Composition Studies on some Varieties of Tobacco and their Smoke

I. Major Components in Smoke Condensate*

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INTRODUCTION

One type of commercial cigarette is made up by blending different varieties of tobacco, i.e. flue-cured, Burley and Turkish tobacco. Each variety of tobacco generates its own aroma and taste when smoked. Many investigations have been carried out in order to elucidate the compositional differences of smoke components from the different varieties of tobacco. β -Methylvaleric acid has been found to be present in large quantity in Turkish tobacco smoke (1) while pyridines are higher in concentration in Burley and Turkish tobacco smoke (2). Kaburaki et al. performed a series of analyses on the volatile components in tobacco smoke and determined the phenolic (3), basic (4) and acidic (5) compositional differences between varieties of tobacco. Ishiguro et al. compared the smoke components from lamina and midrib of flue-cured tobacco smoke (6–11).

We have undertaken comprehensive investigations in order to elucidate the relationship between organoleptic properties and the composition of tobacco smoke.

In this paper, we report on the major smoke semi-volatiles, which significantly contribute to aroma, and the non-volatiles, which are mainly associated with taste, from three varieties of tobacco described above as determined by glass capillary column gas chromatography.

The aroma of tobacco smoke is especially affected by its content of N-containing compounds in the semi-volatile phase (12, 13). We have therefore also studied the content of such compounds in tobacco smoke by the use of a gas chromatograph equipped with an alkali flame detector.

EXPERIMENTAL

Sample Cigarettes

The cigarettes used in this study were 70 mm in length, 25 mm in circumference, and 65 ± 5 mm H₂O in pressure drop. The weights of the sample cigarettes were as follows:

flue-cured (American Type 11a, leaf position) 1.09 ± 0.05 g, flue-cured (American Type 11a, cutter position) 0.95 ± 0.05 g, Burley (Kentucky, leaf position) 0.92 ± 0.05 g, Burley (Kentucky, cutter position) 0.79 ± 0.05 g, Turkish (Basma-M) 1.05 ± 0.05 g, Turkish (Samsun) 0.96 ± 0.05 g. All cigarettes were conditioned at 20 °C and 60% relative humidity prior to use.

Sample Preparation for Analysis for N-containing Compounds

Smoke from three cigarettes was collected on a glass fiber filter pad (Toyo Roshi Co., GB-100R, 37 mm diameter and 0.7 mm thick) under the standard smoking conditions (1 puff/min, 2 s/puff, 35 ml/puff, 30 mm butt length). Immediately after smoking was completed, a circle of ca. 29 mm diameter was cut from the part of the filter pad retaining condensate and was placed in a 0.5 ml vial. Dichloromethane (300 μ l) and 2-(β -hydroxyethyl)pyridine (100 μ g) as internal standard were added and the vial was kept at room temperature for 15 min. The filter pad in the vial was agitated by a micro-spatula and 1 μ l of the dichloromethane solution was injected into the gas chromatograph.

Gas chromatographic conditions:

Instrument:	Shimadzu (Model 7 AG).
Column:	30 m $\times$ 0.25 mm inside diameter glass capillary column coated with PEG-20M.
Carrier gas:	Helium at a flow rate of 1.0 ml/min.
Column temperature:	50–170 °C at 2 °/min.
Detector:	Alkali flame detector (flame thermionic detector)
Split ratio:	1/50.

Sample Preparation for Semi-Volatile Phase Analysis

A glass fiber filter pad, on which smoke from three cigarettes was collected in the same manner as that described

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Table 1. Levels of selected major components in flue-cured (American Type 11a, leaf position) tobacco smoke.

