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Comparison of the volatile flavor compounds in different tobacco types by different extraction method

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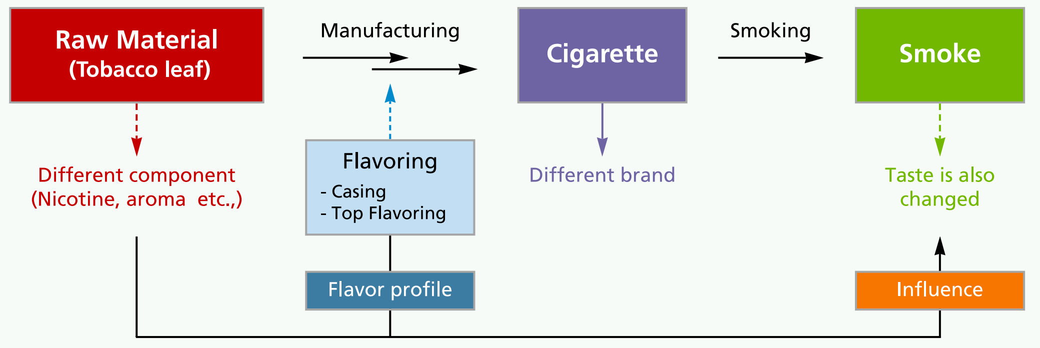
Abstracts

Traditional simultaneous distillation extraction(SDE) and solid-phase micro extraction(SPME) methods using GC/MS were compared for their effectiveness in the extraction of volatile flavor compounds from different tobacco leaves types(flue-cured, burley, oriental). The major volatile flavor compounds of flue-cured and burley tobacco were similar such as neophytadiene, solanone, megastigmatrienone isomers, β-damascenone and β-ionone. On the other hand, volatile flavor compounds such as norambreinolide, sclareolide were specifically identified in oriental tobacco. Each method was used to evaluate the responses of some analytes from real samples and standards in order to provide sensitivity comparisons between two techniques. Among three types of SPME fibers such as PDMS(Polydimethylsiloxane), PA(Polyacrylate) and PDMS/DVB (Polydimethyl-siloxane/Divinylbenzene) which were investigated to determine the selectivity and adsorption efficiency, PDMS/DVB fiber was selected for the extractions of the volatile flavor compounds due to its effectiveness. The qualitative analysis showed that the total amount of volatile flavor compounds in SDE method(130 species) was much more than that in SPME method(85 species). SPME method was more efficient for all the highly volatile compounds than SDE method, but on the other hand, low-volatile compounds such as fatty acids or high-molecular hydrocarbons were detected in SDE method. SPME method based on a short-time sampling can be adjusted to favor a selected group compounds in tobacco. Furthermore this results could be used to estimate the aroma characteristics of cigarette blending by using a different type of tobacco with more effectiveness and convenience.

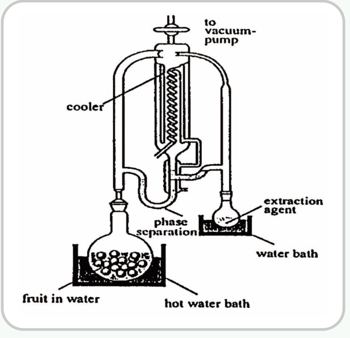
Introduction



- The character of flavor is quite important to the end product of tobacco industry
- Flavoring material is attributed to tobacco taste in some extend
- So understanding of flavor profile in tobacco is quite helpful for the development of product

Experimental

• Simultaneous Distillation Extraction (SDE)

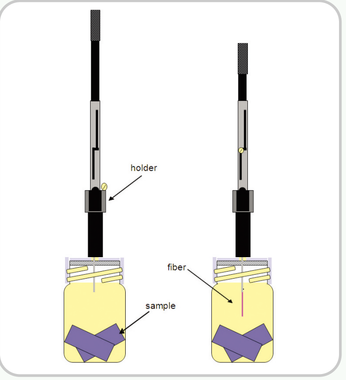


- Solvent is immiscible with and less dense than water
- Extraction time 2 to 3 hours
- Extracts is dried then concentrated before analysis
- Solvent (n-Hexane : Diethyl ether = 1:1)

