.8	2027	2027	3,6-Dimethylphenanthrene	0.01	MS,RI	2027; Lee 337-338

Table 1. Identified Constituents of Fire-Cured Tobacco Supercritical CO_2 Extract (Sample 1)

CAS #	R.T. (Min.)	Lee RI	DB-5 Kovats RI	DB-5 LRI	Compounds	TIC %	Ident.	Lit. KI-LRI
20291-74-1	95.841	341.6	2053	2052	1,6-Dimethylphenanthrene	0.02	MS, RI	Lee 340.8-342.4
25269-17-4	96.467	343.6	2066	2065	Thunbergol (Isocembrol) [(1R,2E,4S,7E,11E)-4-isopropyl-1,7,11-trimethylcyclotetradeca-2,7,11-trien-1-ol]	0.52	MS,RI	2048-2073
206-44-0	96.873	344.8	2074	2074	Fluoranthene	0.12	MS,RI	2060-2085
629-94-7	98.128	348.7	2100	2100	Heneicosane	0.59	MS,RI	Lee 348.4
150-86-7	99.224	352.1	2124	2123	(E)-Phytol[b] [(E)-3,7,11,15-tetramethylhexadec-2-en-1-ol]	2.68	MS,RI	2111-2135
*112-61-8 **129-00-0	99.498	352.9	2130	2129	Methyl stearate* (Methyl octadecanoate) + Pyrene**	0.78	MS,RI	*2117-2130 / **2120-2140
	101.009	357.6	2162	2161	A Duvatrienediol isomer + unknowns	3.03	MS	
60-33-3	101.337	358.6	2169	2168	(Z,Z)-Linoleic acid [(Z,Z)-9,12-Octadecadienoic acid]	2.32	MS,RI	2140-2173
57-11-4	102.339	361.7	2190	2189	Stearic acid (Octadecanoic acid) + unknowns	0.46	MS,RI	2158-2200
1599-67-3	102.551	362.3	2194	2194	1-Docosene	0.38	MS,RI	2190-2196
629-97-0	102.843	363.2	2200	2200	Docosane	1.09	MS,RI	Lee 362.6
	103.401	365.0	2213	2213	A Duvatrienediol isomer	1.80	MS	
10236-16-5*	103.826	366.3	2223	2223	Phytyl acetate* + A Duvatrienediol isomer	1.53	MS,RI*	*2218-2223
57605-80-8 / 57605-81-9	106.009	373.0	2274	2273	α- and/or β-4,8,13-Duvatriene-1,3-diol (α- and/or β-12-isopropyl-1,5,9-trimethylcyclotetradeca-4,8,13-triene-1,3-diol)	8.14	MS, RI[Note]	2240[(30)]
629-96-9	106.830	375.6	2293	2292	1-Eicosanol	0.10	MS,RI	2287-2292
18835-32-0	106.919	375.8	2295	2294	1-Tricosene	0.18	MS,RI	2289-2296
638-67-5	107.157	376.6	2300	2300	Tricosane	0.34	MS,RI	Lee 376.3
1120-28-1	108.392	380.4	2330	2329	Methyl eicosanoate + unknown	0.08	MS,RI	2329-2339
18256-53-6	109.105	382.6	2347	2346	1,2-bis(3-methoxy-4-hydroxyphenyl)ethane[c] [4,4'-ethane-1,2-diylbis(2-methoxyphenol)]	0.11	MS	MS = 137, 274, 122
6809-52-5	110.903	388.1	2389	2389	6,10,14,18-tetramethylnonadeca-5,9,13,17-tetraen-2-one (5E,9E,13E-isomer?)	0.28	MS	
10192-32-2	111.159	388.9	2395	2395	1-Tetracosene	0.36	MS,RI	2391-2396
646-31-1	111.352	389.5	2400	2400	Tetracosane	0.12	MS,RI	Lee 389.4
25385-24-4; 24762-58-1	112.487	393.0	2428	2428	4,4'-propane-1,3-diylbis(2-methoxyphenol[c]	0.03	MS	MS = 137, 288, 151
6064-90-0	112.522	393.1	2429	2429	Methyl heneicosanoate[c] + unknown	0.17	MS,RI	2429-2430
217-59-4	114.321	398.7	2474	2473	Triphenylene	<0.01	MS,RI	Lee 398.7-400.0
218-01-9	114.752	400.0	2484	2484	Chrysene	<0.01	MS,RI	2472-2496
	115.007		2491	2490	A methylenebis(2,6-dimethoxyphenol) isomer[tent]	0.11	MS	MS = 320, 167
661-19-8	115.218		2496	2496	1-Docosanol	0.37	MS,RI	2494-2501
629-99-2	115.394		2500	2500	Pentacosane	0.15	MS,RI	
929-77-1	116.597		2531	2531	Methyl docosanoate[c]	0.10	MS,RI	2529-2535
117-81-7	117.466		2554	2553	Di(2-ethylhexyl)phthalate	0.09	MS,RI	2546-2563
108853-14-1	118.344		2576	2576	4-[2-(4-hydroxy-3-methoxyphenyl)ethyl]-2,6-dimethoxyphenol[c] (Moscatilin)	0.04	MS	MS = 167, 137, 304
18835-33-1	119.116		2596	2596	1-Hexacosene	0.04	MS,RI	2593
630-01-3	119.283		2600	2600	Hexacosane	0.03	MS,RI	

