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Tobacco Chemistry

4*: Chemical and Ciliotoxic Studies of Smoke from Freeze-Dried Tobacco**

by

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INTRODUCTION

The technique of freeze-drying tobacco, which provides a means of altering its volume and filling capacity and also represents a new way of curing, has recently been discussed by one of us (1). In view of the potential the method carries in altering and controlling certain physical and chemical characteristics of cigarettes, it was of considerable interest to examine the chemical composition and ciliotoxic effects of smoke derived from cigarettes made exclusively from such tobacco. The present study was therefore undertaken and deals with differences observed between tobacco freeze-dried during curing and corresponding conventionally cured material as regards particulate matter, nicotine, phenol and ciliotoxic activity of the smoke. Moreover, a detailed gas chromatographic and mass spectrometric examination of the gas phase of fresh smoke from these two differently cured tobaccos has been performed.

MATERIALS AND METHODS

Tobacco and Cigarettes

Tobacco (*Nicotiana tabacum* L., cv. Coker 254) was grown for this study at the Tobacco Research Station, Oxford, N. C., during 1968. Normal cultural practices were followed. Tobacco was harvested at maturity by selective priming at 2-day intervals over a 6-week period to enable continuous use of the freeze-dryer.

The harvested leaves were randomly divided into two groups for processing by [a] the conventional flue-curing process and [b] freeze-drying after completion of the yellowing phase. Yellowing for both groups was accomplished at 35° C and 85–90 % r. h. Conventional curing then involved progressive drying under gradually elevated temperatures to 78° C over a 48-hour period. Samples to be freeze-dried were [a] turgor conditioned by immersing leaf petioles in water for several hours, [b] shredded into 2-inch strips across the midribs, and [c] packed in drying trays to a weight of 1200 to 1800 g/ft². A Vir-Tis laboratory freeze-dryer was used for shelf freezing and freeze-drying. Details of the freeze-drying process have been given elsewhere (1). Following drying by the two methods, samples were conditioned for handling without breakage and midribs were removed.

Prior to cigarette manufacture, tobacco from various leaf positions within each treatment was composited and uniformly blended. Cigarettes (70×8 mm) were prepared by a cooperating tobacco manufacturer using 32 cuts/inch and a regular type of cigarette paper. In manufacturing the cigarettes, the machine was set to achieve cigarettes from the two tobacco lots having the same draw resistance.

Smoking Procedure and Chemical Examination of the Smoke

On arriving in Sweden the cigarettes were moisture-equilibrated at 65 per cent relative humidity and 25° C. The cigarettes prepared from the freeze-dried tobacco were selected to within ± 20 mg of an average weight of 535 mg and the cigarettes made from the conventionally cured tobacco to within ± 30 mg of an average weight of 840 mg.

The two types of cigarettes were smoked mechanically on a Phipps & Bird machine (2) using Cambridge filters for trapping the particulate matter (puff volume 35 ml, puff duration 2 sec., puff frequency 1 puff/min., butt length 23 mm). The determination of the amount of total particulate matter and its water content were performed according to the *Coresta* standard method no. 10 (3). The amount of nicotine was determined by the *Coresta* standard method no. 12 (4). The determination of "phenol" in the particulate matter, which accounts for 97 per cent of total smoke "phenol" value according to earlier investigations, was accomplished using a method of *Williamson* (5) modified by *Carlson* (6), which is based on spectroscopic determination

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(480 nm) after treatment of the separated acidic material with diazotized p-nitroaniline. The results of these determinations are given in Table 3.

Ciliotoxicity of the Smoke

The ciliotoxicity of the smoke derived from the two types of cigarettes was examined by passing the fresh smoke over rabbit trachea *in vitro* and observing the number of puffs required to complete ciliostasis using conditions discussed in detail elsewhere (7). In the present investigation six determinations using six pieces of trachea were performed with the cigarettes prepared from freeze-dried tobacco. In the case of the cigarettes prepared from conventionally cured tobacco, five pieces of trachea were examined. The results of these studies are detailed in Table 1.

Table 1. Number of cigarettes and puffs required to complete ciliostasis of rabbit trachea in vitro using cigarettes of freeze-dried tobacco (FD) and of conventionally cured tobacco (Control).