Benefits
- Provides efficient stripping of aroma volatiles from samples
- Multiple analyses can be obtained from one extract → Analysis by GC, GC-MS etc.
- Complete extraction is possible
- Extract can be fractionated by column chromatography to give increased resolution

Drawbacks
- Losses of low-boiling volatiles
- Artifacts can be formed
- Not suitable for polar volatiles

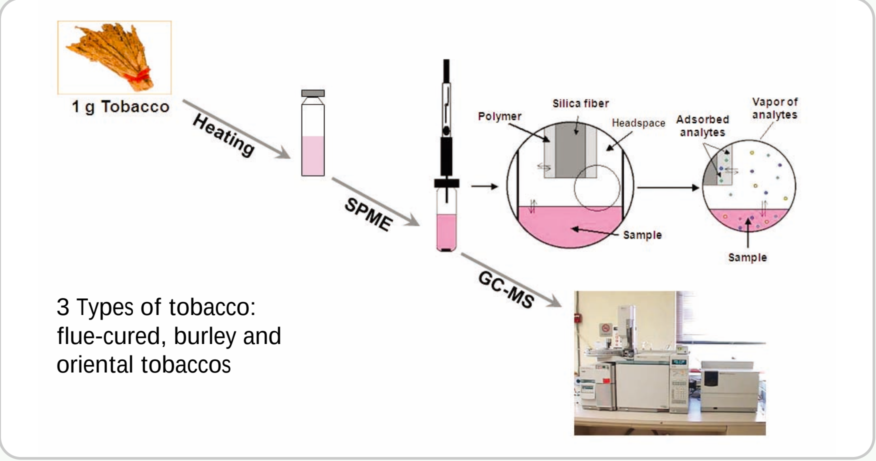
• Solid-Phase Microextraction (SPME)



- Collected onto a fiber coated with adsorbant or absorbant polymer
- Equilibrium between sample, fiber and headspace
- Needle can pierce septum in collection vessel and in GC injection port.
- Several types of fiber available with different properties.

Benefit
- Suitable for any sample or odorous material
- Minimal artefact formation
- Moderate sensitivity
- Suitable for low-boiling compounds
- Relatively good results with polar compounds (adsorption)
- Uses conventional splitless injector
- Simple to use

• SPME experimental procedure



• SPME and GC-MS Conditions

SPME Condition	
Extraction	30 min at 90℃
Fiber	65 ㎛ PDMS/DVB (Polydimethylsiolxane/divinylbenzene)
Adsorption	3 min
Desorption	2 min
GC/MS Condition	
Instrument	Agilent 6890N/5973i MSD
Scan range	m/z 40~400
Column	DB-WAX(30 m X 0.25 mm id, 0.25 ㎛ thickness)
Temp. program	50℃(3min) → 2℃/min → 240℃(30 min)
Ionization	Electron Impact
Injection	Split ratio(30:1), 1 ㎕
Carrier gas	Helium 1.1 mL/min

Study object

- To obtain useful information about flavor profile of different tobacco types and choosing adequate flavor analysis method is important
- To estimate the aroma characteristics of cigarette blending by using a different type of tobacco with more effectiveness and convenience.