Compound	Analysis			Coefficient of variation (%)
	1	2	3	
(µg/cigarette)				
SEMI-VOLATILES				
Limonene	34.0	38.2	32.3	7.1
2-Furfural	25.8	32.7	33.0	10.9
γ-Butyrolactone	25.3	29.6	27.6	6.4
2-Hydroxy-3-methyl-2-cyclopentenone	54.5	51.1	59.6	6.3
p-Cresol	28.9	32.6	27.3	7.5
NON-VOLATILES				
Ethylene glycol	18.3	21.4	20.5	6.5
Catechol	100.8	102.4	100.3	0.9
Levoglucozan	187.2	205.9	218.3	6.3

above, was put into a vial. Dichloromethane (300 µl) and ethyl laurate (150 µg) as internal standard were added and the vial was kept at room temperature for 15 min. The filter pad in the vial was agitated by a micro-spatula

and 1 µl of the dichloromethane solution was injected into the gas chromatograph.

Gas chromatographic conditions:

Instrument:	Shimadzu (Model 5A).
Column:	30 m × 0.25 mm inside diameter glass capillary column coated with PEG-20M.
Carrier gas:	Helium at a flow rate of 1.0 ml/min.
Column temperature:	50–170 °C at 2 °/min.
Detector:	Flame ionization detector.
Split ratio:	1/50.

Sample Preparation for Non-Volatile Phase Analysis

A glass fiber filter pad, on which smoke from three cigarettes was collected in the same manner as that described above, was put into a vial. BSTFA [bistrimethylsilyltri-fluoroacetamide] (300 µl) and 1,3-butylene glycol (150 µg) as internal standard were added and the vial was allowed to stand for one hour at 80 °C. The filter pad in the vial was agitated by a micro-spatula and 0.4 µl of the BSTFA solution was injected into the gas chromatograph.

Table 2. Quantitative estimates of the major N-containing compounds in cigarette smoke (µg/puff).

Peak No.	Compounds	Flue-cured (American Type 11a)		Burley (Kentucky)		Turkish	
		leaf position	cutter position	leaf position	cutter position	Basma-M	Samsun
19	Pyridine	1.8	1.1	1.2	0.75	0.5	0.9
23	α-Picoline	1.2	1.1	1.2	1.0	0.9	1.2
29	Methylpyrazine	1.7	1.6	1.6	1.3	1.1	1.9
33	β-Picoline	1.4	1.2	1.4	1.2	0.9	1.0
34	γ-Picoline	0.8	0.9	1.0	0.9	0.7	0.8
40	2,5-Lutidine	0.4	0.4	0.4	0.4	0.3	0.6
41	2,5-Dimethylpyrazine	1.4	1.3	1.0	1.2	0.6	0.9
51	3-Ethylpyridine	0.3	0.2	0.4	0.2	0.2	0.8
62	3-Vinylpyridine	0.9	0.5	0.9	0.6	0.3	0.3
74	Pyrrole	2.9	1.6	2.5	1.8	1.0	1.8
98	3-Cyanopyridine	0.2	0.1	0.2	0.2	0.1	+
105	3-Acetylpyridine	0.7	0.6	0.8	0.6	0.5	0.7
107	Nicotine	381	315	486	400	272	85
142	Myosmine	1.8	1.0	6.5	5.5	1.6	1.3
153	3-(Dimethylpyrryl)pyridine	1.1	0.6	1.3	1.2	0.8	1.1
156	Nicotyrine	2.3	1.6	2.9	2.8	2.6	1.0
166	3-Hydroxypyridine	7.8	5.6	12	9.6	6.0	8.0
169	Indole + 2,3'-bipyridyl	5.3	3.5	5.7	6.0	1.3	0.5
178	5-Methyl-2,3'-bipyridyl	1.2	0.8	1.1	1.1	+	+
Total amounts (mg/puff)		0.41	0.34	0.53	0.44	0.29	0.11
Total amounts (mg/puff) (excluding nicotine)		0.033	0.024	0.042	0.036	0.019	0.023
Condensate (mg/puff)		2.82	2.64	3.11	2.72	2.56	2.28
Total puff number		11.1	8.8	8.3	6.4	11.8	9.4

+ : at trace level

Gas chromatographic conditions:

Instrument: Shimadzu (Model mini-1).
Column: 60 m × 0.25 mm inside diameter glass capillary column coated with OV-101.
Carrier gas: Helium at a flow rate of 1.1 ml/min.
Column temperature: 50–190 °C at 2 °/min.
Detector: Flame ionization detector.
Split ratio: 1/45.

