Beiträge zur Tabakforschung International · Volume 14 · No. 2 · October 1988 DOI: 10.2478/cttr-2013-0589

Pattern Recognition of Tobacco Headspace GC Profiles

A Potential New Analytical Tool for the Classification of Raw Tobaccos *

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SUMMARY

The first part of the paper describes a new method of obtaining reproducible and meaningful headspace profiles of tobacco lamina by using a modified closed loop stripping apparatus. The complex chromatograms are obtained by high-resolution glass capillary gas chromatography.

The second part summarizes the results of a chemometric approach to interpret the chromatograms obtained from a series of nine Virginia flue-cured tobaccos from different origins and belonging to different quality groups, each one analyzed three times by the method described above. After the elimination of peaks containing redundant information, the resulting data set, consisting of 27 × 17 data points, was analyzed to detect natural groupings by using an in-house program (in BASIC) for principal component analysis. A subsequent discriminant analysis yielded two discriminant functions capable of separating the nine Virginia tobaccos into three quality groups as defined by a conventional organoleptic analysis carried out by a smoking panel. All the tobaccos could be classified correctly (100%).

A first attempt to classify, by the procedure described above, a group of six Virginia tobaccos whose organoleptic scores were not known, did not yield clearly interpretable results, possibly because the performance of the capillary column used for analysis had slightly deteriorated during the experiment with resultant changes in retention characteristics, which led to wrong identifications of certain peaks.

ZUSAMMENFASSUNG

Der erste Teil der Arbeit beschreibt eine neuartige Headspace-Probenahmeapparatur (closed-loop stripping analyzer / CLSA), die es ermöglicht, reproduzierbare und aussagekräftige Headspace-Profile von Rohtabaken zu gewinnen. Die komplexen Chromatogramme werden mittels hochauflösender Kapillar-Gaschromatographie erhalten.

Im zweiten Teil wird über den Versuch berichtet, mit chemometrischen Methoden die Headspace-Chromatogramme von neun „flue-cured"-Virginia-Tabaken verschiedener Herkunft und Qualität zu interpretieren und zu klassieren. Jede Tabakprobe wurde dabei dreimal untersucht. Nach Eliminierung von redundanten Peaks ergab sich eine Datenmatrix der Dimension 27 × 17, welche mit einem selbstentwickelten Programm (in BASIC) für die Hauptkomponentenanalyse auf natürliche Gruppierungen untersucht wurde. Eine anschließend durchgeführte Diskriminanzanalyse klassierte die neun Virginia-Tabake 100 %ig korrekt in drei Qualitätsgruppen, die ein Degustationspanel zu Beginn der Arbeiten auf herkömmliche Weise definiert hatte.

* Presented, in part, as a short communication at the 8th International Tobacco Science Congress (CORESTA) held in Vienna, Austria, in 1984.

Received: 10th December 1986 – accepted: 12th January 1988.

Ein erster Versuch, eine Gruppe von sechs unbekannten Virginia-Tabaken nach dem oben beschriebenen Verfahren qualitativ zu klassieren, ergab keine eindeutig interpretierbaren Resultate. Grund dieser Schwierigkeiten war möglicherweise die Tatsache, daß sich die Trenncharakteristik der verwendeten Kapillarsäule im Verlaufe des Experimentes etwas verschlechterte, was zwangsläufig zu Fehlerkennungen von Peaks führte.

RESUME

La première partie du travail décrit une nouvelle méthode permettant d'obtenir des profils «headspace» significatifs et reproductibles de tabacs bruts, au moyen d'un appareil modifié pour l'extraction par entraînement gazeux (closed-loop stripping analyzer / CLSA). L'analyse est effectuée par chromatographie capillaire à haute résolution.

La deuxième partie compile les résultats analytiques obtenus sur une série de neuf tabacs de Virginie «fluecured» appartenant à trois groupes d'origine et de qualité différentes. L'analyse de chacun des tabacs a été effectuée à trois reprises, et les chromatogrammes complexes ont été interprétés et classés par des méthodes chimiométriques. Après élimination des pics corrélés, la matrice des données obtenue contenait encore 27 × 17 points. Dans le but de détecter des groupes naturels parmi les neuf tabacs, les auteurs l'ont analysée en utilisant un logiciel élaboré dans leur laboratoire (BASIC) pour déterminer les composants principaux. L'analyse discriminante des mêmes données effectuée ensuite a conduit à des fonctions discriminantes dont les deux premières ont déjà permis le parfait classement (100 %) des neuf tabacs dans les trois groupes de qualité qui avaient été définis au préalable par le panel de dégustation.