Result

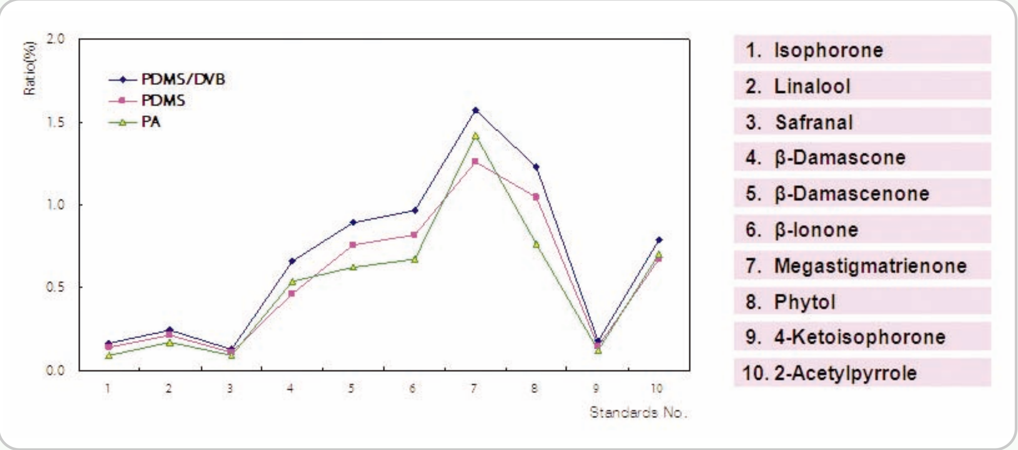


Figure 1. Response of the three SPME fibers to 10 standards.

The PDMS/DVB fiber was shown to be the most efficient at extracting the selected standards. The parameters were optimized : 90℃ adsorption temperature, 3 min of adsorption time, 250℃ desorption temperature and 2 min of desorption time.

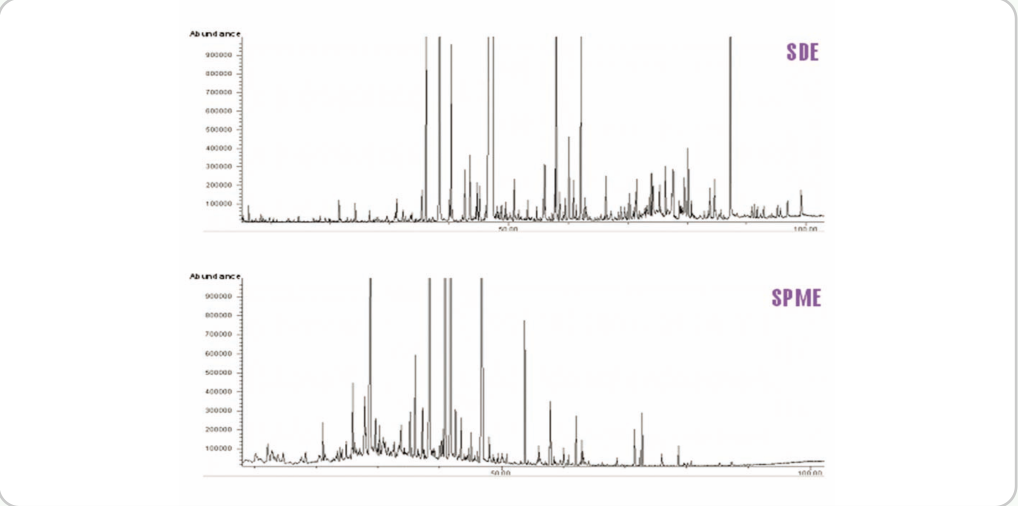


Figure 2. GC/MS chromatograms of the volatile compounds in flue-cured tobacco by different extraction methods.

Table 1. Volatile compounds identified in three-types tobacco leaf by different extraction methods.