	Control		FD	
	Number of cigarettes	Number of puffs	Number of cigarettes	Number of puffs
	7	73	17	98
	12	120	21	129
	9	89	15	91
	6	61	12	82
	8	82	16	84
			15	85
$\bar{M}$	8.4	85.0	16	94.8

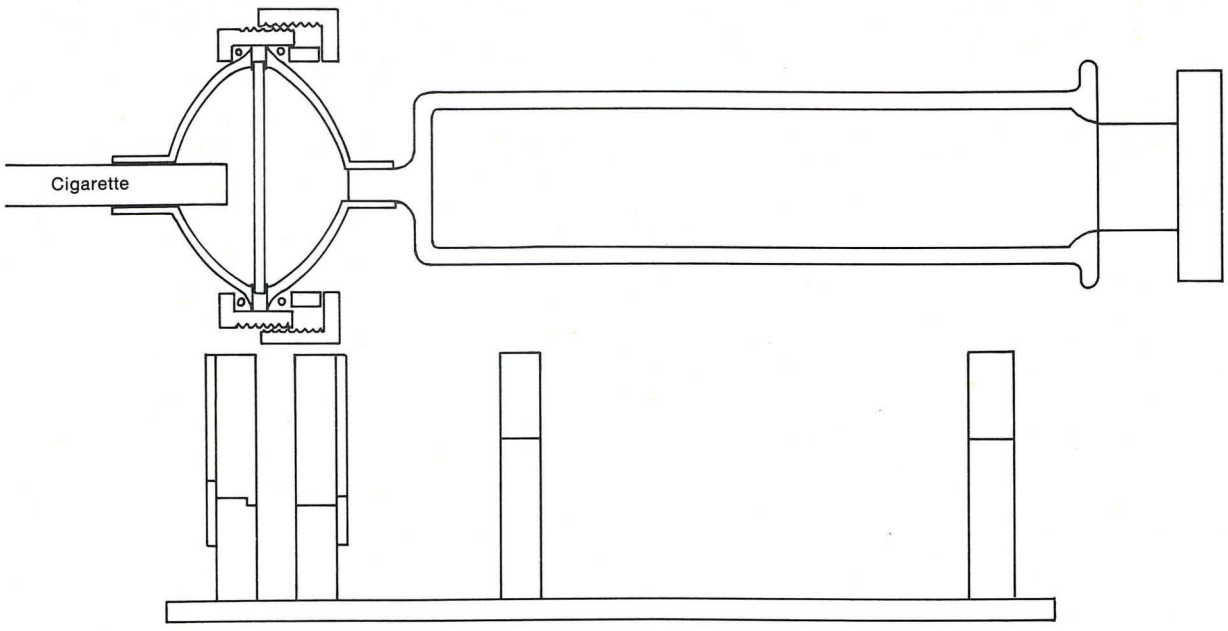
Gas Chromatographic and Mass Spectrometric Examination of the Gas Phase

The smoking was accomplished with the aid of the device shown in Fig. 1. The Cambridge filter was inserted between the two hemispherical glass parts, which were then clamped together with two threaded metal rings lined with rubber rings to achieve an even pressure. The cigarette to be smoked was inserted in one of the two hemispherical glass parts and an all-glass Hamilton syringe (50 ml) in the other. The smoking was carried out by pulling the plunger at an even speed to the 35 ml mark during two seconds. This procedure was repeated at one-minute intervals and the syringe was vented between puffs by disconnecting it from the hemispherical glass part and pushing the plunger to the bottom. In the present investigation all GC and GC-MS analyses were performed on the third puff. While the 50 ml syringe was initially also used for direct injection in the gas chromatograph, this proved difficult on account of the high pressure in the inlet system. The method was therefore modified in that the luertip of the 50 ml syringe was rapidly covered with a silicon membrane after the third puff and a 10-ml sample withdrawn with a 10 ml Hamilton syringe, equipped with a teflon-covered plunger and a steel cannula. The sample in the smaller syringe was immediately injected onto the column.

The gas chromatographic separation was carried out on a previously described, home-made instrument, which has a very small dead volume in the inlet-split-to-column and column-to-detector connections (8). In the present investigation the instrument was modified in such a way that a linear temperature programming between −70° C and 130° C could be achieved. A glass capillary column (110 m, i. d. 0.2 mm) coated with SF 96 and having an efficiency of above 500,000 theoretical plates (measured for toluene at room temperature) was used, both when a flame ionisation detector and an LKB 9000 mass spectrometer were employed for detection.

The analyses were performed by injecting 10 ml of gas phase using a split ratio of 1:40 and increasing the temperature of the column oven from −70° to 130° C linearly at 2°/min. The injection block was kept

Figure 1. All-glass smoking device.



at 100° and the detector at 150° C. Nitrogen (inlet pressure 1.9 kg/cm², flow rate 0.30 ml/min at 20° C) was used as carrier gas when the flame ionisation detector was utilised and the results obtained are given in Figures 2 and 3.