Table 1. Identified Constituents of Fire-Cured Tobacco Supercritical CO_2 Extract (Sample 1)

CAS #	R.T. (Min.)	Lee RI	DB-5 Kovats RI	DB-5 LRI	Compounds	TIC %	Ident.	Lit. KI-LRI
822-26-4	119.722		2612	2612	1-Docosyl acetate[c]	0.11	MS,RI	2609-2613
2433-97-8	120.467		2632	2631	Methyl tricosanoate	0.05	MS,RI	2628-2632
593-49-7	123.049		2700	2700	Heptacosane	0.31	MS,RI	
2442-49-1	124.219		2732	2732	Methyl tetracosanoate[c]	0.16	MS,RI	2725-2731
537-35-9	126.669		2798	2798	1,2-bis(3,5-dimethoxy-4-hydroxyphenyl)ethane[c] [4,4'-ethane-1,2-diylbis(2,6-dimethoxyphenol)]	0.02	MS	MS = 167, 334
630-02-4	126.761		2800	2800	Octacosane	0.11	MS,RI	
55373-89-2	128.017		2831	2831	Methyl pentacosanoate[c]	<0.01	MS,RI	2823-2825
59681-06-0	128.114		2833	2832	10-Demethylsqualene (2,6,10,19,23-pentamethyltetracosa-2,6,10,14,18,22-hexaene)	0.39	MS,RI	2830[(31)]-2831[(22)]
111-02-4	128.756		2848	2848	Squalene (2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexaene)	0.18	MS,RI	2847
	129.055		2855	2855	A Polyisoprenoid (Farnesol type)	0.22	MS	
1560-98-1	129.336		2862	2862	2-Methyloctacosane[tent]	0.26	MS,RI	2857-2864
630-03-5	130.927		2900	2900	Nonacosane	0.11	MS,RI	Lee 454.9
5802-82-4	132.472		2943	2943	Methyl hexacosanoate[c]	0.04	MS,RI	2942; Lee 474.9
638-68-6	134.545		3000	3000	Triacontane	0.42	MS,RI	Lee 467.0
630-04-6	139.303		3100	3100	Hentriacontane	0.30	MS,RI	Lee 478.9
57-88-5	143.912		3162	3162	Cholest-5-en-3β-ol (Cholesterol)	0.49	MS 99%	3122.3[(32)]; 3192[(33)]; 3075
630-05-7	146.771		3300	3300	Tritriacontane[tent]	0.20	MS,RI	
	148.705				A Polyisoprenoid	0.36	MS	
	149.476				A Polyisoprenoid	0.40	MS	
	151.991				A Phytosterol	0.66	MS	
	152.509				A Phytosterol	0.23	MS	
	153.609				A Polyisoprenoid	0.22	MS	
	155.071				A Phytosterol	1.91	MS	
	157.354				A Polyisoprenoid	0.28	MS	
	158.054				A Polyisoprenoid	0.33	MS	
	159.111				A Polyisoprenoid	0.28	MS	
	159.875				A Phytosterol	0.57	MS	
	161.361				A Phytosterol	0.22	MS	
	167.025				A Phytosterol	0.29	MS	
	167.854				A Polyisoprenoid	0.22	MS	
					Total % Identified	**71.75**		

a) - also reported as a Fire-Cured tobacco constituent by Davis et al., Reference 7.

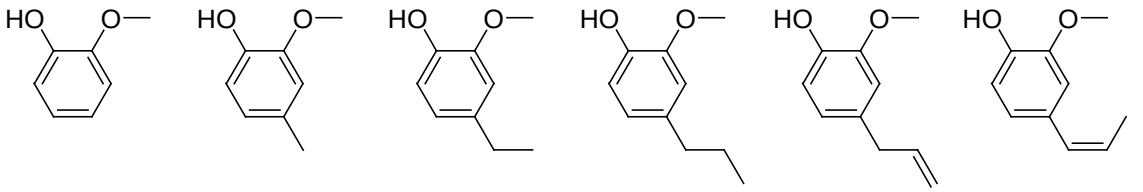
b) - also reported as a Fire-Cured tobacco constituent by Wahlberg et al., Reference 8.

c) New tobacco isolate; i.e., not reported in tobacco or tobacco smoke (Reference 34).

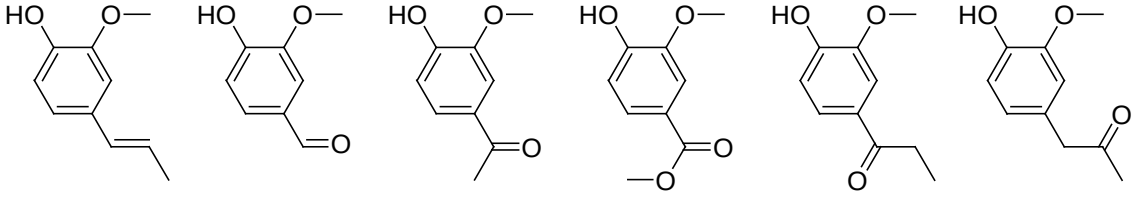
Note - In sample # 1 the KI for 4,8,13-Duvatriene-1,3-diol (broad peak) was from 2254-2273; In sample #2 the KI was from 2239-2244

Charts 1 & 2 show the major guaiacol and syringol components found in the Fire-cured extracts (percentages are FID % from sample #2).