No.	Compounds	R.T (min)	SDE Response ratio(%) Flue-cured	SDE Burley	SDE Oriental	SPME Flue-cured	SPME Burley	SPME Oriental
1	Citral	7.41	0.1258	0.0004	0.5191			
2	Limonene	8.25	0.1007	0.1092	0.0038	0.0685	0.0709	0.0026
3	n-Dodecane	8.44	0.0423	0.0368	0.1652			
4	Isopentyl alcohol	8.62	0.2491	0.2242	0.1120			
5	2-Hexenal	9.00	0.0603	0.0318	0.1298			
6	2-Hexanol	9.20	0.2293	0.0034	0.0011			
7	2-Pentyl furan	9.74	0.2495	0.2036	0.9473	0.1697	0.1323	0.6536
8	6-Methyl-2-heptanone	9.86	0.1009	0.1234	0.1851			
9	n-Hexanal diethyl acetal	9.80	0.2613	0.6430				
10	2,6-Dimethypyridine (2,6-Lutidine)	10.08	0.0059	0.0214	1.6453			
11	2,6-Dimethylphenol	10.44	0.1209	0.0718	0.1850			
12	2-Methyldihydro-3(2H)-furanone	10.98	0.1817	0.1534	0.0905	0.1235	0.0997	0.0624
13	1,2,4-Trimethylbenzene	11.69	0.0243	0.0122	0.0964	0.0165	0.0079	0.0665
14	4-Methylpyridine	11.92	-	-	0.0689			
15	2-Isopropylpyridine	12.30	-	-	0.2348			
16	n-Tridecane	12.95	0.1388	0.1136	0.1173	0.0944	0.0738	0.0809
17	cis-2-(2-Pentenyl)furan	13.01	0.0464	0.0689	0.1766	0.0316	0.0448	0.1219
18	2,2,6-Trimethylcyclohexanone	13.19	0.0721	0.0431	0.1296	0.0490	0.0280	0.0894
19	2-(1-Methylbutyl)oxirane	13.54	0.0518	0.0502	0.1258			
20	3-Methyl-2-butenol(Prenyl alcohol)	13.86	0.1482	0.0242	0.1885	0.1008	0.0157	0.1301
21	6-Methyl-5-heptene-2-one	14.63	0.1900	0.1521	1.1294	0.1292	0.0989	0.7793
22	1-Hexanol	15.55	0.0681	0.1055	0.1155	0.0463	0.0686	0.0797
23	Cis-3-Hexenol	17.13	0.2163	0.1058	0.2329	0.1471	0.0688	0.1607
24	4-Methyl-3-penten-1-ol	17.41	0.0599	0.0264	0.1021			
25	3-Pentyl furan	17.59	0.0760	0.0487	0.1522	0.0516	0.0316	0.1050
26	Citronellyl 2-butenoate	18.01	0.0956	0.0071	0.7624			
27	n-Tetradecane	18.25	0.2837	0.3150	0.1776	0.1929	0.2047	0.1226
28	1,4-Dimethyl-3-cyclohexenyl methyl ketone	19.16	0.3209	0.0123	0.1394			
29	Furfural	21.45	2.3998	2.0326	2.8451	2.6319	2.3212	2.9631
30	Acetic acid	21.52	0.3072	0.5273	1.6418	0.2089	0.3427	1.1328
31	β-Ionene	21.84	0.1044	0.0021	0.0034	0.0710	0.0014	0.0023
32	2,4-Heptadienal	22.78	0.3593	0.0443	0.4822			
33	2-Ethylhexanol	23.21	0.1004	0.1236	0.0729			
34	2-Ethyl-5-methylfuran	23.52	0.4215	0.3590	0.5596	0.2866	0.2334	0.3861
35	n-Pentadecane	23.91	0.1514	0.1703	0.1313	0.1030	0.1107	0.0906
36	Benzaldehyde	24.21	1.4098	0.6235	3.6243	0.9586	0.4053	2.5007
37	trans-2-Nonenal	25.19	0.0300	0.0175	0.0895			
38	Nonanal diethyl acetal	25.39	0.1674	0.6159	-			
39	2-Methyl-2-nonen-4-one	25.77	0.0851	0.1503	0.1223	0.0578	0.0977	0.0844
40	Linalool	26.55	0.3578	0.1455	0.2072	0.2433	0.0946	0.1430
41	5-Methyl furfural	27.29	0.2920	0.0112	0.4314	0.1986	0.0073	0.2977
42	Isophorone	27.56	0.2462	0.2932	0.3275	0.1674	0.1906	0.2260
43	Hexanoic anhydride	27.75	0.3335	0.0526	1.8777	0.2268	0.0342	1.2956
44	2-Cyclopentene-1,4-dione	27.79	0.2995	-	-	0.2037	-	-
45	6-Methyl-3,5-heptadien-2-one	28.30	0.2188	0.0780	1.3835	0.1488	0.0507	0.9546
46	3,3,5-Trimethylcyclohexene	28.71	0.0473	0.0183	0.2005			