Un premier essai visant à classer qualitativement, selon la même méthode, une série de six tabacs de Virginie inconnus n'a pas abouti à des résultats pouvant être interprétés de manière concluante. Cela tient probablement au fait que la qualité de rétention de la colonne capillaire utilisée s'était quelque peu altérée entre les deux séries d'analyse et que les pics n'ont pas été reconnus correctement par l'intégrateur.

INTRODUCTION

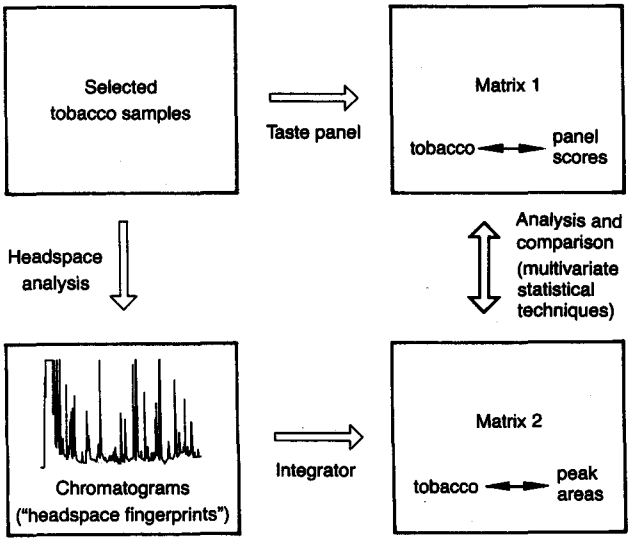
Many articles have recently been published describing pattern recognition techniques to correlate chromatographic profiles with a distinctive property of the analyzed sample.* For instance, the profiles of body fluids or respiratory air correlated to certain diseases, a method which could be used to place medical diagnosis on an objective basis (1). Other fields are enology (headspace profiles of wine correlated with its sensory evaluation (2)), essential oils (3), crude oil (4), many fruit aromas (5) and finally tobacco.

In the tobacco field, only a few papers have been published dealing with pattern recognition techniques related to tobacco aroma analysis (6—10) despite the numerous papers published on the identification of the many thousand components in tobacco leaf and smoke. However, there are numerous possible applications of multivariate statistical analyses of chromatographic fingerprint profiles for the objective classification of tobacco leaf or smoke.

They include:

- evaluation of tobacco leaf quality,
- control of correct blend composition in cigarette production,
- daily cigarette quality control,
- assistance of traditional sensory evaluation by smoking panels,
- identification of compounds present in small amounts which contribute to the distinction of quality groups.

Figure 1.
Schematic representation of the attempted correlation of tobacco headspace and organoleptic scores.



This paper concentrates on possible applications in the field of tobacco leaf as summarized in Fig. 1.

It is felt that a useful application of the pattern recognition analysis of chromatographic profiles of unmanufactured tobaccos should go beyond the problem of distinguishing a Burley from an Oriental tobacco. The real

* Journals covering the field of chemometrics: e.g. CHEMOMETRICS NEWSLETTERS, CHEMOMETRICS AND INTELLIGENT LABORATORY SYSTEMS, TRENDS IN ANALYTICAL CHEMISTRY etc.

problem starts when attempting to assess tobacco quality *within* a given tobacco variety (e.g. Virginia tobaccos only) by means of chromatographic profiles. This paper describes the attempts to use principal component analysis (PCA) to classify nine different *Virginia* tobaccos (Table 1), and discriminant analysis (DA) to distinguish between three quality levels obtained by the classical sensory evaluation of these tobaccos.

The chromatographic profiles of the tobacco leaf investigated were obtained by headspace analysis. Headspace analysis was used as an analytical tool for three main reasons:

a) The headspace technique is probably the simplest technique for reproducibly separating a selected group of compounds and thus providing a reduced amount of information from the several thousand chemical constituents of tobacco leaf.

b) Little sample preparation is necessary for analysis.

c) Since we are dealing by definition with volatile compounds in headspace analysis, we hoped to assure a long lifetime of our capillary columns (experience showed us to be wrong on this point!).