Chart 1. – Major Guaiacol Lignin Depolymerization Products (5.1%)



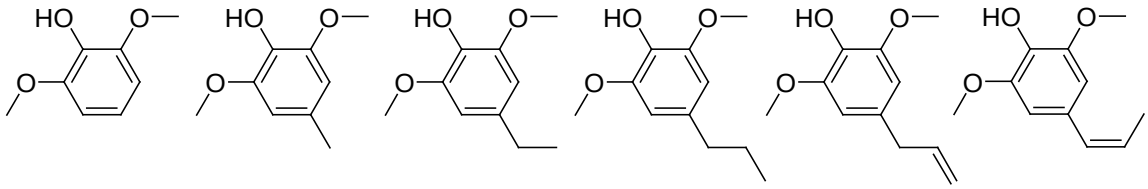
(1) 0.14% (2) 0.33% (3) 0.49% (4) 0.36% (5) 0.37% (6) 0.19%



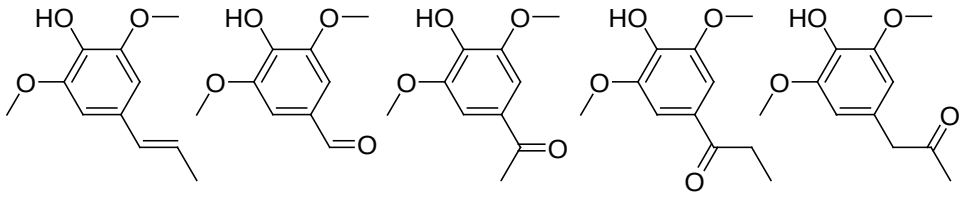
(7) 0.76% (8) 0.68% (9) 0.54% (10) 0.25% (11) 0.19% (12) 0.10%

(1) Guaiacol, (2) 4-Methylguaiacol, (3) 4-Ethylguaiacol, (4) 4-Propylguaiacol, (5) Eugenol, (6) (Z)-Isoeugenol, (7) (E)-Isoeugenol, (8) Vanillin, (9) Acetovanillone, (10) Methyl vanillate, (11) 1-Propiovanillone, (12) 2-Propiovanillone

Chart 2. – Major Syringol Lignin Depolymerization Products (19.2%)



(1) 5.22% (2) 5.87% (3) 3.64% (4) 1.31% (5) 1.27% (6) 0.13%



(7) 0.11% (8) 0.54% (9) 0.52% (10) 0.49% (11) 0.18%

(1) Syringol, (2) 4-Methylsyringol, (3) 4-Ethylsyringol, (4) 4-Propylsyringol, (5) 4-Allylsyringol, (6) (Z)-4-(1-propenyl)syringol, (7) (E)-(Z)-4-(1-propenyl)syringol, (8) Syringaldehyde, (9) Acetosyringone, (10) 1-Propiosyringone, (11) 2-Propiosyringone

Table 2 provides the mass spectral data for the important smoke constituents of the extracts. Table 3 provides the country regulatory status and odor/flavor properties of the smoke flavorant constituents.

Table 2. FID Percent and Mass Spectral Data for Smoke Constituents of Fire-Cured Supercritical CO_2 Extract (Sample 2)

Compound	FID %	Mol. Wt.	MS (m/z) Relative Ion Intensity
Phenol	0.30	94	94 (100), 66 (31), 65 (22), 39 (16)
2-Hydroxy-3-methylcyclopent-2-en-1-one (3-Methyl-1,2-cyclopentanedione) [Cyclotene]	0.05	112	112 (100), 55 (46), 69 (42), 41 (33), 56 (26), 83 (25), 39 (23)
2-Methylphenol	0.20	108	108 (100), 107 (86), 79 (34), 77 (33), 90 (24)
4-Methylphenol	0.22	108	107 (100), 108 (84), 77 (26), 79 (21), 51 (10)
3-Methylphenol	0.43	108	108 (100), 107 (95), 79 (30), 77 (29), 39 (13)
Guaiacol (2-Methoxyphenol)	0.14	124	109 (100), 124 (87), 81 (62), 53 (19)
Maltol (5-Hydroxy-6-methyl-4H-pyran-4-one)	0.10	126	126 (100), 71 (37), 43 (30), 55 (24), 97 (21)
3-Ethyl-2-Hydroxycyclopent-2-en-1-one (3-Ethyl-1,2-cyclopentanedione) [Ethylcyclopentenolone]	0.01	126	126 (100), 55 (52), 69 (42), 83 (40), 43 (38), 41 (36), 39 (33)
2-Ethylphenol	0.04	122	107 (100), 122 (43), 77 (27), 79 (13)
2,4-dimethylphenol	0.19	122	107 (100), 122 (98), 121 (58), 77 (25), 91 (18)
2,5-dimethylphenol	0.16	122	107 (100), 122 (100), 121 (46), 77 (30), 91 (22)
4-Ethylphenol	0.17	122	107 (100), 122 (33), 77 (17)
3-Ethylphenol	0.16	122	107 (100), 122 (49), 77 (25)
3,5-Dimethylphenol	0.38	122	122 (100), 107 (87), 121 (32), 77 (29), 91 (14)
2,3-dimethylphenol	0.19	122	107 (100), 122 (87), 77 (29), 121 (29),91 (22)
4-Methylguaiacol (4-Methyl-2-methoxyphenol)	0.33	138	138 (100), 123 (94), 95 (31), 77 (18), 67 (18), 55 (14)
3,4-Dimethylphenol	0.16	122	107 (100), 122 (74), 121 (44), 77 (23), 91 (15)
2,4,6-Trimethylphenol	0.02	136	121(100), 136 (89), 135 (33), 91 (25, 77 (17)
2-Ethyl-6-methylphenol	0.04	136	121 (100), 136 (43), 91(22), 77 (20)
2-Ethyl-4-Methylphenol	0.02	136	121 (100), 136 (29), 91 (19), 41 (17), 77 (15)
2-Ethyl-5-methylphenol	0.10	136	121 (100), 136 (30), 91 (12, 77 (11)