47	p-Ethoxyanisole	29.44	0.1952	0.0382	1.3408	0.1327	0.0248	0.9251
48	n-Hexadecane	29.57	0.2197	0.2677	0.3026	0.1494	0.1740	0.2088
49	4,5,6,7-Tetramethyl-2H-isoindole	29.87	0.0560	0.0104	0.0380			
50	Safranal	30.80	0.2311	0.1358	0.0641	0.1571	0.0883	0.0443
51	Benzeneacetaldehyde	30.95	2.5398	3.3871	1.5265	1.7271	2.2016	1.0533
52	1-Undecanol	31.20	0.5250	0.0313	0.3142	0.3570	0.0203	0.2168
53	Acetaldehyde ethyl amyl acetal	32.24	0.8180	0.4334	0.1657			
54	2-Furanmethanol(furfural alcohol)	32.64	0.1394	0.1203	0.2453	0.0948	0.0782	0.1692
55	Farnesyl alcohol(fanesol)	33.18	0.1077	0.0399	0.3859	0.0733	0.0259	0.2663
56	4-Ketoisophorone	33.45	0.2583	0.6974	0.8785	0.1756	0.4533	0.6062
57	4-Methyl-Valeric acid,methyl ester	33.52	0.4031	0.0002	1.2035			
58	n-Decyl acetate	33.74	0.1910	0.0651	-			
59	Terpineol	34.23	0.0777	0.0241	0.1097	0.0529	0.0157	0.0757
60	Eucarvone	34.87	0.0570	0.0766	0.1240	0.0388	0.0498	0.0856
61	n-Heptadecane	35.13	0.1253	0.1615	0.3864	0.0852	0.1050	0.2666
62	Phenylacetaldehyde	35.43	1.7846	2.6509	-	1.2135	1.7231	-
63	Neophytadiene	35.56	8.8641	6.4515	4.6645	6.0276	4.1935	3.2185
64	Solanone	36.08	4.1531	3.1241	3.3955	2.8241	2.0307	2.3429
65	3,4,7-Trimethyl-1-indanone	36.65	0.1887	-	0.0458			
66	β-Damascone	40.00	0.9705	0.6300	1.0648	0.6599	0.4095	0.7347
67	β-Damascenone	40.32	10.5588	2.8708	8.0433	0.8953	0.4695	0.7368
68	1,5,8-Trimethyl-dihydronaphthalene	40.52	0.7145	0.0053	0.1732			
69	3-Phenylaniline	41.19	0.0333	0.0155	0.4851			
70	3,3-Dimethyl-6-methylene-1-cycolhexene	42.22	0.1463	0.1496	0.7484			
71	trans-Geranylacetone	42.58	0.9738	0.7748	0.5876	0.6622	0.5036	0.4054
72	n-Hexanoic acid(Caproic acid)	42.75	0.2930	0.0595	1.2606	0.1993	0.0386	0.8698
73	Propanoic acid, 2-methyl- 2-ethyl-3-hydroxyhexyl ester	43.21	0.1079	-	0.2905	0.0733	-	0.2005
74	Terpinolene	43.29	0.0396	-	0.4224	0.0269	-	0.2915
75	Benzyl alcohol	43.47	4.0925	0.0584	2.2376	2.7829	0.0380	1.5439
76	Isolimonene	43.98	0.1264	0.0671	0.2063	0.0860	0.0436	0.1423
77	4-Carene	44.47	0.3554	0.7008	0.5900	0.2417	0.4555	0.4071
78	Fenchene	44.67	1.0663	0.9011	2.0019	0.7251	0.5857	1.3813
79	Phenylethyl Alcohol	45.08	4.1846	0.1752	4.4805	2.8455	0.1139	3.0915
80	Hydroxy(4-hydroxyphenyl) acetic acid	45.94	0.2270	-	0.6100	0.1544	-	0.4209
81	β-Ionone	46.11	1.4176	0.4860	1.1382	0.9640	0.3159	0.7854
82	Pinene	46.75	1.9552	3.3881	4.5352	1.3296	2.2023	3.1293
83	Caryophyllene oxide	47.59	-	-	0.1536			
84	2-Methyl-1,3-benzenediol	47.76	0.1958	-	-			
85	2-Acetylpyrrole	48.02	1.1639	0.0279	0.3265	0.7915	0.0181	0.2253
86	4,4-Dimethyl-2-cyclohexenone	48.25	0.1560	-	-	0.1061	-	-
87	β-Ionone epoxide	48.63	0.7926	0.4200	0.6093	0.5390	0.2730	0.4204
88	4-Hexyloxyaniline	48.84	0.7989	0.3146	1.6498	0.5433	0.2045	1.1384
89	4-(2,6,6-Trimethylcyclohexyl)but-3-en-2-one	49.26	0.2524	0.1090	0.2249	0.1716	0.0708	0.1552
90	4,6-Quinolinediamine	49.43	0.6538	0.2679	1.0434	0.4446	0.1742	0.7200