A preliminary paper (11) showed that a simple static headspace technique (75 °C tobacco temperature / 2 hours equilibration time / injection of 1.0 ml headspace gas) without an enrichment step made it possible to obtain chromatographic profiles which clearly differentiated different tobacco varieties such as Burley, Virginia, Oriental and Latakia (Fig. 2). It was obvious however that these chromatograms were quite poor; there was too little information (not enough peaks) to allow the classification of tobaccos *within* a given variety. In addition, reproducibility and quantitation were difficult. Since further enhancement of peak size by means of an enrichment step is not possible by this static technique, a GC/MS analysis of the small, yet for sensory evaluation probably interesting peaks, is practically excluded. All this led us to develop a new *dynamic* headspace sampling technique based on an enrichment step. We used GROB's closed loop stripping analyzer (CLSA) (12), modified for use with a small tobacco sample. This new method using glass capillary GC analysis gave very good and reproducible headspace profiles as shown in Fig. 3. The complexity of the headspace profiles necessitates the use of capillary columns to obtain satisfactory resolution. Since the multivariate statistical techniques used are based on the relative areas of several dozen peaks as integrated by an electronic integrator, an absolutely reproducible working capillary column is essential for this work. Slight changes in column performance can easily modify the composition of unresolved peaks (migrating peaks) which are difficult to detect and which in turn lead to false peak areas (see Discussion).
Identification of the multitude of peaks obtained in our headspace profiles was not the principal goal in this work, since many papers have already dealt competently with the analysis of tobacco volatiles (13—16). Knowledge of peak identity is not necessary for an *"a priori"* classification of the chromatographic profiles by principal component and discriminant analyses. It was

Figure 2.
Comparison of headspace profiles of different tobacco varieties obtained by static sampling (2 hours equilibration at 75 °C in a closed vial / 1.0 ml headspace gas injected at 0 °C, then heating up at 10°/min to 240 °C / glass capillary column: 50 m; i.d. = 0.27 mm; OV-1701, 1.0 µ).

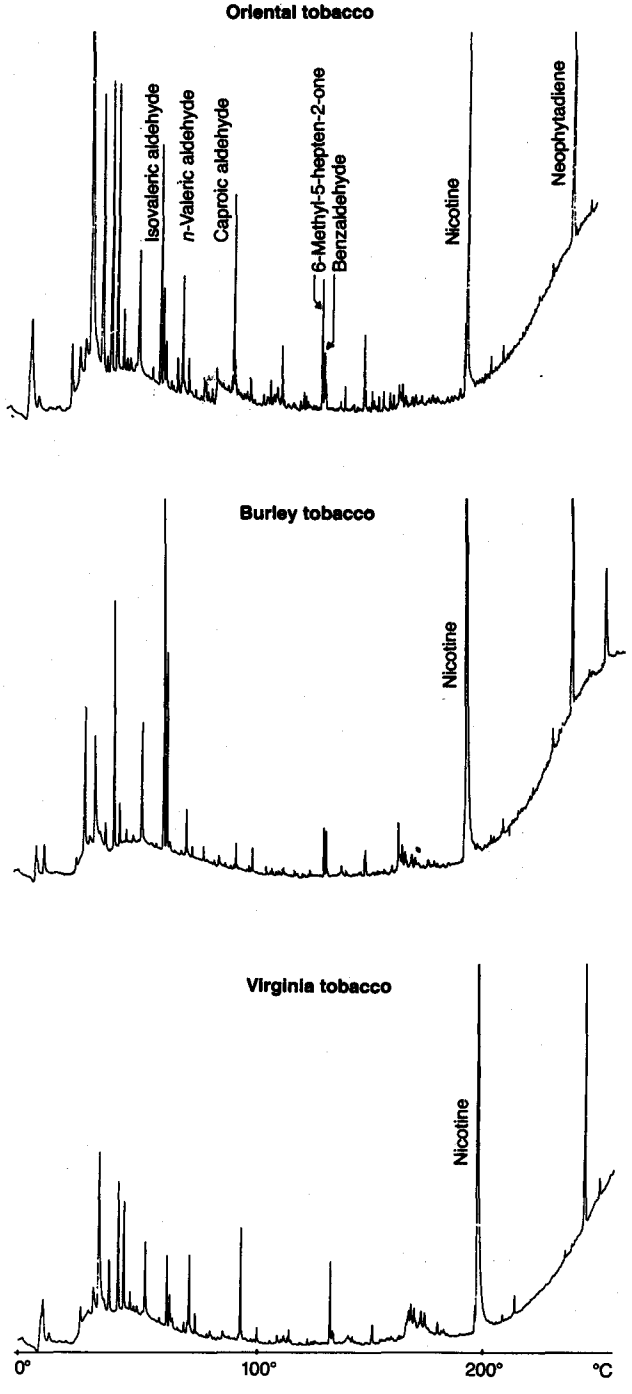


Figure 3.

