Tobacco Chemistry

17: Evaluation of Two Methods for the Collection of Tobacco Head Space Volatiles*

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INTRODUCTION

The volatile constituents of tobacco leaf and their alterations during curing and ageing are of considerable interest since their structures will provide information on the chemical processes during each stage and also on the quality of the product. In this laboratory, investigations of these topics are in progress and the present paper discusses the initial phase constituting selection and evaluation of a suitable collection method.

Previous studies of the volatile constituents of tobacco, reviewed by Stedman (1), have shown the presence of a large number of compounds, and more recent studies employing advanced analytical techniques have established that many of the important aroma compounds occur only in very low concentration (2–5). In view of this, a sampling method was required which would provide sufficient quantities of material to obtain information on the trace components, since these are bound to be of importance when evaluating the alterations occurring on curing and ageing.

Recently, a method was developed in this laboratory for this purpose, employing active charcoal trapping of volatile constituents and their subsequent liberation with dimethyl ether and propane (6). A successful preliminary comparison between fermented and non-fermented Virginia tobacco was achieved. However, since our further studies of the curing and ageing processes will involve the collection of a large number of samples, and the previous method proved to be laborious, its simplification or the adoption of a superior method was considered desirable. Methods involving steam distillation, or solvent extraction followed by distillation of the extract were excluded since they were judged still more laborious and less reproducible (7). Furthermore, the steam distillation procedure is likely to lead to the formation of artefacts (8). It hence became of interest to explore the possible advantages of direct distillation of tobacco by a technique adapted from one previously developed in this laboratory and successfully applied to tobacco extracts (9), involving the use of carbon dioxide as carrier gas and simultaneous condensation of both distillate and carrier gas. The improvements introduced and the advantages and disadvantages of the modified carbon dioxide distillation and charcoal trapping methods are described below.

EXPERIMENTAL

Greek tobacco (Serres, 1968) with 11.6% moisture content was prepared with a 1 mm cut before use. The ether and sodium sulphate used were purified as described earlier (10). The charcoal (Merck's Active Charcoal for Gas Chromatography, 20–35 mesh) was prepared for use by Soxhlet extraction with purified diethyl ether for four days. The extracted charcoal was kept under vacuum (water pump) and slowly heated to 400° C over a period of 6 hr. Remaining traces of impurities were removed by reducing the pressure further (10^{-3} mm) while keeping the temperature at 400° to 500° C. After cooling to room temperature, the charcoal was immediately transferred to glass bottles which were sealed and stored until required.

Analytical gas chromatography was performed on a Varian 2700 instrument equipped with a capillary injector, a flame ionisation detector, and an Infotronics CRS 204 integrator. Gas chromatography in combination with mass spectrometry (GC-MS) was carried out on a modified LKB 9000 instrument operated at 70 eV, in which the original gas chromatograph had been replaced by a home-made unit equipped with a capillary injector, a device for the introduction of make-up gas, a splitter, and a flame ionisation detector (11).

Carbon Dioxide Distillation Method

The apparatus previously described (9) was converted to an "open" system, see Fig. 1. The tobacco was filled into flask A which was immersed in a thermostat-controlled water bath after which the distillation system was evacuated. Carbon dioxide of high purity ($>99.99\%$ CO_2) was passed through a filter of active charcoal and a heat exchanger into flask A and finally condensed in flask B, which was kept at -180°C by means of a liquid nitrogen bath. To check for possible through-system losses, a filter of active charcoal was inserted between the pump and the collection flask. Three distillation runs, employing different water-bath temperatures (20° , 40° , and 85°C) were carried out but only two of them, the 40° and 85°C runs, were found satisfactory since the yield encountered in the 20°C run was very low.