Table 2. FID Percent and Mass Spectral Data for Smoke Constituents of Fire-Cured Supercritical CO_2 Extract (Sample 2)

Compound	FID %	Mol. Wt.	MS (m/z) Relative Ion Intensity
3-Ethyl-5-Methylphenol	0.06	136	121(100), 136 (47), 91 (33), 77 (17)
3-Methoxy-1,2-benzenediol (3-Methoxycatechol)	0.08	140	140 (100), 125 (77), 97 (45), 51 (17), 107 (14), 79 (11)
4-Ethyl-3-Methylphenol	0.22	136	121 (100), 136 (52), 91 (17), 77 (16)
2,3,6-Trimethylphenol	0.16	136	121 (100), 136 (98), 91 (31), 135 (30), 77 (16)
4-Ethylguaiacol (4-Ethyl-2-Methoxyphenol)	0.49	152	137 (100), 152 (42), 122 (9), 138 (9), 91 (9), 77 (7)
2,3,5-Trimethylphenol	0.11	136	121 (100), 136 (82), 91 (33), 135 (23), 77 (22)
4-Methyl-1,2-benzenediol (4-Methylcatechol)	0.03	124	124 (100), 123 (68), 78 (44), 77 (25), 51 (13), 107 (11)
2,4,5-trimethylphenol	0.05	136	121 (100), 136 (65), 135 (32), 91 (23), 77 (16)
Carvacrol [2-Methyl-5-(1-methylethyl)phenol]	0.02	150	135 (100), 150 (41), 91 (31), 77 (19), 107 (18), 79 (12)
3,4,5-trimethylphenol	0.07	136	121 (100), 136 (59), 135 (24), 77 (23), 91 (22)
3-Methoxy-5-methylphenol (Orcinol methyl ether)	0.09	138	138 (100), 109 (28), 107 (23), 108 (11)
Syringol (2,6-Dimethoxyphenol)	5.22	154	154 (100), 139 (47), 111 (23), 96 (22), 93 (17), 65 (13)
Eugenol [2-Methoxy-4-(2-propenyl)phenol]	0.37	164	164 (100), 149 (33), 77 (30), 91 (28), 131 (26), 103 (26)
4-Propylguaiacol (4-Propyl-2-Methoxyphenol)	0.36	166	137 (100), 166 (25), 138 (13), 122 (9), 77 (7),
Vanillin (4-Hydroxy-3-methoxybenzaldehyde)	0.68	152	151 (100), 152 (92), 81 (21), 123 (17), 109 (15)
(Z)-Isoeugenol [(Z)-2-Methoxy-4-(prop-1-enyl)phenol]	0.19	164	164 (100), 149 (34), 77 (27), 91 (27), 103 (23), 131 (21)
4-methylsyringol (4-Methyl-2,6-dimethoxyphenol)	5.87	168	168 (100), 153 (45), 125 (44), 53 (11)
(E)-Isoeugenol [(E)-2-Methoxy-4-(prop-1-enyl)phenol]	0.76	164	164 (100), 149 (32), 77 (24), 91 (22), 103 (22), 131 (20)
Acetovanillone [1-(4-hydroxy-3-methoxyphenyl)ethanone]	0.54	166	151 (100), 166 (49), 123 (22), 152 (9)
Methyl vanillate (Methyl 4-hydroxy-3-methoxybenzoate)	0.25	182	151 (100), 182(59), 123 (16), 152 (12)

Table 2. FID Percent and Mass Spectral Data for Smoke Constituents of Fire-Cured Supercritical CO_2 Extract (Sample 2)