Typical headspace profile of a flue-cured tobacco obtained by the new closed loop stripping analyzer (CLSA) method (see text; the numbering of the peaks corresponds to the numbering in Fig. 7 and Fig. 9). Glass capillary column: 35 m; i.d. = 0.3 mm; SE-54, 0.5 µ. Temperature program: injection at 25 °C, after 1.5 min rapid heating to 45 °C, then at 5°/min to 220 °C, finally isothermal at 220 °C for 6 min.

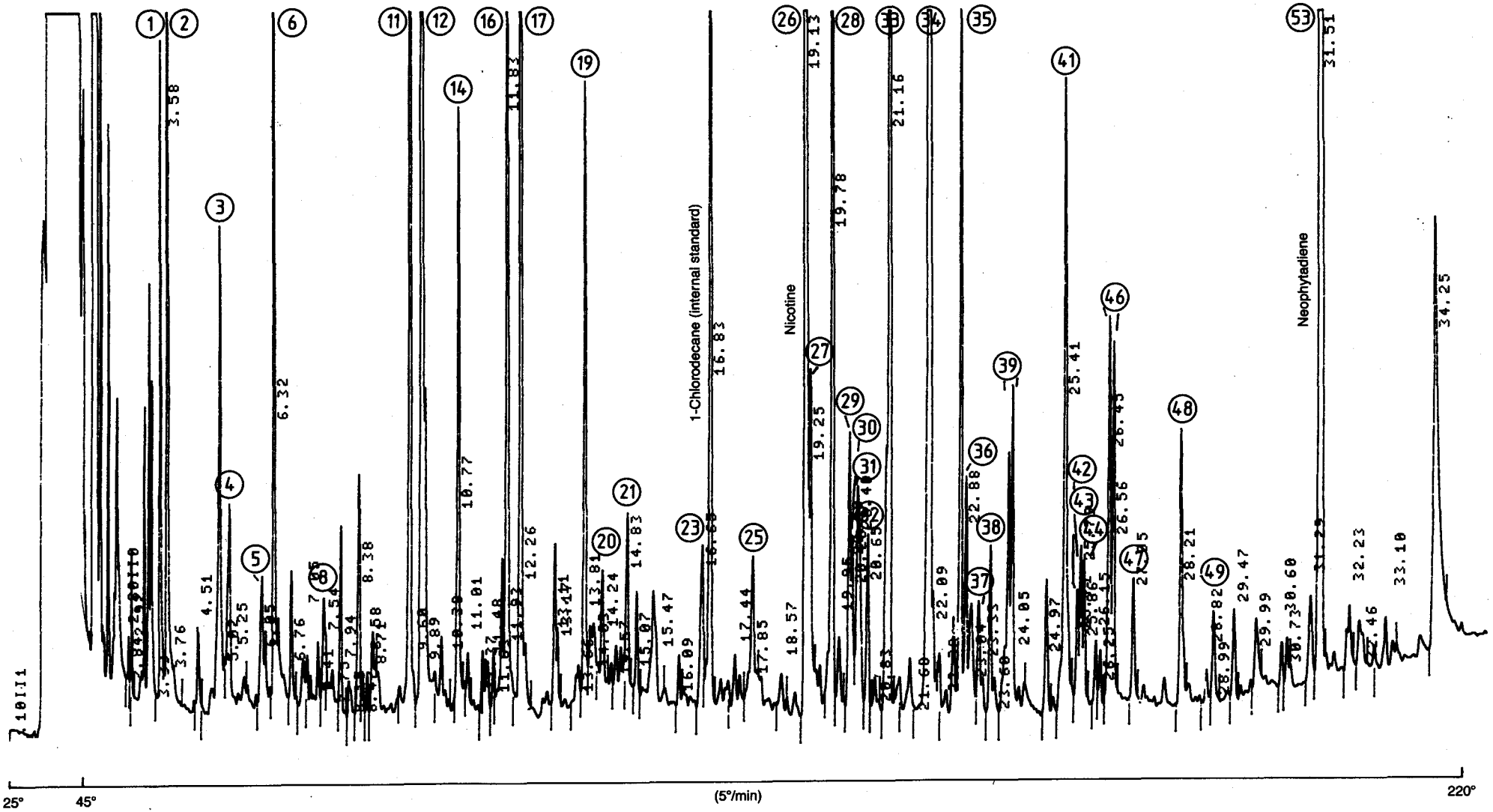
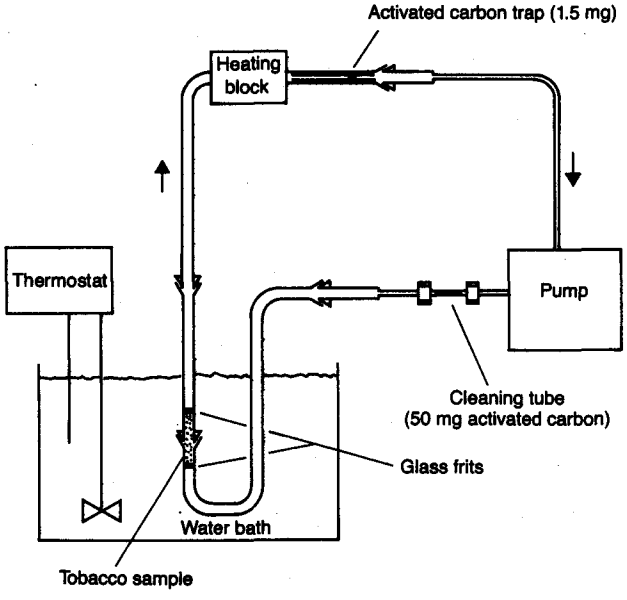