When the column was attached to the separator of the mass spectrometer, which was kept at 220° C, the coupling previously described by *Novotny* (9) was used. Nitrogen was substituted for helium as carrier gas and the inlet pressure increased to 3 kg/cm². The mass spectra were recorded at 70 eV with the aid of a data-acquisition system developed by *Bergstedt* et al. (10), which was coupled on line to the mass spectrometer. A small computer with computing routines for background subtraction and normalization was employed to facilitate and improve the accuracy of the analysis of the recorded spectra. The compounds identified or representing the best fit are given in Table 5, which also details the retention times and molecular weights of the majority of the compounds encountered.

RESULTS AND DISCUSSION

The freeze-drying process, recently discussed by *Johnson* (1), may be utilized for modification of the physical structure and other attributes of tobacco at two distinct stages of processing. Freeze-drying of yellowed, high moisture leaf during curing offers potential improvements with respect to filling capacity, enzymatic activity, color control, and dry matter losses. On the other hand, freeze-drying of cigarette rag, turgor conditioned to afford significant cellular expansion, provides a major opportunity for modification of the physical structure. The present study deals with the former stage of processing since it was considered that the more pronounced differences experienced here should be examined in the first instance.

Before discussing the chemical and biological results, certain properties achieved by freeze-drying or in cigarette manufacture should be noted. Turgor conditioning of yellowed tobacco, when followed by freeze-drying, establishes near maximum cellular volume which appreciably increases filling capacity. Unfortunately, shredding to cigarette rag dimension prior to freeze-drying was not possible and the compressive action during cutting for cigarettes is therefore considered to have reduced somewhat the effect achieved by the freeze-drying. Nevertheless, the tobacco still retained sufficiently high specific volume to permit a marked reduction of the cigarette weight, cf. Table 3. A second noticeable difference in the tobaccos was that of color. Freeze-dried tobacco was distinctly yellow and clear whereas the conventionally cured material exhibited a normal yellow-to-orange color with evidence of slight oxidative browning. The reason for this difference is readily accounted for as, under the vacuum conditions of freeze-drying, oxidation and further color change do not occur.

Smoke panel evaluations of the processed samples, made in cooperation with five tobacco companies, have provided ratings for mildness and preference and these are summarized in Table 2.

The results indicate that the freeze-dried tobacco is milder [31 vs. 13], but that preference was shown for the control [18 vs. 26]. Since the cigarettes of 100% freeze-dried tobacco burn considerably faster than the control cigarettes (fewer puffs per cigarette, cf. Table 3), it is evident that future smoke panel evaluations should preferably involve a blend or reduction of the burn rate by some means. The freeze-dried cigarettes retained normal tobacco flavor with no off-flavors detected.

The results of the determinations of total particulate matter (TPM), dry condensate (TPM minus water), nicotine, "phenol" and ciliotoxic activity of the smoke derived from the two types of tobacco are summarized in Table 3, which also gives the average weight per cigarette and the average number of puffs per cigarette. It follows from these figures that only about half the amount of TPM, dry condensate and nicotine and only about one third of the amount of phenol are encountered for the FD-cigarettes relative to the control cigarettes.

Since however the FD-cigarettes contain much less tobacco and give noticeably fewer puffs per cigarette, on the average 5 versus 9 for the control cigarettes, it is of interest to compare recalculated figures for the FD-cigarettes based on equal amounts of tobacco or

Table 2. Ratings for cigarettes of freeze-dried tobacco (FD) versus cigarettes of corresponding conventionally cured tobacco (Control).

	Ratings			
	Milder		Prefer	
Company	FD	Control	FD	Control
1	12	2	7	7
2	2	4	0	6
3	7	1	6	2
4	4	2	3	3
5	6	4	2	8
Total	31	13	18	26

Table 3. Differences between cigarettes of freeze-dried tobacco (FD) and of conventionally cured tobacco (Control).

	Control	FD
Average weight, mg/cigarette	835	530
Average number of puffs, puffs/cigarette	9.0	5.0
Total particulate matter (TPM), mg/cigarette	46.7	27.0
Dry condensate (TPM-H_2O), mg/cigarette	41.2	21.7
Nicotine, mg/cigarette	3.4	1.7
"Phenol", µg/cigarette	345	123
Ciliostasis subsequent to:		
number of cigarettes	8.4	16
number of puffs	85.0	94.8

Table 4. Comparison of recalculated values for the FD-cigarettes with the values for the control cigarettes.