Compound	FID %	Mol. Wt.	MS (m/z) Relative Ion Intensity
4-Ethylsyringol (4-Ethyl-2,6-dimethoxyphenol)	3.64	182	167 (100), 182 (59), 168 (10)
2-Propiovanillone [1-(4-hydroxy-3-methoxyphenyl)propan-2-one]	0.10	180	137 (100), 180 (24), 122 (12)
1-Propiovanillone [1-(4-hydroxy-3-methoxyphenyl)propan-1-one]	0.19	180	151 (100), 180 (29), 123 (17)
4-Allylsyringol [2,6-Dimethoxy-4-(2-propenyl)phenol]	1.27	194	194 (100), 91 (23), 119 (18), 131 (14), 77 (12), 179 (12)
4-Propylsyringol (4-Propyl-2,6-dimethoxyphenol)	1.31	196	167 (100), 196 (35), 168 (12)
Syringaldehyde (3,5-Dimethoxy-4-hydroxybenzaldehyde)	0.54	182	182 (100), 181 (60), 167 (12), 111 (11)
(Z)-4-(1-propenyl)syringol (2,6-dimethoxy-4-[(1Z)-prop-1-en-1-yl]phenol)	0.13	194	194 (100), 179 (44), 91 (25), 77 (18), 193 (16)
(E)-4-(1-propenyl)syringol (2,6-dimethoxy-4-[(1E)-prop-1-en-1-yl]phenol)	0.11	194	194 (100), 179 (17), 91 (17), 77 (14), 119 (13)
Acetosyringone [1-(4-Hydroxy-3,5-dimethoxyphenyl)ethanone]	0.52	196	181 (100), 196 (52), 153 (11), 43 (10), 182 (10)
2-Propiosyringone (Syringylacetone) [1-(4-Hydroxy-3,5-dimethoxyphenyl)propan-2-one]	0.18	210	167 (100), 210 (26), 168 (19), 123 (12)
Syringic acid hydrazide	0.47	212	212 (100), 181 (88), 197 (12), 213 (11), 141 (11), 182 (10), 153 (9)
1-Propiosyringone [1-(4-hydroxy-3,5-dimethoxyphenyl)propan-1-one]	0.49	210	181 (100), 210 (31), 182 (10), 153 (8)
1,2-bis(3-Methoxy-4-hydroxyphenyl)ethane [4,4'-ethane-1,2-diylbis(2-methoxyphenol)]	0.08	274	137(100), 274 (25), 122 (9), 138 (8)
4-[2-(4-Hydroxy-3-methoxyphenyl)ethyl]-2,6-dimethoxyphenol (Moscatilin)	0.10	304	167(100), 137 (43), 304 (33)
Percent of Extract	**28.13**		

Table 3. FID Percent and Country Approvals as food flavorants for Constituents (in Fire-Cured Extract Sample 2)

Compound	FID %	FEMA #	EU FLAVIS #	JAPAN	Odor/Flavor
Phenol	0.30	3223	04.041	Yes	Phenolic medicinal odor, somewhat sweet, burnt, tarry
2-Hydroxy-3-methylcyclopent-2-en-1-one [Cyclotene]	0.05	2700	07.056	Yes	Caramellic-maple, sweet, burnt, lovage notes
2-Methylphenol	0.20	3480	04.027	Yes	Phenolic, medicinal, ink-like, somewhat musty, smoky
4-Methylphenol	0.22	2337	04.028	Yes	Tarry, smoky, phenol-medicinal, animal-like character
3-Methylphenol	0.43	3530	04.026	Yes	Dry-tarry, medicinal-phenolic, leathery odor
Guaiacol	0.14	2532	04.005	Yes	Sweet, smoke-like, vanilla, spicy, phenolic-medicinal notes
Maltol	0.10	2656	07.014	Yes	Sweet, caramellic, cotton candy odor; strawberry, fruity, berry notes
3-Ethyl-2-Hydroxycyclopent-2-en-1-one [Ethylcyclopentenolone]	0.01	3152	07.057	Yes	Sweet, caramel-maple, burnt sugar like notes
2-Ethylphenol	0.04	--	04.070	Yes	Sweet, tarry phenolic-medicinal notes
2,4-dimethylphenol	0.19	--	04.066	Yes	Burnt, roasted, phenolic, leathery notes
2,5-dimethylphenol	0.16	3595	04.019	Yes	Phenolic, sweet, smoky notes
4-Ethylphenol	0.17	3156	04.022	Yes	Sweet, woody-phenolic, animalic, smoky notes
3-Ethylphenol	0.16	--	04.021	Yes	Phenolic, medicinal, animal-leather notes
3,5-Dimethylphenol	0.38	--	04.020	Yes	Phenolic, spicy, woody-orris, somewhat balsamic
2,3-dimethylphenol	0.19	--	04.065	Yes	Leathery, caryophyllene, spicy, animalic, smoky notes
4-Methylguaiacol	0.33	2671	04.007	Yes	Sweet, spicy, vanilla-smoky, phenolic notes
3,4-Dimethylphenol	0.16	3596	04.048	Yes	Dry phenolic, earthy, animal-like notes
2,4,6-Trimethylphenol	0.02	4329	04.095	No	Phenolic, smoky, leathery, Latakia tobacco, tarry, medicinal, spicy notes
2,3,6-Trimethylphenol	0.16	3963	04.085	Yes	Smoky, phenolic, tarry, medicinal, spicy, woody, leathery notes
4-Ethylguaiacol	0.49	2436	04.008	Yes	Spicy, smoky, clove, vanilla, phenolic, mildly bacon like notes
Carvacrol	0.02	2245	04.031	Yes	Medicinal, phenolic, Origanum, smoky herbaceous notes