Table 1.
Characteristics and results of panel testing of nine Virginia tobaccos used for headspace analysis. Classification into three quality groups.

No.	Origin	Harvest year	Characteristics, colour	Panel score	Quality group
1	U.S.A. (Georgia)	1982	Ripe smoking leaf, light brown	Full flavoured, mild, clean, pleasant, mature	I
2	U.S.A. (Old Belt)	1982	Mature smoking leaf, light brown	Full flavoured, pungent but quite sweet, mature	
3	U.S.A. (Georgia)	1978	Lemon, bright orange leaf	Full flavoured, quite strong, pleasant, clean, typical Virginia	
4	U.S.A. (Old Belt)	1983	Orange leaf	Full flavoured, pungent, a little irritating, clean, typical Virginia	
5	Brazil (Blumenau)	1983	Deep orange leaf	Quite aromatic, quite pungent but sweet, clean	II
6	Brazil (Blumenau)	1983	Ripe smoking leaf, light brown	Quite aromatic, subdued strength, quite mild	
7	Poland	1982	3rd quality, lemon, light green	Light, little aromatic, woody, filler	III
8	Yugoslavia	1982	Leaf, lemon/orange	Quite pungent, not typical Virginia, clean, filler	
9	Thailand	1983	Low stalk, low grade filler	Light, neutral, flat, earthy, filler	

worthwhile to concentrate primarily on classifying the chromatographic fingerprints as a whole and then attempt, with chemometric methods, to detect underlying patterns useful for classifying the tobaccos. Next, we intend to analyze by mass spectrometry specifically those peaks which have been selected by discriminant analysis and which contribute to the distinction between the different quality groups (to be published in a subsequent paper).

Figure 4.
Principle of the modified closed loop stripping analyzer (CLSA). See as well Materials and Methods.



MATERIALS AND METHODS

Tobacco Samples

Nine different samples of Virginia tobaccos (different origins, crops, and characteristics) were analyzed for their organoleptic scores by a traditional smoking panel. The results are listed in Table 1: tobacco Nos. 1—4 represent typical (full flavoured) Virginia, Nos. 5 and 6 less typical yet quite aromatic, while Nos. 7, 8 and 9 are filler-type flue-cured tobaccos. For closed loop stripping analysis, the tobacco samples were finely cut with a simple vegetable cutter. This avoids overheating the tobacco sample by grinding.