The distillations were carried out at reduced pressure (0.01 mm Hg) and each batch of tobacco kept under the selected distillation conditions for five successive ninety-

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Figure 1. Carbon dioxide distillation apparatus.

minute periods, during which the carbon dioxide flow (ca. 2 l/min.) was alternately on and off for three-minute intervals. The solidified carbon dioxide was removed by sublimation under cold conditions (-20°C /760 mm Hg) and the distillate, containing water and organic materials, subsequently extracted with ether ($2 \times 25\text{ ml}$). The combined ether solutions were dried (Na_2SO_4) and concentrated by three-stage distillation as described below. In the 40° and 85°C runs, the required volatiles were obtained in fair yields (0.12 g and 0.85 g, uncorrected for residual solvent) from the tobacco (340 g and 310 g, respectively).

In each case, ether extraction of the active charcoal filter attached to flask B to check for possible through-system losses, followed by concentration using the three-stage distillation method yielded only negligible quantities of material.

Charcoal Trapping Method

The apparatus, shown in Figure 2, was adapted from that previously described (6) by conversion to a "closed-loop" system and by the inclusion of a third charcoal filter in the gas-stream after the silicone-rubber lined

Figure 2. Charcoal trapping apparatus.

membrane pump. Each filter ($3.3 \times 9\text{ cm}$) contained purified activated charcoal (25 g).

For each collection, the column ($17 \times 100\text{ cm}$) was packed with tobacco (3.5 kg) and the charcoal filters set into the system which was then purged with nitrogen before sealing. In operation, a gas flow of 7.5 l/min. was achieved and the ambient temperature could be adjusted to 20° or 40°C by means of a polystyrene environmental cabinet equipped with a thermostat-controlled fan heater. After fourteen days, the tobacco moisture content was unchanged in each case. The bottom filter was removed for Soxhlet extraction with purified ether (600 ml, 60 hr.). The solution thus obtained was concentrated in three stages as described below. The bottom filter concentrates from the 20° and 40°C collections were clear viscous yellow-brown liquids (2.07 g and 4.34 g, respectively, uncorrected for residual solvent). Repeated ether extraction of the bottom filter, and also of the top and scrubbing filters, yielded only negligible amounts of material.

Charcoal trapping at 85°C was carried out but the tobacco degraded rapidly and extensively as judged by a marked colour change later shown to be associated with structural alteration. The resulting volatile concentrates could not, therefore, be regarded as representative and were discarded.

Three-Stage Concentration Method

Each ether extract obtained was concentrated in three stages, initially employing a vacuum-jacketed Vigreux column ($60 \times 1.5\text{ cm}$) packed with nickel Dixon rings (3 mm) and equipped with a reflux head (reflux ratio 5:1, waterbath temperature 40°C). The resulting concentrate (40 ml) was further distilled using a silvered vacuum-jacketed column ($25 \times 1.5\text{ cm}$) packed with nickel Dixon rings (3 mm) and equipped with a reflux head (reflux ratio 5:1, water-bath temperature slowly increased during the distillation to 55°C).

The final concentration was accomplished by means of a home-made Dufton-type column (30 cm) consisting of a sealed central glass tube (6 mm o. d.), wrapped with a 1 cm-pitch spiral of stainless steel wire (1 mm) which in turn is enclosed by an outer glass tube (8 mm i. d.) with teflon-sleeved B-10 joints at top and bottom. The outer glass tube, ensuring a tight fitting of the spiral between glass surfaces, is itself wrapped with three separate heating sections of Kanthal wire ($2 \times 0.1\text{ mm}$) and fitted with a 3 cm air-jacket (closed with asbestos at top and bottom) and a semi-micro reflux head. Each heating section is equipped with a variable current supply and a teflon-insulated thermocouple. The currents are adjusted to give outer surface temperatures of 38° , 37° , and 36°C at the bottom, middle, and top sections respectively. The bottom of the column and the waterbath itself are enclosed in an environmental cabinet held at 45°C to eliminate condensation problems at the lower B-10 joint.