Table 3. FID Percent and Country Approvals as food flavorants for Constituents (in Fire-Cured Extract Sample 2)

Compound	FID %	FEMA #	EU FLAVIS #	JAPAN	Odor/Flavor
3-Methoxy-5-methylphenol	0.09	--	--	Yes	Natural oakmoss, sweet, phenolic; reminiscent of oak- and treemoss notes
Syringol	5.22	3137	04.036	Yes	Phenolic-woody-medicinal, tarry, spicy, smoky (bacon) notes
Eugenol	0.37	2467	04.003	Yes	Spicy, clove-like, dry, pungent, smoky, ham & bacon notes
4-Propylguaiacol	0.36	3598	04.049	Yes	Sweet, spicy, balsamic, clove, eugenol-like, smoky notes
Vanillin	0.68	3107	05.018	Yes	Sweet, creamy, vanilla-like
(Z)-Isoeugenol	0.19	2468	04.004	Yes	Spicy, clove, phenolic, smoky notes
4-methylsyringol	5.87	3704	04.053	Yes	Smoky, spicy, phenolic, medicinal & woody notes
(E)-Isoeugenol	0.76	2468	04.004	Yes	Spicy, clove, phenolic, smoky & floral (carnation) notes
Acetovanillone	0.54	--	07.142	No	Sweet, reminiscent of Vanillin, but less spicy
Methyl vanillate	0.25	--	09.799	Yes	Spicy vanilla notes, with a fresh, green and flowery background
4-Ethylsyringol	3.64	3671	04.052	No	Phenolic, burnt, smoky, spicy notes
4-Allylsyringol	1.27	3655	04.051	Yes	Spicy, burnt, smoky, phenolic notes; smoked meat (bacon) note
4-Propylsyringol	1.31	3729	04.056	No	Spicy, burnt, smoky, slightly phenolic notes
Syringaldehyde	0.54	4049	05.153	No	Sweet, balsamic-vanilla-floral with some smoky, phenolic, woody notes
(Z)-4-(1-propenyl)syringol	0.13	3728	04.055	No	Sweet, smoky, spicy, phenolic notes
(E)-4-(1-propenyl)syringol	0.11	3728	04.055	No	Sweet, smoky, spicy, phenolic notes
Acetosyringone	0.52	--	07.164	No	Weak, sweet, balsamic, vanilla phenolic spicy notes
1-Propiosyringone	0.49	--	07.154	No	Weak, sweet, mild, somewhat floral, balsamic-vanilla-smoky notes
Percent of Extract (Sample # 2)	**26.25**				

FEMA # - Flavor Extract Manufacturers Association GRAS Lists 3-26 (USA)

EU FLAVIS # - As included in REGULATION (EU) No 872/2012 of 1 October 2012

JAPAN - As per the Japanese Food Sanitation Act as designated by the Minister of Health, Labour and Welfare as food additives.

For those interested in additional information on the chemistry of smoke flavors, see the article by Guillen (17) and references 35-42.

References:

(1) Crop Production, National Agricultural Statistics Service (NASS), Agricultural Statistics Board, United States Department of Agriculture (USDA), ISSN:1936-3737, October 11, 2012; retrieved on March 15, 2013 from http://usda.mannlib.cornell.edu/usda/nass/CropProd/2010s/2012/CropProd-10-11-2012.pdf

(2) 2012 Supply & Demand Report, Universal Leaf Tobacco Company, Inc.; retrieved on March 15, 2013 from http://www.universalcorp.com/Operations/Tobacco/Tobacco-WorldLeafProduction.asp?Menu=

(3) Project (April 2009). ITC Netherlands Survey Summary, University of Waterloo, Waterloo, Ontario, Canada, and STIVORO, The Netherlands; retrieved on March 15, 2013 from http://www.itcproject.org/download/keyfindi/itcnetherl?attachment=1

(4) Vereniging Nederlandse Kerftabakindustrie; retrieved on March 15, 2013 from http://en.kerftabak.nl/item.html&objID=576

(5) Crystal Dawn Hege Byrd et al., Fire-Cured Tobacco Extract and Tobacco Products Made Therefrom, U. S. Patent Application 20120125354, May 24, 2012.

(6) J.C. Leffingwell, E.D. Alford, Phenolic Aroma Constituents of Kentucky Fire-Cured Tobacco, 52nd Tobacco Science Research Conference, Atlanta, Georgia, Sept. 14, 1998, Paper No. 17.