Closed loop stripping analysis (CLSA)

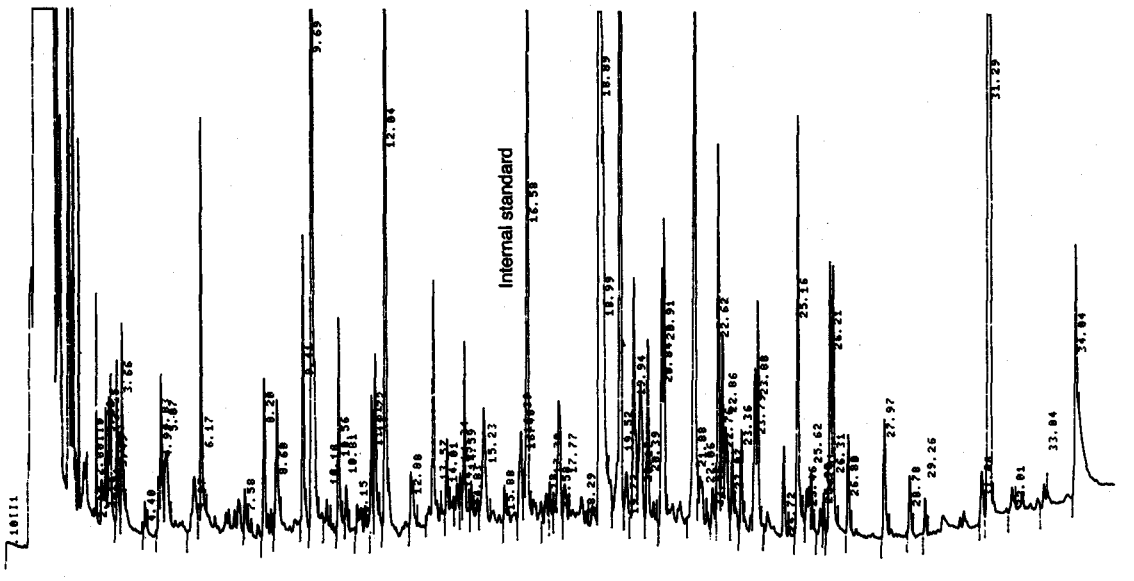
Closed loop stripping analysis was performed on a standard apparatus (Brechbühler A.G., Schlieren, Switzerland) containing two modifications (Fig. 4):

- a sample holder made in our laboratories, consisting of a glass tube (inside diameter 9 mm, length 45 mm) between two glass frits, designed to receive the fine cut tobacco (250 mg);
- a piece of stainless steel tubing (inside diameter 6 mm), incorporated between pump and sample, which contains 50 mg activated carbon between glass wool plugs in order to eliminate residual effects from the pump due to previous substances breaking through the trap.

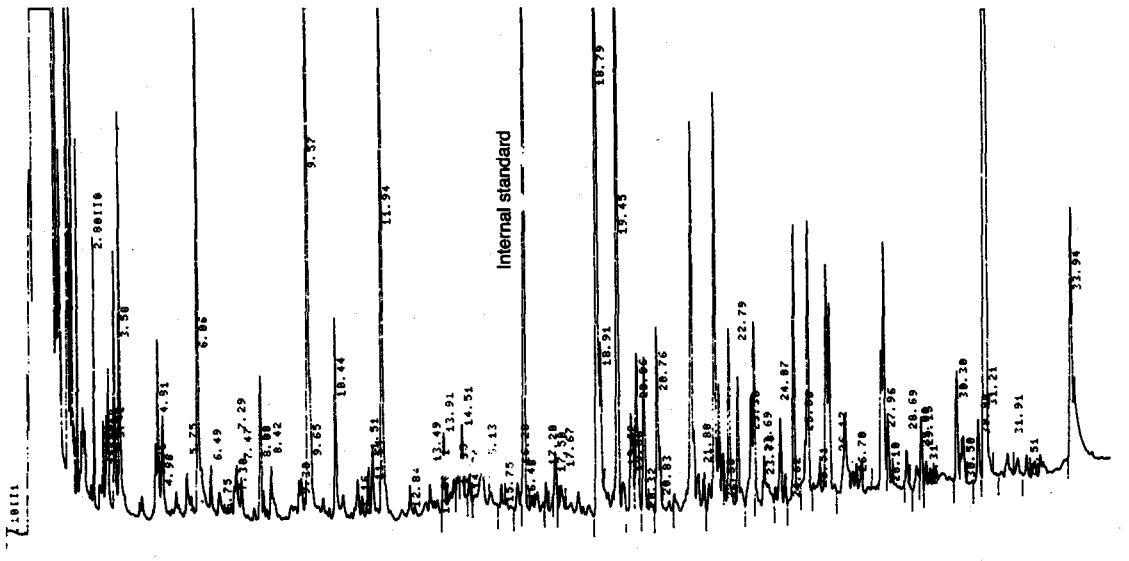
Figure 5.