	Control	Recalculated values for FD-cigarettes on the basis of equal amounts (numbers) of			
		Tobacco	Puffs	Nicotine	TPM
Weight of tobacco, mg	835	835	954	1060	915
Number of puffs (no. of ct.)	9.0	7.9 (1.6)	9.0 (1.8)	10 (2.0)	8.6 (1.7)
TPM, mg	46,7	42.5	48.6	54.0	46.7
Dry condensate, mg	41.2	34.2	39.0	43.4	37.5
Nicotine, mg	3.4	2.7	3.1	3.4	2.9
"Phenol", µg	345	194	222	246	212

equal number of puffs with the figures for the control cigarettes. It follows from these results (Table 4) that the TPM, dry condensate and nicotine values are somewhat lower for the FD-cigarettes even when the amount of tobacco is taken to be the same. On the other hand these values are nearly the same when the number of puffs are taken as equal. Similar figures are also obtained if the calculations are made on the basis of equal amounts of nicotine or TPM. Moreover, it is evident that the ratio of nicotine to dry condensate is about the same in the smoke from the two types of cigarettes.

An interesting and unexpected difference, however, is that the amount of "phenol", irrespective of the basis on which the comparison is made, is considerably lower for the FD-cigarette. Although this finding could be associated with a number of factors, it seems probable that changes in the combustion process due to the altered tobacco-to-oxygen ratio plays a dominant role. A further obvious factor which may be taken into consideration is that certain components may vary or may be differently bound in the two types of tobacco on account of the milder treatment of the freeze-dried tobacco.

Examination of the ciliotoxic effect of the smoke from the two types of tobacco on rabbit trachea in vitro shows, as indicated in Tables 1 and 3, that 16 FD-cigarettes are required to achieve complete ciliostasis, whereas only 8.4 control cigarettes are necessary to obtain the same effect. However, if the number of puffs are taken into consideration, it is demonstrated that there is no statistically significant difference between the freeze-dried and the conventionally cured material. The latter findings indicate that there is little difference in ciliotoxicity of the smoke from the two types of tobacco and this is consistent with the fact that an equal number of puffs from the control and FD-cigarettes give very nearly the same amount of TPM. It is also obvious that the difference with respect to the "phenol" content does not seem to effect the ciliotoxic properties (cf. Tables 3 and 4).

In order to establish if the gas phases of the smoke from freeze-dried and conventionally cured tobaccos are different, analyses were performed with the aid of a high resolution gas chromatographic system described in a previous paper in this series (8). The results of these studies, which also involved gas chromatography in combination with mass spectrometry, are given in Figures 2 and 3 and in Table 5.

All analyses were performed on fresh smoke to suppress secondary reactions. The gas phase of the third puff was analysed and, as detailed in the experimental part, 10 ml of this material were injected directly onto the cooled column without prior condensation or concentration. The reproducible conditions required to allow meaningful comparison between the gas chromatograms of the gas phases from different types of tobaccos were achieved after redesigning the previously described instrument in such a way that the temperature could be increased linearly from −70° to 130° C. The limiting factor of reproduceability, as demonstrated by repeated analysis of the same tobacco, was now the inhomogenity of the material analysed. These and similar experiments also revealed certain variations in intensity, notably of the peaks encountered at the retention-time intervals 17–19 min. [peaks 8–10], 28–34 min. [peaks 18–21] and 40–43 min. [peaks 40–43], and moreover that these effects were randomly distributed and could not be associated with any special type of tobacco. All examinations were performed on a 110-meter glass capillary column coated with SF 96. This stationary phase was selected because it retains the separation efficiency even at very low temperatures [−70° C] and is well suited for the separation of hydrocarbons and compounds of similar polarity, which according to the pioneering work of *Grob* (11, 12) represent the most prominent group of the gas phase constituents. However, it has the disadvantage that it is less satisfactory for polar compounds and this seems to be a main reason for the observed elevations of the baselines in certain parts of the chromatograms. When taking these factors into account it may be concluded that there are no significant differences between the chromatograms of the gas phase from the freeze-dried and conventionally cured tobaccos, cf. Figures 2 and 3.

When coupled to the mass spectrometer, the high resolution of the gas chromatographic system was retained after altering the Becker-Ryhage separator as described by *Novotny* (9). While the sensitivity of the flame ionisation detector was adequate under the conditions used, the concentration of several gas phase components were too low to give satisfactory spectra. A further limitation was the complexity of the gas phase, which restricted the recording of background spectra required to compensate for all bleeding and overlapping effects encountered. The analysis of the mass spectra, recorded with the aid of a data acquisition

system, was however simplified and improved by the use of a small computer with computing routines for background subtraction and normalization.

The mass spectrometric results, summarized in Table 5, indicate that the vast majority of the compounds in the gas phase from the freeze-dried tobacco are identical to those of corresponding gas chromatographic properties present in the gas phase from the conventionally cured material. Although minor differences are encountered [cf. Table 5], these evidently lack significance. It should be noted that the structural assignments made in Table 5 are at different levels of confidence on account of the inherent limitations of mass spectrometry and that many of the assignments only constitute the best fit of the available reference data (13–16). To indicate when the presence of a compound is taken as well established the name has been underlined.