In operation, the concentrate (10 ml) obtained from the second stage was put into a pear-shaped flask (15 ml) and attached to the column. The required currents were

supplied to the heating sections and the flask immersed in a thermostat-controlled water-bath. With the reflux ratio held at 10:1, the water-bath temperature was gradually increased from 40° to 65° C until the distillation slowed markedly. The cabinet, water-bath, and reflux head were removed and the column stoppered at the top prior to immersion of the distillation flask in a Dry Ice/acetone bath. With the system thus under partial vacuum and the column heating still in operation, the result was a downward distillation of volatiles held in the spiral. After *ca.* ten minutes, the flask was removed, purged with nitrogen, and sealed for storage at -90° C.

In a test of the efficiency of the distillation system, a mixture (470 mg) of n-pentane, n-hexane, n-heptane and n-octane in equal proportions by weight was dissolved in ether (1000 ml) and the solution concentrated as above. The concentrate was analysed using a 2 m squalane packed column and the recoveries, evaluated from integrator data, were as follows: n-pentane 0%, n-hexane 85%, n-heptane 100%, and n-octane 100%.

Similarly, in a test of the overall efficiency of the system and an evaluation of the possible instability of various classes of compounds during distillation, use was made of the previously described (6) six-component mixture (equal parts by weight of isopropyl acetate, n-pentane-1-ol, 2E-hexene-1-al, linalool, isovaleric acid, and carvone). The mixture (614 mg) was dissolved in ether (1000 ml) and the solution concentrated as above prior to estimation of the individual recoveries by GC-integration using a glass capillary column (50 mm × 0.35 mm i. d.) coated with Ucon HB 5100 oil. All six components were recovered in quantitative yield and, in particular, the trace impurities observed before distillation were present in the same concentrations and no artefacts were observed.

Overall Recovery Test of the Charcoal Trapping Method

The six-component mixture (695 mg) was transferred onto purified activated charcoal (20 g) as previously described (6). The charcoal was then Soxhlet-extracted for two 48-hour periods with ether (2 × 200 ml) and the solutions separately concentrated as above. For each concentrate, quantitation of the individual and total

Table 1. Percentage recoveries of six-component mixture from charcoal.

Compound	First extract concentrate*	Second extract concentrate**	Total
Isopropyl acetate	86	9.7	76
n-Pentane-1-ol	69	11	80
2E-Hexene-1-al	58	8.6	67
Linalool	81	2.1	83
Isovaleric acid	82	11	93
Carvone	87	6.1	93

* - contained 80 % by weight of ether

** - contained 39 % by weight of ether.

recoveries was carried out (Table 1) based on the zero distillation losses demonstrated above. No artefact formation could be detected and the combined recoveries of the mixture amounted to 74% for the first, and to 8.1% for the second extraction, for a total of 82%. The residual solvent in the concentrates amounted to 39% and to 60% by weight for the two extracts respectively, calculated from gas chromatographic analyses.

GC and GC-MS Analysis of Tobacco Volatile Concentrates

Gas chromatography of the carbon dioxide distillation and the charcoal trapping concentrates was performed on a glass capillary column (50 m × 0.35 mm i. d.) coated with Ucon oil HB-2000 (carrier gas: 2 ml/min. of N₂; program rate: 4°/min.). Evaluation of integrator data and subsequent GC-MS studies provided the results detailed in Table 2.

RESULTS AND DISCUSSION

Prior to comparison, the two selected methods were improved with respect to the present aims. Thus, the carbon dioxide distillation method (9) now applied directly to tobacco, was converted to an "open" system (Fig. 1). This modification, which involved connection of the pump to the condenser flask to allow continuous pumping during the distillation, was introduced to eliminate the pressure increase which otherwise occurs during prolonged operation times and leads to reduced yields. Similarly, the charcoal trapping system, previously used for studying fermented and non-fermented Virginia tobacco (6), was modified to a "closed-loop" three-filter form (Fig. 2) and nitrogen was adopted as carrier gas to limit exogenous contamination and alteration of trapped compounds. Also, the method previously used for extraction of the volatile compounds collected on the charcoal employing propane and dimethyl ether under pressure (6) was replaced by a simpler procedure using ether.