(7) D.L. Davis, B.H. Song & W.W. Weeks, Chemical composition of dark air-cured and fire-cured tobacco. Paper presented at 35th Tob. Chem. Res. Conf., Winston-Salem, NC, Oct. 6-9, 1981 (abstract 30); D . L . Davis, B. H . Song, G. Everett, W. W. Weeks, Chemical Characterization of Dark Tobacco Semi-Volatile Oils, Coresta 7th International Tobacco Conference, Manila, Nov. 10-14, 1980; retrieved on March 15, 2013 from http://legacy.library.ucsf.edu/tid/kzl18a99

(8) Inger Wahlberg, Tommy Ringberger, Smokeless Tobacco in Tobacco : Production, Chemistry and Technology, D.L. Davis, Ed., Blackwell Publishing Ltd., 1999, Chapter 14 pp. 452-460.

(9) Adam Muller, Clear tobacco aroma oil, a process for obtaining it from a tobacco extract, and its use, U.S. Patent 4,506,682 (March 26, 1985)

(10) S.E. Stein, director, NIST Mass Spec Data Center, "Retention Indices", in NIST Chemistry WebBook, NIST Standard Reference Database Number 69 (P.J. Linstrom and W.G. Mallard, eds.), National Institute of Standards and Technology, Gaithersburg MD, 20899, http://webbook.nist.gov, (retrieved September 20, 2010).

(11) ESO 2000 (Update 2006) - The complete database of essential oils, Boelens Aroma Chemical Information Service. 2006, Leffingwell & Associates, Pub., Canton GA, USA

(12) R.P. Adams. Identification of essential oil components by gas chromatography/quadrapole mass Spectrometry, 4th Ed. Allured Pub. Corp., Carol Stream IL (2007)

(13) E. Kovats. Helv. Chim. Acta 41, 1915-32 (1958)

(14) H. van den Dool, P. D. Kratz. J. Chromatogr. 11, 463–471 (1963)

(15) Andy Bailey, Harvesting, Curing, and Preparing Dark Fire-Cured Tobacco for Market, Kentucky Cooperative Extension Service, University of Kentucky – College of Agriculture, Publication AGR 152, April 2006; retrieved on March 17, 2013 from http://www.ca.uky.edu/agc/pubs/agr/agr152/agr152.pdf.

(16) Igor Jerkovic, Dragan Kovacevic, Drago Subaric, Zvomimir Marijanovic, Krešimir Mastanjevic, Kristina Suman, Authentication study of volatile flavour compounds composition in Slavonian traditional dry fermented salami "kulen", Food Chemistry, Volume 119, Issue 2, 15 March 2010, Pages 813–822.

(17) Marıa D. Guillen and Marıa L. Ibargoitia, New Components with Potential Antioxidant and Organoleptic Properties, Detected for the First Time in Liquid Smoke Flavoring Preparations, J. Agric. Food Chem., 1998, 46(4), 1276–1285.

(18) Kelly Ross and Giuseppe Mazza, Comparative Analysis of Pyrolysis Products from a Variety of Herbaceous Canadian Crop Residues, World Journal of Agricultural Sciences 7 (6): 763-776, 2011.

(19) Qiang Lu, Zhi-Fei Zhang, Chang-Qing Dong and Xi-Feng Zhu, Catalytic Upgrading of Biomass Fast Pyrolysis Vapors with Nano Metal Oxides: An Analytical Py-GC/MS Study, Energies, 2010, 3, 1805-1820.

(20) Igor Z. Stojanovic, Niko S. Radulovic, Tatjana Lj. Mitrovic, Slavisa M. Stamenkovic, Gordana S. Stojanovic, Volatile constituents of selected Parmeliaceae lichens, J. Serb. Chem. Soc. 76 (7) 987–994 (2011).

(21) Nam-Chan Kim and Min-Seop Eom, Fast Pyrolysis of Biomass for Generation of Value Added Chemicals in a Bubbling Fluidized Bed Pyrolyzer, J. of the Korean Society for Environmental Analysis, 11, No. 1, 75-81, 2008.

(22) John C Leffingwell and E. D. Alford, Volatile constituents of perique tobacco, Electronic Journal of Environmental, Agricultural and Food Chemistry, 4(2), 2005. March - April Issue.

(23) Obrenija Kalamanda, Zoran Kukric, Miroslava Nikolic, Isolation, Chemical and Microbiological Characterization of Essential Oils from Tobacco Dust, Acta Agriculturae Serbics, Vol. VI, 12, 65-72, 2001.

(24) Wang Dongshan, Peng Qianrong, Yang Zaibo, Tang Ke, Cai Yuanqing, Analysis and Comparison of the Important Aroma Components in Flue-cured Tobacco Leaves from Different Regions and Different Grades, Flavour Fragrance Cosmetics, Dec. 2005, No. 6, pp. 14-18.

(25) Niko Radulovic, Milan Dekic, Zorica Stojanovic Radic, Radosav Palic, Chemical composition and antimicrobial activity of the essential oils of Geranium columbinum L. and G. lucidum L. (Geraniaceae), Turk. J. Chem., 35, 499-512, 2011.

(26) Kosuke Ikeya, Shuichi Yamamoto, Akira Watanabe, Semiquantitative GC/MS analysis of thermochemolysis products of soil humic acids with various degrees of humification, Organic Geochemistry 35, 583–594, 2004.

(27) J.C. Leffingwell, Identified in Latakia Tobacco headspace analysis (unpublished results).