91	5-Amino-1-phenylpyrazole	49.63	0.2333	0.0369	0.2794	0.1587	0.0240	0.1928
92	2-Methyl-2,3-dihydrobenzofuran	50.25	0.3140	0.2090	0.3579	0.2135	0.1359	0.2470
93	Methyl myristate	50.54	0.1433	0.0756	0.2128	0.0974	0.0491	0.1468
94	5-Ethyl-m-xylene	50.90	1.4724	0.0277	0.3499	1.0013	0.0180	0.2414
95	Nerolidol	52.09	0.1094	0.0754	0.2047	0.0744	0.0490	0.1412
96	n-Octanoic Acid (Caprylic acid)	53.18	1.1137	0.2571	1.4795	0.7573	0.1671	1.0209
97	N,N-Dimethyl-4-aniline	54.73	0.5417	0.0835	0.2397	0.3684	0.0543	0.1654
98	Methyl pentadecanoate	55.41	0.0938	0.0697	0.0795	0.0638	0.0453	0.0549
99	Hexahydro farnesyl acetone	55.83	0.5910	4.0329	3.6263	0.4018	2.6214	2.5022
100	Megastigmatrienone 1	56.06	1.3497	0.3806	0.5451	0.9178	0.2474	0.3761
101	3-Amino-2-cyclohexenone	57.31	0.3968	0.2619	0.9303	0.2699	0.1703	0.6419
102	Megastigmatrienone 2	57.95	4.9653	0.0985	0.7757	3.3764	0.0640	0.5352
103	Myristic alcohol	58.20	0.1460	0.1346	0.3234	0.0993	0.0875	0.2232
104	4-Vinylguaiacol	58.52	2.4652	-	-	1.6763	-	-
105	3-(4,8,12-trimethyltridecyl) furan	59.49	1.8985	7.9817	12.1338	1.2909	5.1881	8.3723
106	Palmitic acid, methyl ester	60.08	4.3597	0.7982	1.4071	2.9646	0.5188	0.9709
107	Megastigmatrienone 3	60.84	1.0953	0.0148	0.1043	0.7448	0.0097	0.0720
108	1-Vinylcyclododecanol	61.05	0.0008	0.7306	0.6066			
109	3-Isopropenyl-5-methylcyclohexene	61.29	0.2663	0.1516	0.1116			
110	Palmitic acid, ethyl ester	61.76	0.2066	0.3514	0.4291			
111	Megastigmatrienone 4	62.10	5.2565	0.0095	0.1214	3.5744	0.0062	0.0837
112	n-Decanoic acid (Capric acid)	62.73	0.6986	0.1291	0.9816	0.4750	0.0839	0.6773
113	2(4H)-Benzofuranone, 5,6,7,7-tetrahydro-4,4,7-trimethyl	63.02	0.8249	0.1210	1.6801			
114	Isophytol	63.49	0.2987	0.9806	0.6421	0.2031	0.6374	0.4430
115	Cuminyl acetate	63.71	0.0659	-	-	0.0448	-	-
116	7,10,13-Hexadecatrienoic acid, methyl ester	65.45	0.1160	-	-			
117	Farnesyl acetone	66.33	1.3031	1.5025	1.4837			
118	Indole (Benzopyrrole)	68.48	0.4743	0.2460	1.6975	0.3225	0.1599	1.1713
119	Heptadecanoic acid, methyl ester	68.89	0.2810	0.0590	0.0934	0.1911	0.0384	0.0645
120	11-Octadecenoic acid,methyl ester	69.49	0.1685	0.0976	-			
121	8,11-Octadecadienoic acid, methyl ester	71.36	0.4338	0.0689	0.0964			
122	Vanillin	73.23	0.7180	0.0499	0.1107			
123	Thunbergol	73.64	0.3077	0.0826	1.0013			
124	Linolenic acid, methyl ester	73.97	1.0934	0.0799	0.3804	0.7435	0.0520	0.2625
125	Phytol	76.36	1.8064	0.4717	4.9336	1.2284	0.3066	3.4042
126	3-Methyloctadecane	79.92	0.7389	0.3275	2.8630			
127	5-Hydroxy-3-methyl-1-indanone	80.71	1.1057	-	-			
128	Pentadecanoic acid	83.52	0.1903	0.0274	0.5142			
129	Sclaral (sclareolide lactol)	84.24	-	-	0.2260			
130	5-Methyl-1-cyclopentene-1-carboxylic acid	85.66	0.1375	-	0.0722			
131	Palmitic acid	87.26	7.7973	0.3658	5.9471			
132	Norambreinolid	87.61	-	-	1.3670	-	-	0.9432
133	Oleic Acid	95.21	0.2324	-	0.1188			
134	Diphenyl sulfone	95.74	1.0490	1.5694	-			
135	Linoleic acid	96.88	0.4904	0.0416	0.1792			