Comparison of headspace profiles of Virginia grades of different origins and belonging to different quality groups, as obtained by the closed loop stripping analyzer (CLSA) method (for sampling and analytical details see Materials and Methods). Glass capillary column: 35 m; i.d. = 0.3 mm; SE-54, 0.5 µ. Same temperature program as in Fig. 3.

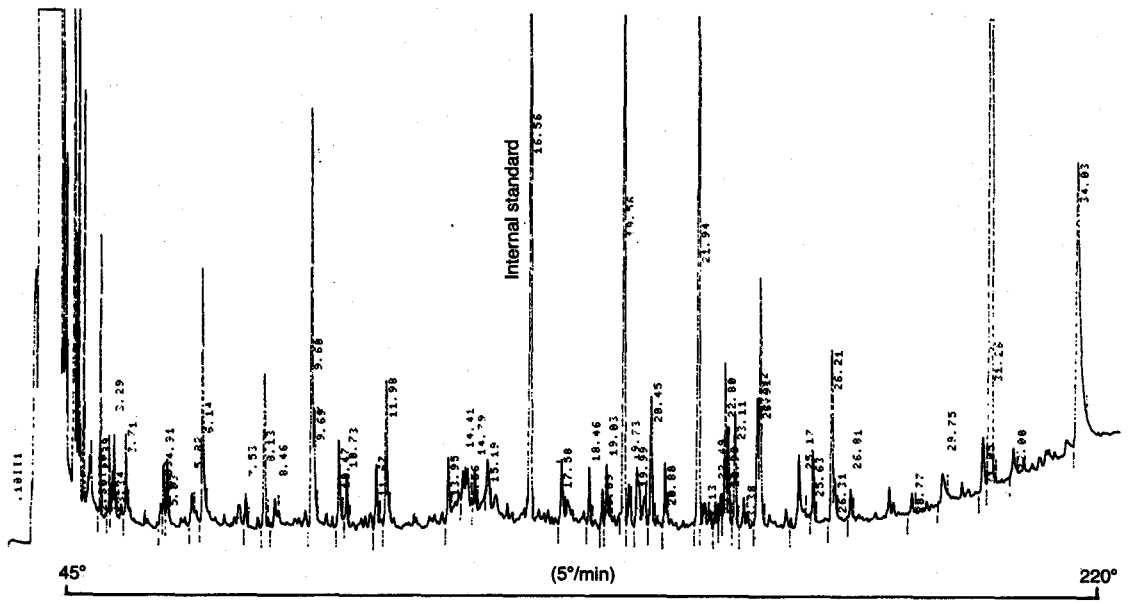
Virginia U.S.A., Old Belt (mature smoking leaf)



Virginia Brazil, Blumenau (ripe smoking leaf)



Virginia Yugoslavia (leaf, lemon/orange)



Sampling was performed for 15 minutes at 22 °C. When the whole system was installed for sampling, the gas flow in the sampling circuit was about 1.4 l/min. Immediately after sampling, the glass tube holding the activated carbon disc (precision charcoal filter made by Brechbühler A.G., Schlieren, Switzerland, containing 1.5 mg activated carbon) was disconnected, the sample tube removed and extracted with 1 µl internal standard solution and 4 µl CS_2, followed by 4 × 5 µl CS_2, resulting in a total of ca. 20 µl extract solution, of which 2 µl were directly injected "on column" into the gas chromatograph. As internal standard 1-chlorodecane (Fluka) in CH_2Cl_2 (100 µg/ml) was used.

Gas Chromatography

The instrument was a modified Carlo Erba Fractovap 2400, equipped with a GROB-type on-column injector, FID and an in-house glass capillary column (17) (35 m, 6.75% SE-54, film thickness 0.5 µ). Gas flows: carrier (H_2) 1.2 atm, make-up gas for FID detector (N_2) 1.25 atm.

Temperature program: 25 °C (injection); rapid heating to 45 °C after 1.5 min, then at 5°/min to 220 °C, finally isothermal at 220 °C for 6 min.