(28) Claudio F. Lima, Luiz C. A. Barbosa, Cristiana R. Marcelo, Flaviano O. Silverio, and Jorge L. Colodette, Comparison between analytical pyrolysis and nitrobenzene oxidation for determination of syringyl/guaiacyl ratio in Eucalyptus spp.. Lignin. Bioresour. 3, 701–712, 2008.

(29) José C. del Río, Jorge Rencoret, Gisela Marques, Jiebing Li, Göran Gellerstedt, Jesús Jiménez-Barbero, Ángel T. Martínez and Ana Gutiérrez, Structural Characterization of the Lignin from Jute (Corchorus capsularis) Fibers, J. Agric. Food Chem., 57 (21), 10271–10281, 2009.

(30) Sladjana Alagic , Ivica Stancic , Radosav Palic , Gordana Stojanovic & Zika Lepojevi, Chemical Composition of the Supercritical CO_2 Extracts of the
Yaka, Prilep and Otlja Tobaccos, J. Essent. Oil Res., 18, 185-188, March/April 2006.

(31) Oluwatosin K. Yusuf and Clement O. Bewaji, GC-MS of volatile components of fermented wheat germ extract, Journal of Cereals and Oilseeds, Vol. 2(3), 38-42, 2011.

(32) Jiaojiao Zheng, Yicun Chen, Fen Yao, Weizhou Chen and Ganggang Shi, Chemical Composition and Antioxidant/Antimicrobial Activities in Supercritical Carbon Dioxide Fluid Extract of Gloiopeltis tenax, Marine Drugs, 10, 2634-2647, 2012.

(33) Source: Pherobase (www.pherobase.com) citing Allison C. Alberts, Thomas R. Sharp, Dagmar I. Werner, Paul J. Weldon, Seasonal variation of lipids in femoral gland secretions of male green iguanas (Iguana iguana), Journal of Chemical Ecology, 18, 703-712, 1992.

(34) Alan Rodgman and Thomas A. Perfetti, The chemical components of tobacco and tobacco smoke, CRC Press, Boca Raton, 2008.

(35) A. O. Lustre, Phillip Issenberg, Volatile components of hardwood sawdust smoke. Components of phenolic fraction, J. Agric. Food Chem., 17 (6), 1387–1393, 1969.

(36) Walter Fiddler, Robert C. Doerr, Aaron E. Wasserman, Composition of an ether-soluble fraction of a liquid smoke solution, J. Agric. Food Chem., 18 (2), 310–312, 1970.

(37) Denis E. Hruza, Michel. Van Praag, Howard Heinsohn, Isolation and identification of the components of the tar of hickory wood smoke, J. Agric. Food Chem., 22 (1), 123–126, 1974.

(38) Susi Soldera, Nerina Sebastianutto and Renzo Bortolomeazzi, Composition of Phenolic Compounds and Antioxidant Activity of Commercial Aqueous Smoke Flavorings, J. Agric. Food Chem., 56 (8), 2727–2734, 2008.

(39) Naim Montazeri, Alexandra C. M. Oliveira, Brian H. Himelbloom, Mary Beth Leigh, Charles A. Crapo , Chemical characterization of commercial liquid smoke products. Food Science & Nutrition, 1: 102–1, 2013.

(40) Brígida Fernández de Simón, Enrique Esteruelas, Ángel M. Muñoz, Estrella Cadahía and Miriam Sanz, Volatile Compounds in Acacia, Chestnut, Cherry, Ash, and Oak Woods, with a View to Their Use in Cooperage, J. Agric. Food Chem., 57 (8), 3217–3227, 2009.

(41) Joseph A. Maga, Contribution of Phenolic Compounds to Smoke Flavor in Phenolic Compounds in Food and Their Effects on Health I, ACS Symposium Series, Vol. 506, Chapter 13, 1992, pp. 170-179.

(42) S.C. Moldoveanu, Chapter 9. Analytical pyrolysis of lignins in Techniques and Instrumentation in Analytical Chemistry, Analytical Pyrolysis of Natural Organic Polymers, Volume 20, (1998), Elsevier B.V, pp. 327-351.

(39) Naim Montazeri, Alexandra C. M. Oliveira, Brian H. Himelbloom, Mary Beth Leigh, Charles A. Crapo , Chemical characterization of commercial liquid smoke products. Food Science & Nutrition, 1: 102–1, 2013.

(40) Brígida Fernández de Simón, Enrique Esteruelas, Ángel M. Muñoz, Estrella Cadahía and Miriam Sanz, Volatile Compounds in Acacia, Chestnut, Cherry, Ash, and Oak Woods, with a View to Their Use in Cooperage, J. Agric. Food Chem., 57 (8), 3217–3227, 2009.

(41) Joseph A. Maga, Contribution of Phenolic Compounds to Smoke Flavor in Phenolic Compounds in Food and Their Effects on Health I, ACS Symposium Series, Vol. 506, Chapter 13, 1992, pp. 170-179.

(42) S.C. Moldoveanu, Chapter 9. Analytical pyrolysis of lignins in Techniques and Instrumentation in Analytical Chemistry, Analytical Pyrolysis of Natural Organic Polymers, Volume 20, (1998), Elsevier B.V, pp. 327-351.