Table 2. Comparison of number of functional groups identified in three-types tobacco by different extraction methods.

Functional groups	SDE Flue-cured	SDE Burley	SDE Oriental	SPME Flue-cured	SPME Burley	SPME Oriental
Ketones	24	21	22	21	19	18
Nitrogenous	11	10	12	7	7	7
Alcohols	22	21	21	15	14	14
Aldehydes	13	12	10	6	5	5
Esters	13	10	9	7	5	6
Acids	11	8	11	5	4	5
Hydrocarbons	17	16	17	11	9	10
Miscellaneous	19	19	20	13	13	14
Total	130	117	122	85	76	79

Conclusion

This study clearly shows that comparison for their effectiveness in the extraction method of volatile flavor compounds from different tobacco leaves types (flue-cured, burley, oriental)

Traditional SDE analysis of volatile compounds is a widely used technique. However the SDE method lacked the sensitivity and convenience needed to perform adequately.

Comparison of SDE and SPME showed that SPME determinations of flavor volatile compounds were more sensitive under the conditions employed in this study. Different SPME fiber were investigated to determine the selectivity of the various fiber. The PDMS/DVB fiber was shown to be the most efficient at extracting the selected standards. Consequently, SPME was suitable for simple, rapid, routine screening, while SDE was used for proper quantitative analysis.