Comparison of the present results with those obtained earlier by *Grob* (12) reveals obvious differences in the observed composition of the gas phase, noticeably in the respect that fewer oxygenated compounds are encountered in the present study. Such differences may be expected since *Grob* used a different sampling technique, which involved the condensation of the gas phase of all puffs from ten cigarettes and subsequent concentration of the condensate to a small part of the trap followed by transfer of the condensate with the aid of ether to a storage flask before injection of the ether solution.

The condensation of the gas phase evidently increases the chances of artefact formation, as does the use of ether as a transfer and storage medium, noticeably because complete exclusion of peroxides on handling ether in the open is difficult. It is clear, however, that further differences involving gas chromatographic conditions, types of cigarettes and different smoking conditions, e. g. puff duration and number of puffs per cigarette, make any attempt to evaluate the possible importance of such effects unfruitful.

SUMMARY

Freeze-dried and corresponding conventionally cured tobacco have been subjected to comparative studies. It is shown that the cigarettes manufactured from the freeze-dried tobacco have a noticeably lower average weight due to the higher filling capacity of this tobacco and that this has little or no influence on the taste, but affects the burning rate considerably. Determinations of the amounts of total particulate matter, dry condensate, and nicotine in the smoke shows that these are about half in the case of the freeze-dried material when an equal number of cigarettes are smoked; they differ much less when an equal amount of tobacco or an equal number of puffs are used as the bases for comparison. The "phenol" content of the smoke is however in all cases found to be noticeably lower for the freeze-dried cigarettes. Examination of the ciliotoxic effect of the smoke from the two types of tobacco on rabbit trachea *in vitro* shows that there is no significant difference between the number of puffs required to achieve complete ciliostasis. A detailed gas chromatographic-mass spectrometric study using a high resolution glass capillary column and computerised data-acquisition demonstrates that there are no significant differences between the gas phases of the smoke derived from the two differently treated tobaccos.

ZUSAMMENFASSUNG

Durch Gefrieren und entsprechende durch herkömmliche Verfahren getrocknete Tabake werden miteinander verglichen. Wegen der höheren Füllfähigkeit von gefrier-getrocknetem Tabak haben die daraus gefertigten Cigaretten im Durchschnitt ein merklich niedrigeres Gewicht. Die Gefriertrocknung hat keinen oder nur einen geringen Einfluß auf den Geschmack der Cigaretten; die Verbrennungsgeschwindigkeit wird jedoch erheblich beeinträchtigt. Bezogen auf gleiche Cigarettenzahl finden sich im Rauch von Cigaretten, deren Tabak nach der herkömmlichen Methode getrocknet wurde, doppelt so hohe Mengen an Partikelphase, trockenem Kondensat und Nikotin wie bei Cigaretten aus gefriergetrocknetem Tabak. Bezogen auf die gleiche Tabakmenge oder eine gleiche Anzahl von Zügen, sind diese Unterschiede viel geringer. Der Phenolgehalt des Rauches ist jedoch in jedem Fall bei den gefriergetrockneten Cigaretten merklich niedriger. Die ziliotoxische Wirkung des Rauches beider Tabakarten, die in vitro an der Trachea von Kaninchen geprüft wurde, war praktisch gleich. Die Zugzahlen, die jeweils vollkommene Ziliostase erzeugen, unterscheiden sich nicht. Durch Computer ausgewertete Untersuchungen mit einer Kombination von Gaschromatographie und Massenspektrometrie und dem Einsatz einer hochauflösenden Glaskapillarsäule ergaben Analysenwerte, nach denen sich die Gasphasen des Rauches aus den beiden verschiedenartig getrockneten Tabaken nicht signifikant voneinander unterscheiden.

RESUME

Une étude comparative, portant sur du tabac lyophilisé d'une part et sur du tabac séché de façon conventionelle de l'autre, a été faite. Il est démontré que l'on réduit notablement le poids moyen d'une cigarette fabriquée à partir de tabac lyophilisé grâce à la capacité de remplissage plus grande de ce tabac; ceci n'influence que peu ou pas le goût mais change considérablement la vitesse de combustion. La détermination de la quantité de résidu sec d'une part, et du taux de nicotine dans la fumée de l'autre, montre que, à nombre de cigarettes fumées égal, ces valeurs sont réduites de moitié dans le cas de tabac lyophilisé. La différence est moins accentuées si une même quantité de tabac ou un même nombre de bouffées sont pris comme bases de comparaison. Cependant, la teneur en phénol de la fumée de cigarettes faites à partir de tabac lyophilisé apparait être notablement plus faible, indépendamment de la base de comparaison. L'examen de l'effet ciliotoxique de

Figure 2. Gas chromatogram of gas phase from freeze-dried tobacco.