Gas Chromatographic – Mass Spectrometric Conditions:

Instrument: Nippon Electric Varian (Model TE-600) mass spectrometer.
Ionization voltage: 70 eV.
Ion source temperature: 170 °C.

Identification

Peaks on the gas chromatograms of semi-volatiles and non-volatiles were tentatively identified by comparison of the gas chromatographic retention times with those of authentic compounds and confirmed by gas chromatography – mass spectrometry.

N-containing compounds were identified by comparison of gas chromatographic retention times with those of authentic compounds and by the gas chromatographic profiles reported by *Ishiguro* and *Sugawara* (8) for N-containing compounds.

Quantitative Analysis

Calibration curves were prepared on the basis of the relation between concentration and peak area of authentic compounds and that of an internal standard. Levels of the major components were calculated from the calibration curves.

RESULTS AND DISCUSSION

The satisfactory reproducibility of the analytical methods used in this study is illustrated in Table 1.

N-Containing Compound Analysis

A gas chromatogram of N-containing compounds in Burley (Kentucky, leaf position) tobacco smoke condensate is illustrated in Fig. 1, while levels of major components are listed in Table 2. The major N-containing components from the three varieties of tobacco were compared in terms of their deliveries per puff, which should be closely related to the organoleptic properties. The total amount (µg/puff) of the 18 determined components excluding nicotine was higher in Burley tobacco smoke than in the smoke from other varieties of tobacco. Again excluding nicotine, 3-hydroxypyridine was present in the highest concentration in all smokes analysed. 2,5-Dimethylpyrazine was present in high concentration in flue-cured tobacco smoke. 3-Cyanopyridine, nicotine and myosmine

Figure 1. Gas chromatogram of N-containing compounds in Burley (Kentucky, leaf position) tobacco smoke.

Table 3. Quantitative estimates of the major components in the semi-volatile phase of cigarette smoke ($\mu\text{g/puff}$).

Peak No.	Compounds	Flue-cured (American Type 11a)		Burley (Kentucky)		Turkish	
		leaf position	cutter position	leaf position	cutter position	Basma-M	Samsun
24	Limonene	3.1	2.8	1.8	1.8	1.2	0.6
41	1-Hydroxy-2-propanone (Acetol)	4.3	5.0	2.5	1.9	2.2	2.8
51	2-Cyclopentenone	1.4	1.7	1.1	1.4	0.8	1.3
55	2-Methyl-2-cyclopentenone	1.0	1.2	1.1	1.4	0.7	1.1
75	Acetic acid	53	52	16	12	11	24
79	2-Furfural	2.7	2.9	1.1	1.2	0.6	0.9
87	3-Methyl-2-cyclopentenone	1.3	1.2	1.1	1.0	0.6	1.2
92	2,3-Dimethyl-2-cyclopentenone	0.9	0.9	0.7	0.7	0.5	1.0
94	Propionic acid	3.1	2.9	1.1	0.9	1.0	1.8
101	5-Methylfurfural	1.9	1.8	0.4	0.3	0.7	0.6
111	γ -Butyrolactone	2.5	2.3	1.5	1.0	1.4	2.3
115	Butyric acid	2.7	2.4	1.3	1.4	1.8	+
124	Furfuryl alcohol	4.7	4.4	1.5	1.0	1.9	2.1
145	Cyclopentan-1,2-dione	2.2	1.9	0.9	0.7	1.0	1.3
154	β -Methylvaleric acid	+	+	+	+	9.8	7.8
160	2-Hydroxy-3-methyl-2-cyclopentenone	5.0	5.3	2.8	2.4	2.5	3.8
188	Neophytadiene	23	35	31	34	12	17
193	Phenol	10	9.4	6.7	5.1	5.8	7.6
198	4-Hydroxy-2,5-dimethyl-3(2H)-furanone	7.4	7.5	2.8	2.2	2.8	3.5
205	2,5-Dihydroxyvaleric acid γ -lactone (?)	2.4	2.3	1.1	0.7	0.9	1.5
209	p-Cresol	2.7	2.3	2.3	1.9	1.9	2.6
211	m-Cresol	1.4	1.3	1.3	0.9	0.8	1.1
222	2,4-Dihydroxybutyric acid γ -lactone	4.5	4.2	3.3	2.2	3.1	3.5
239	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	32	28	4.2	2.2	3.7	13
280	5-Hydroxymethylfurfural	11	10	+	+	+	+
Total amounts (mg/puff)		0.18	0.19	0.09	0.08	0.07	0.10