The higher boiling point of ether would lead to serious losses of volatile material during a one-stage concentration of the extracts and the distillation was therefore carried out in three stages to permit suitable adjustment of the extract volume to the optimal capacity of the distillation columns. The first two steps consisted of concentration with the aid of columns packed with Dixon rings. The final concentration was achieved with a micro Dufton column equipped with three separately adjustable heating coils. This procedure was also used for concentrating the ether extracts obtained from the carbon dioxide distillation.

The effects of these modifications were evaluated using procedures employed previously (6). A test of the distillation system using a very dilute ether solution of an n-alkane mixture showed that n-hexane was recovered in 85 per cent yield and the higher homologues

quantitatively, while n-pentane, only differing from ether in boiling point by one degree, was completely lost. When compared to the reported method, previously tested by separating the same n-alkane mixture from a very dilute propane solution, the new system provides a sharper "cut-off" and higher yields of hexane and heptane but not of pentane.

It has previously been demonstrated (12) that severe losses can easily occur when concentrating very dilute solutions. One reason for the successful recoveries with the present system may be that the heating bath temperatures have been kept as low as possible, and therefore the distillation slows markedly when the solvent concentration becomes sufficiently low. The result is that a fair amount of solvent is always present in the final concentrate, in contrast to the previous method (6). This is, however, of minor importance for the analyses of the extracts.

The overall efficiency of the charcoal trapping technique was evaluated using a six-component mixture (isopropyl acetate, n-pentane-1-ol, 2E-hexene-1-al, linalool, isovaleric acid and carvone). A separate three-stage distillation test was carried out with this mixture, and quantitative recovery of all the components was achieved. After repeated Soxhlet extraction with ether of charcoal carrying the six-component mixture, the individual constituents were analysed by means of gas chromatography. This experiment revealed, as indicated in Table 1, that by far the major portion of the adsorbed compounds was extracted within the first two days and that the concentrates, as in the case of the earlier method, con-

tained no observable artefacts. The total yields of the individual compounds after four days extraction were on the average above 80%. The present procedure, therefore, besides being more convenient than that reported previously, provided uniformly higher recoveries.

With these favourable results for the modified procedures available, comparison of the two collection methods was undertaken using sun-cured Greek tobacco, which was selected since the analysis of its solvent-extractable constituents is already a well-advanced project in this laboratory. Each method was evaluated at two temperature levels, namely 40° and 85° C for the carbon dioxide distillation and 20° and 40° C for the charcoal trapping to obtain comparative distribution data. Initially, both methods were employed at room temperature (20° C), but the carbon dioxide distillation method yielded very small amounts of material. Since previous experience from carbon dioxide distillation of tobacco extracts at 85° C (10) showed that this was the upper temperature limit yielding a suitable distillate for gas chromatography, the 85° C operating temperature level was selected. However, charcoal trapping at this temperature resulted in rapid degradation of the tobacco, and the 40° C operating temperature was adopted to eliminate this problem. Examination of the samples by gas chromatography in combination with mass spectrometry showed that the two methods gave material of qualitatively similar composition (cf. Table 2), while significant quantitative differences could be observed, particularly with respect to the more volatile components. This result may be due to the fact that water-soluble compounds are partly or completely lost in the

Table 2. Yields of individual tobacco volatiles in elution order (p. p. m., ex integrator values, of original tobacco weight, corrected for residual solvent).