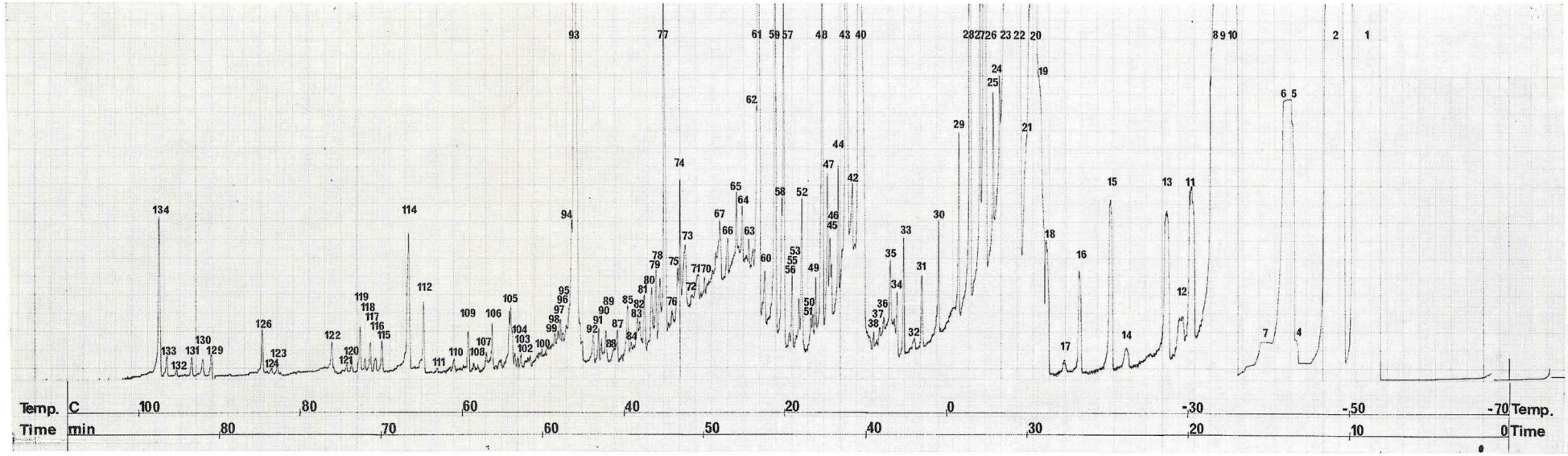


Figure 3. Gas chromatogram of gas phase from corresponding conventionally cured tobacco.

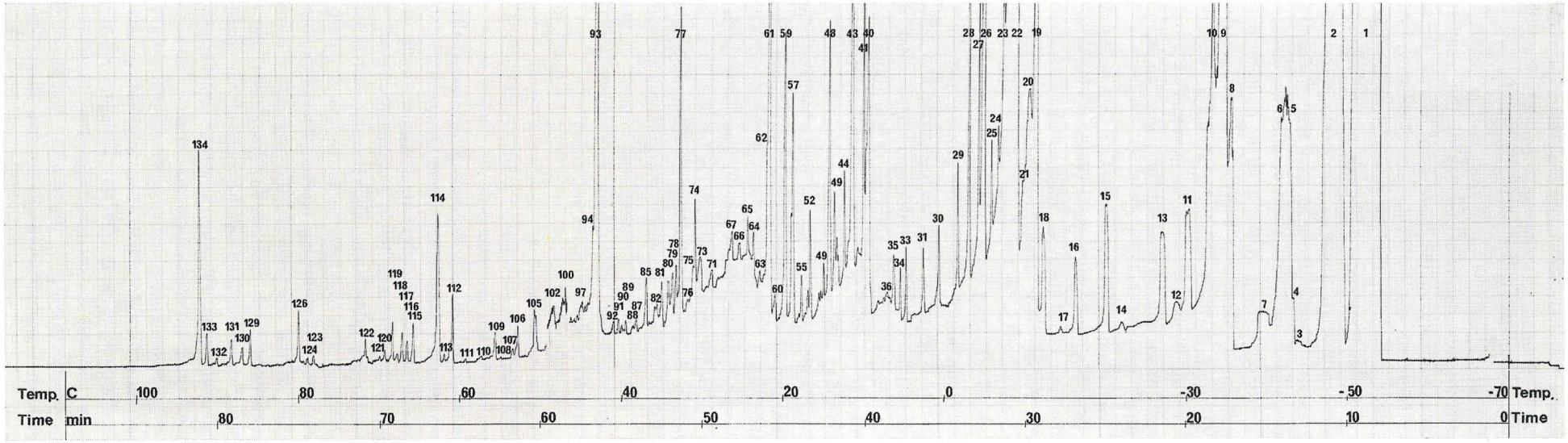


Table 5. Gas chromatographic and mass spectrometric results obtained from the gas phases of freeze-dried tobacco and a conventionally cured check.