+ : at trace level

were found in higher concentration in Burley tobacco smoke whereas β -picoline, 3-vinylpyridine, indole + 2,3'-bipyridyl and 5-methyl-2,3'-bipyridyl were present in lower concentration in Turkish tobacco smoke.

Semi-Volatile Phase Analysis

Gas chromatograms of the semi-volatiles of flue-cured (American Type 11a, cutter position) and Turkish (Basma-M) tobacco smoke condensates are illustrated in Figures 2 and 3, respectively, while levels of the major components are shown in Table 3.

The total amounts ($\mu\text{g/puff}$) of the 25 determined components in flue-cured tobacco smoke were about twice as much as those in the smoke from other varieties of tobacco.

In flue-cured tobacco smoke, compounds which were pre-

sent in higher concentrations than in the smoke from other varieties of tobacco were as follows: carbohydrate pyrolysates (1-hydroxy-2-propanone, 2-furfural, 5-methylfurfural, γ -butyrolactone, furfuryl alcohol, cyclopentan-1,2-dione, 2-hydroxy-3-methyl-2-cyclopentenone, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one and 5-hydroxymethylfurfural) (14–17), a terpenoid breakdown product (limonene) (18) and others (acetic, propionic and butyric acid). 5-Hydroxymethylfurfural was present in especially high concentration. In Burley tobacco smoke, carbohydrate pyrolysates (5-methylfurfural and furfuryl alcohol) were present in lower concentration. In Turkish tobacco smoke, β -methylvaleric acid was present in much higher concentration while terpenoids (limonene and neophytadiene) were lower.

Figure 2. Gas chromatogram of the semi-volatile phase of flue-cured (American Type 11a, cutter position) tobacco smoke.

Figure 3. Gas chromatogram of the semi-volatile phase of Turkish (Basma-M) tobacco smoke.

Table 4. Quantitative estimates of the major components in the non-volatile phase of cigarette smoke ($\mu\text{g}/\text{puff}$).

Peak No.	Compounds	Flue-cured (American Type 11a)		Burley (Kentucky)		Turkish	
		leaf position	cutter position	leaf position	cutter position	Basma-M	Samsun
41	Ethylene glycol	1.8	1.8	1.7	0.9	1.8	1.8
62	Lactic acid	7.1	7.1	2.8	2.8	4.5	4.7
66	Glycolic acid	4.9	4.7	1.7	1.5	2.1	1.6
121	4-Vinylphenol	2.8	3.0	1.5	1.2	2.5	2.5
131	Glycerol	9.1	8.3	4.7	2.2	7.6	13
138	Catechol	16	17	3.5	3.6	8.6	14
155	4-Methylcatechol	2.1	2.3	0.9	0.8	0.8	0.7
158	Hydroquinone	17	20	8.6	7.5	4.5	9.0
173	4-Ethylcatechol	3.6	4.3	1.0	0.9	1.8	1.4
185	4-Vinylcatechol	5.0	7.1	0.9	0.8	3.1	2.1
242	Levogluconan	18	15	10	7.5	14	7.7
254	Glucometasaccharinic acid γ -lactone	5.4	7.5	1.0	0.7	1.7	1.1
255	Quinic acid γ -lactone	27	30	13	7.6	15	14
Total amounts (mg/puff)		0.12	0.13	0.05	0.04	0.07	0.07

Figure 4. Gas chromatogram of the non-volatile phase of flue-cured (American Type 11a, leaf position) tobacco smoke (trimethylsilyl derivatives).