Compound \ Method	Charcoal trapping, 20° C	Charcoal trapping, 40° C	Carbon dioxide, 40° C	Carbon dioxide, 85° C
1. Benzaldehyde	1.5	26	0.2	7.0
2. Unknown	6.3	51	0.2	2.4
3. Pentadecane	1.5	2.9	0.5	2.0
4. Benzyl alcohol	+	14	—	16
5. Solanone	20	32	17	20
6. β-Phenylethanol	6.8	32	5.6	+
7. Nicotine	14	343	40	848
8. Geranylacetone	3.4	18	7.7	14
9. Heptadecane	0.4	3.4	—	+
10. 1,3,7,7-Tetramethyl-2-oxa-bicyclo (4.4.0)-dec-5-en-9-one	0.4	2.2	1.1	5.9
11. 1,3,7,7-Tetramethyl-2-oxa-bicyclo (4.4.0)-decan-9-one	0.3	3.4	0.9	8.0
12. Megastigma-4,6Z,8E-trien-3-one [§]	—	5.1	1.4	20
13. Neophytadiene	18	162	9.4	466
14. Dihydroactinidiolide	1.5	6.8	5.7	18
15. Megastigma-4,6E,8E-trien-3-one [§]	0.4	7.1	5.6	16
16. Hexahydrofarnesylacetone	—	11	3.7	20
Total volatiles	262	896	115	1580

+ = present but obscured

— = not detectable

§ = for structure, see ref. 13.

Figure 3. Gas chromatogram of 40° C charcoal trapping concentrate.

carbon dioxide distillation procedure when removing the tobacco-derived water from the distillate.

The mixtures showed extreme complexity and only a limited number of compounds could be identified, since most overlapped each other in the gas chromatographic analyses. An example of one of the gas chromatograms demonstrates this (Fig. 3). In order to obtain information on the increased yields associated with the higher sampling temperatures, as well as to observe distribution differences between the compounds in the four concentrates, the amount of the identified compounds which occurred in at least three of them were calculated from the gas chromatographic analysis, and are shown in Table 2.

As can be seen from the table, large distribution differences are observable between the two methods and also when the sampling temperature is raised. Although evaluation of other experimental parameters is essential for both methods, an increase in operating temperature greatly increases the total yield of volatiles mainly due, however, to increases in nicotine and neophytadiene. The effect is not simple, since the yields of individual components are not increased in the same proportions. Accordingly, accurate temperature control is essential for both techniques.

When taking other important factors into consideration such as product composition, time requirement, and applicability on a large scale, the following points may be noted. In the carbon dioxide distillation procedure, the composition of the distillate is somewhat distorted if the water-soluble components, as in the present study, are not recovered from the aqueous portion. In fact, equilibration of an ether-diluted aliquot of the charcoal trapping concentrate with water followed by recovery and concentration of the ether-soluble volatiles demonstrated the complete loss of several components, *e. g.* acetic acid, compound 2, and nicotine. Complete recovery of such compounds from the carbon-dioxide distillate would be both time-consuming and difficult. Although the collection time is much greater for char-

coal trapping, its application provides extracts of low water content, making such solvent extraction unnecessary. However, division of the concentrate into *e. g.* acidic, basic, and neutral fractions using aqueous media would eliminate this apparent advantage of the charcoal trapping method.

A further scaling up of the methods, to obtain approximately 30 g of concentrate, our minimum requirement for a more detailed trace component study, was found to be greatly in favour of the charcoal trapping method. It appears likely that this situation would persist on increasing the scale further. Charcoal-trapping at 40° C was considered most favourable and is presently used in this laboratory for studying alterations of tobacco volatiles during curing and ageing. In this connection, rigorous tests of the reproducibility of this technique, on a large scale, are currently in progress.

SUMMARY

A vacuum distillation method, using carbon dioxide as a transport medium, was applied to tobacco and compared with a charcoal trapping technique with the ultimate aim of studying the alterations of tobacco volatiles during curing and ageing. Examination by gas chromatography in combination with mass spectrometry of the four samples obtained after concentration in three stages showed that they were qualitatively similar, especially with respect to the more abundant constituents. The relative concentration of certain more volatile substances was, however, higher in the case of charcoal trapping, primarily because water-soluble components were not completely recoverable after carbon dioxide distillation. Evaluation of each method at two temperature levels showed that the yields increased markedly with temperature, mainly arising from unproportionally large increases in the concentrations of the major components, nicotine and neophytadiene. Thus, precise operation temperature control is required, especially in terms of

reproducibility. The total yield for the carbon dioxide distillation procedure (85° C) was greater than that for the charcoal trapping (40° C), due to the higher yields of both nicotine and neophytadiene in the former case. Notably because of the greater ease with which the method could be scaled up to allow trace component studies, charcoal trapping at 40° C was accepted as the method of choice for further work.