Peak no.	GC-Results: Ret. time (min. × 10^{-1}) Freeze-dried tobacco	GC-Results: Ret. time (min. × 10^{-1}) Check to freeze-dried tobacco	MS-Results: Mol. wt.	MS-Results: Substance (best fit)	References
1	80.0–97.5	78.5– 94.5	–	–	–
2	103.5–114.5	100.0–111.0	42	propene	17
			44	propane	17
3	–	431.0	–	–	–
4	132.5	129.5	–	–	–
5	134.5–140.5	133.0	50	chlormethane	12, 17
6	134.5–140.5	137.0	–	–	–
7	151.5	150.0	58	isobutane	17
8	169.5–184.0	166.5	56	1-butene	17
9	169.5–184.0	171.5–176.0	54	1,3-butadiene	12, 17
10	169.5–184.0	179.5	58	n-butane	17
11	197.5	194.0	56	2-butene (trans)	17
12	204.5	201.5	–	acetaldehyde	8, 12, 17
13	213.0	210.5	56	2-butene (cis)	17
14	238.0	237.0	–	–	–
15	248.0	247.5	70	1,1-dimethyl-cyclopropane	8
16	267.0	268.0	72	2-methylbutane	17
17	277.0	277.0	–	–	–
18	288.0	288.5	56	acrolein a)	12, 17
			70	n-pentane	
19	294.0–298.5	294.0	68	furan a)	12, 17
20	294.0–298.5	296.5–304.0	70	2-methyl-2-butene b)	12, 17
			56		
21	294.0–298.5	296.5–304.0	72	n-pentane	12, 17
			68	2-pentyne	
22	304.0–315.0	305.0–310.5	68	isoprene	12, 17
23	304.5–315.0	315.5	68	1,2-dimethylcyclopropane	8
			58	acetone a)	17
24	317.0	319.0	84	3,3-dimethyl-1-butene	12, 17
			58	acetone	
25	321.5	323.5	70	cis-2-pentene	12, 17
26	326.5	329.0	70	2-methyl-2-butene	
27	329.5	331.0	68	trans-1,3-pentadiene	17
28	335.5	337.0	66	cyclopentadiene	17
29	342.5	344.5	68	1,2-pentadiene a)	
30	355.0	356.5	68	cyclopentene	
31	365.0	366.0	84	C_6H_{12}	
32	370.0	370.5	–	–	
33	376.5	376.5	84	2,3-dimethyl-1-butene	12, 17
34	380.5	380.0	84	C_6H_{12}	
			86	2-methylpentane	
35	385.0	384.0	84	trans-4-methyl-2-pentene	
36	389.0	388.0	70		
			72	mixture a)	
37	391.5	391.0	70	crotonaldehyde a)	
38	395.0	394.0	68		
			72	mixture	
39	398.0	398.0	86	2,3-butanedione a)	
40	404.0	399.5	70		
			84	methyl-cyclopentane	
41	407.0	402.5	84	1-hexene	
			86	C_6H_{14} a)	
42	408.5	406.0	86	–	
43	412.5	409.5	82	2-methylfuran	12, 17
44	417.5	414.0	86	n-hexane	12, 17
45	421.0	419.0	–	–	

a: Substance only found in the freeze-dried tobacco.

b: Substance only found in the check to freeze-dried tobacco.

Table 5 (cont.).