Non-Volatile Phase Analysis

A gas chromatogram of the non-volatile phase from flue-cured (American Type 11a, leaf position) tobacco smoke is illustrated in Fig. 4, while the levels of the major components are summarized in Table 4. The total amounts ($\mu\text{g/puff}$) of the 13 determined components in flue-cured tobacco smoke were about twice as much as those in the smoke from other varieties of tobacco.

In flue-cured tobacco smoke, compounds which were present in higher concentrations than those in the smoke from other varieties of tobacco were as follows: chlorogenic acid pyrolysates (catechol, 4-methylcatechol, 4-ethylcatechol, 4-vinylcatechol and quinic acid γ -lactone) (19, 20), carbohydrate pyrolysates (hydroquinone, levoglucosan and glucometasaccharinic acid γ -lactone) (21–23) and others (lactic and glycolic acid). In Burley tobacco smoke, glycerol, which is thought to be directly transferred from tobacco, 4-vinylphenol, lactic acid and chlorogenic acid pyrolysates (catechol, 4-vinylcatechol and 4-ethylcatechol) were present in lower concentrations. In Turkish (Samsun) tobacco smoke, glycerol was present in high concentration.

The concentration of each compound in the smoke condensate, which would also contribute to the organoleptic properties, showed a similar tendency as described above, because the levels of condensate in a puff were approximately the same for all varieties of tobacco studied.

It must be noted that the blended cigarette smoke components are not identical with the total of those from the unblended cigarette. Further investigations will be necessary to elucidate the relationship between the smoke composition from the blended cigarette and that from the unblended cigarette.

SUMMARY

The relative proportions of the major smoke components and N-containing components in the semi-volatile phase and the non-volatile phase from some varieties of tobacco, i.e. flue-cured, Burley and Turkish tobacco, were determined. In flue-cured tobacco smoke, 2-furfural, 5-methylfurfural, 5-hydroxymethylfurfural and other carbohydrate pyrolysates, catechols and organic acids were present in higher concentrations than in the smoke from the other tobaccos. In Burley tobacco smoke, myosmine was present in much higher concentration than in the smoke from the other tobaccos. In Turkish tobacco smoke, glycerol and β -methylvaleric acid were both present in much higher concentrations.

ZUSAMMENFASSUNG

Der relative Anteil der hauptsächlichen Rauchinhaltsstoffe und der stickstoffhaltigen Komponenten wurde in der halbflüchtigen und in der nichtflüchtigen Phase des Rauches von „flue-cured“- und Burleytabak sowie von türkischem Tabak untersucht. Folgende Verbindungen fanden sich im Rauch von „flue-cured“-Tabak in größeren Mengen als im

Rauch der anderen Tabake: 2-Furfural, 5-Methylfurfural, 5-Hydroxymethylfurfural und andere Pyrolyseprodukte von Kohlehydraten, Katecholen und organische Säuren. Der Myosmingehalt war im Rauch von Burley-Tabak wesentlich größer als in dem der anderen Tabake. Die Glycerin- und β -Methylvaleriansäurekonzentration war im Rauch von türkischem Tabak vergleichsweise erheblich größer.

RÉSUMÉ

En partant des variétés de tabacs «flue-cured», Burley et Orient, les proportions relatives des composés principaux et composés azotés ont été déterminées dans les phases semi-volatile et non-volatile de la fumée. Dans la fumée du tabac «flue-cured», les concentrations de 2-furfural, 5-méthylfurfural, 5-hydroxyméthylfurfural et autres substances obtenues par pyrolyse d'hydrates de carbone, pyrocatechines et acides organiques sont supérieures à celles trouvées dans la fumée des autres tabacs. Dans la fumée du tabac Burley, la concentration de myosmine était plus élevée que dans la fumée des autres tabacs. On a trouvé des concentrations beaucoup plus importantes de glycérine et d'acide β -méthylvalérienique dans la fumée du tabac turc.

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