ZUSAMMENFASSUNG

Mit dem Ziel, die Veränderungen der flüchtigen Verbindungen des Tabaks während der Trocknungs- und Alterungsprozesse zu untersuchen, wurde eine Vakuum-Destillationsmethode, die Kohlendioxid als Transportmedium benutzt, mit einem auf Aktivkohle basierenden Abscheidungsverfahren verglichen. Gaschromatographische und massenspektrometrische Untersuchungen der vier Tabakproben, die in drei Konzentrierungsschritten gewonnen worden waren, zeigten eine qualitative Ähnlichkeit der Proben vor allem hinsichtlich der häufiger vorkommenden Inhaltsstoffe. Die relative Konzentration bestimmter Substanzen höherer Flüchtigkeit war jedoch bei der Abscheidung mittels Aktivkohle stärker, vor allem weil wasserlösliche Bestandteile nach der Destillation mit Kohlendioxid nicht vollständig wiederzugewinnen waren. Ein Vergleich der beiden Verfahren bei zwei Temperaturstufen zeigte, daß die Ausbeuten deutlich mit der Temperatur zunahmen, was vor allem darauf zurückzuführen ist, daß die Konzentrationen der hauptsächlichen Bestandteile, Nikotin und Neophytadien, in unverhältnismäßig starkem Maße zunahmen. Präzise Temperaturkontrolle ist also vor allem im Hinblick auf die Reproduzierbarkeit erforderlich. Die Vakuum-Destillationsmethode mit Kohlendioxid (85°) ergab durch die größeren Erträge an Nikotin und Neophytadien eine höhere Gesamtausbeute als die Abscheidung mittels Aktivkohle (40°). Da das letztgenannte Verfahren im Vergleich zu der Vakuum-Destillationsmethode in verhältnismäßig einfacher Weise für die Untersuchung sehr kleiner Substanzmengen anwendbar ist, wurde es als Untersuchungsmethode (40°) für zukünftige Arbeiten gewählt.

RESUME

Nous avons comparé l'application de deux méthodes au tabac, la première basée sur la distillation sous vide avec le dioxyde de carbone comme véhicule, et la deuxième basée sur la technique de fixation sur charbon de bois, avec le but final d'étudier les changements des volatiles du tabac durant le séchage et le vieillissement du tabac. L'examen par chromatographie en phase gazeuse, combinée à la spectrométrie de masse des quatre échantillons obtenus après concentration à trois étages, démontre une similitude qualitative, surtout en ce qui concerne les constituants plus abondants. Par contre, la concentration relative de certaines substances plus volatiles

s'est avérée plus élevée dans le cas de fixation sur charbon de bois; ceci principalement dû au fait que la fraction soluble dans l'eau n'a pas pu être récupérée complètement après distillation au dioxyde de carbone. L'évaluation de chaque méthode à deux niveaux de température a démontré que le rendement augmentait sensiblement avec la température, principalement par une augmentation disproportionnée des concentrations des composants majeurs, la nicotine et le néophytadiène. Donc, afin de garder la reproductibilité, il faut un contrôle précis de la température de travail. Le rendement total par distillation au dioxyde de carbone (85° C) est plus élevé que par fixation sur charbon de bois (40° C). Ceci est dû aux rendements plus élevés en nicotine et en néophytadiène dans le premier cas. Nous avons adopté la méthode de fixation sur charbon de bois à 40° C pour nos expériences ultérieures, spécialement parce qu'il est plus facile de la développer pour l'étude de composés en trace.

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