Peak no.	GC-Results: Ret. time (min. × 10^{-1}) Freeze-dried tobacco	GC-Results: Ret. time (min. × 10^{-1}) Check to freeze-dried tobacco	MS-Results: Mol. wt.	MS-Results: Substance (best fit)	References
46	422.5	419.0	72	2-butanone a)	12, 17
47	424.0	420.5	82	1-hexyne a)	
48	427.0	424.0	84	2-methyl-2-pentene	12, 17
49	431.0	427.5	82	2-methyl-1,3-pentadiene	
50	433.0	429.5	82	2-hexyne	
51	434.0	431.0	–	–	
52	440.0	436.0	84	3-hexene	
53	441.5	437.5	–	C_6H_{10}	
54	443.0	439.0	–	C_6H_{10}, C_6H_{12}	
55	445.5	441.5	78	butadienylacetylene	
56	447.5	443.0	–	–	
57	451.5	447.0	80	1,3-cyclohexadiene	
58	452.5	448.0	82	3-hexyne	
59	456.5	451.5	80	methylcyclopentadiene	
60	463.0	458.0	82	2-methyl-1,3-pentadiene	
61	467.0	461.5	78	benzene	12, 17
62	468.0	463.0	82	C_6H_{10} a)	
63	473.0	468.0	82	C_6H_{10} a)	
			84	thiophene ? a)	17
64	478.0	471.0	80	1,3,5-hexatriene	
65	481.0	475.0	80	methylcyclopentadiene	
66	486.5	480.0	86	C_6H_{14}	
67	491.5	484.5	–	–	
68	–	–	–	–	
69	–	–	–	–	
70	500.0	–	–	–	
71	505.0	497.0	130	trichloroethylene a)	
72	509.0	–	–	–	
73	513.0	505.0	–	–	
74	516.5	507.5	96	–	
			98	1,2-dimethylcyclopentane	
75	517.5	509.0	–	–	
76	521.0	–	–	–	
77	526.0	516.0	96	2,5-dimethylfuran	12, 17
78	528.5	519.0	96		
			98	mixture	
			100		
79	531.0	521.0	98	2-methyl-2-hexene	
80	534.0	524.0	96	mixture	
			98		
81	538.5	528.0	94	phenol a)	17
			98	C_7H_{14}	
82	541.0	531.0	–	–	
83	542.5	532.5	–	1,5-heptadiene-3-yne	
				C_7H_{14}	
84	546.5	536.0	–	–	
85	548.5	537.5	81	1-methylpyrrole a)	17
86	–	541.0	–	–	
87	555.0	543.5	–	–	
88	557.0	545.5	–	–	
89	562.0	549.5	–	–	
90	564.5	552.0	–	–	
91	566.5	553.5	92	1,3,5-cycloheptatriene	
			94		
92	570.0	557.0	–	–	
93	581.0	567.0	92	toluene	12, 17
94	583.5	569.0	94	–	

a: Substance only found in the freeze-dried tobacco.

b: Substance only found in the check to freeze-dried tobacco.

Table 5 (cont.).

Peak no.	GC-Results: Ret. time (min. × 10^{-1}) Freeze-dried tobacco	GC-Results: Ret. time (min. × 10^{-1}) Check to freeze-dried tobacco	MS-Results: Mol. wt.	MS-Results: Substance (best fit)	References
95	586.5	572.0	–	–	
96	588.5	574.0	98	methylthiophene	
97	590.0	576.0	96	1-methylcyclohexene	
98	591.5	578.5	112	2,5-dimethyl-2-hexene a)	
99	593.5	582.5	114	2,5-dimethylhexane	
100	602.0	587.0	–	–	
101	609.0	593.0	–	–	
102	614.5	598.0	–	–	
103	616.5	601.0	–	–	
104	618.0	–	–	–	
105	621.0	604.0	110 112	mixture	
106	632.5	614.5	112 114	C_8H_{18}	
107	636.0	618.0	112	2-octene	
108	644.0	625.0	–	–	
109	647.5	628.5	110	2,5-dimethyl-2,4-hexadiene	
110	656.0	637.0	110 120	mixture	
111	666.5	646.5	120 122	mixture	
112	675.0	654.5	106	ethylbenzene	12, 17
113	–	659.0	–	–	
114	684.5	663.0	106		12, 17
115	700.5	678.5	104	styrene	12, 17
116	705.0	682.0	106	o-xylene	12, 17
117	708.0	685.0	124	7-methyl-2,4-octadiene	
118	711.0	688.5	124	C_9H_{16}	
119	714.5	691,0	122	o-ethylphenol	18
120	719.5	696.0	126	2-methyl-1-octene	
121	722.5	699.0	–	–	
122	732.5	708.0	138	2,5-dimethyl-2,7-octadiene	
123	765.5	739.5	–	–	
124	769.0	743.5	–	–	
125	772.0	746.0	–	–	
126	775.0	748.5	136	sylvestrene	
127	–	767.0	–	–	
128	–	773.0	–	–	
129	806.0	778.5	138	dihydroterpene	
130	811.0	783.0	138	dihydroterpene	
131	818.0	790.0	138	dihydroterpene	
132	827.0	799.0	134	tert. butylbenzene	
133	833.5	805.0	138	m-menth-3(8)-ene	
134	839.0	810.0	136	limonene	17

a: Substance only found in the freeze-dried tobacco.

b: Substance only found in the check to freeze-dried tobacco.

la fumée des deux sortes de tabac effectué »in vitro« sur une trachée de lapin montre qu'il n'y a pas de différence significative entre le nombre de bouffées des deux sortes de tabac requis pour obtenir une ciliostase complète. L'analyse détaillée par chromatographie en phase gazeuse couplée à un spectromètre de masse, utilisant une colonne capillaire en verre de haute résolution et le traitement informatique des données du spectromètre de masse, a démontré qu'il n'existe pas de différence notable entre la phase gazeuse de la fumée obtenue à partir de tabacs séchés par l'une ou l'autre des méthodes.

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