Integration was performed on a Spectra Physics SP 4270 integrator. Qualitative identification of the peaks was obtained from their retention time and, where questionable, by GC/MS analysis (HP 5970B mass selective detector). Quantitation was accomplished with 1-chlorodecane as internal standard.

Multivariate Statistical Analysis

Principal component analysis and discriminant analysis were performed using programs written in our laboratory in BASIC on a Hewlett-Packard HP 85 computer. Calculations were based on peak surfaces as variables corrected by the internal standard, with triplicate analysis of each tobacco sample taken through the complete procedure: closed loop stripping apparatus, extraction, and GC analysis. This gave a total of 9×3=27 chromatograms as the data base. From each chromatogram the 40 most intense or obvious peaks were selected manually (numbered peaks in Fig. 3) to give an array of 27×40 data points. Given the limited capacity of the HP 85 computer only 20 peaks could be treated at once (27×20 data points); thus two sets had to be formed: the first matrix contained peak Nos. 1—20, matrix 2 peak Nos. 21—40. After elimination of correlated peaks containing redundant information by using a correlation matrix, 22 and finally 17 peaks were retained on which to perform principal component analysis and discriminant analysis.

* The individual samples are reproducible (coefficient of variation of peak areas for three repetitive analyses: < 20%).

RESULTS

A typical chromatogram from a flue-cured tobacco obtained by our CLSA method is shown in Fig. 3. At first glance the complexity of these aromatograms is evident, 150—200 completely or partially resolved peaks can easily be detected. Interestingly enough, high molecular weight substances such as neophytadiene (molec. wt. 278) are found in large amounts when the headspace is collected at room temperature with the CLSA method compared to the static headspace analysis. The latter yielded only very volatile compounds even at elevated sampling temperature (75 °C). As pointed out previously, we did not attempt to identify the numerous peaks in the chromatograms. Typical chromatographic fingerprints in Fig. 5 (one each of the three quality groups as defined in Table 1) show slight but clearly visible differences between the individual types * consisting mainly of different relative intensities among the peaks. It can also be easily seen from Fig. 5 that the chromatograms stemming from tobaccos at the bottom of Table 1 (low quality) are generally less complex than those from the middle or top (medium quality and typical Virginias). To interpret and analyze in more detail the less salient slight differencies in relative peak intensities between the 9 samples, a computer is an absolute necessity.

By performing a principal component analysis on the 17 selected variables (peaks) after correlation analysis (see Methods), a series of principal components could be obtained (by linear combination of the variables) whereby the first three, F 1, F 2 and F 3 together, still retained 73.2% of the original information. These principal components F 1, F 2 and F 3 allowed a projection of the tobacco sample vectors originally defined in 40-dimensional space (40 peaks) into two planes defined by F 1 and F 2 on the one hand und F 1 and F 3 on the other hand. These plots are shown in Figs. 6a and 6b; Fig. 6a is based on the principal components F 1 and F 2 (retaining together 57.0% of the original information) and Fig. 6b on F 1 and F 3, which still retain 51.4% of the initial information. The close vicinity of the same numbers (replicate analyses of the same tobacco sample) confirms the above-mentioned fact that the headspace chromatograms are very well reproducible. Close inspection of the axes F 1, F 2 and F 3 shows that F 1 and F 3 in particular seem to differentiate quite nicely the three quality classes of the nine tobaccos analyzed.

The contribution of the individual variables (peaks) to the representation of the nine Virginia tobaccos investigated is shown in Figs. 7a and 7b based on the same principal components F 1, F 2 and F 3 respectively, used in Fig. 6a/b.

In attempting to detect peaks or combinations of peaks able to separate the nine tobaccos into the three quality groups I, II and III as defined by subjective panel testing (see Table 1), a discriminant analysis was performed based on the areas of the 17 peaks retained. The perfect separation of the three groups is shown in Fig. 8 (plot

Figure 6.

Results of principal component analysis — Scatterplot of the nine Virginia tobaccos (three measurements each) **on (a) the principal components F 1** (x-axis) **and F 2** (y-axis) **and (b) the principal components F 1** (x-axis) **and F 3** (y-axis).

The three replicate analyses of each tobacco are indicated by the same three numbers; those correspond to the tobacco numbering in Table 1. On the axes the percentage of original information retained is given.

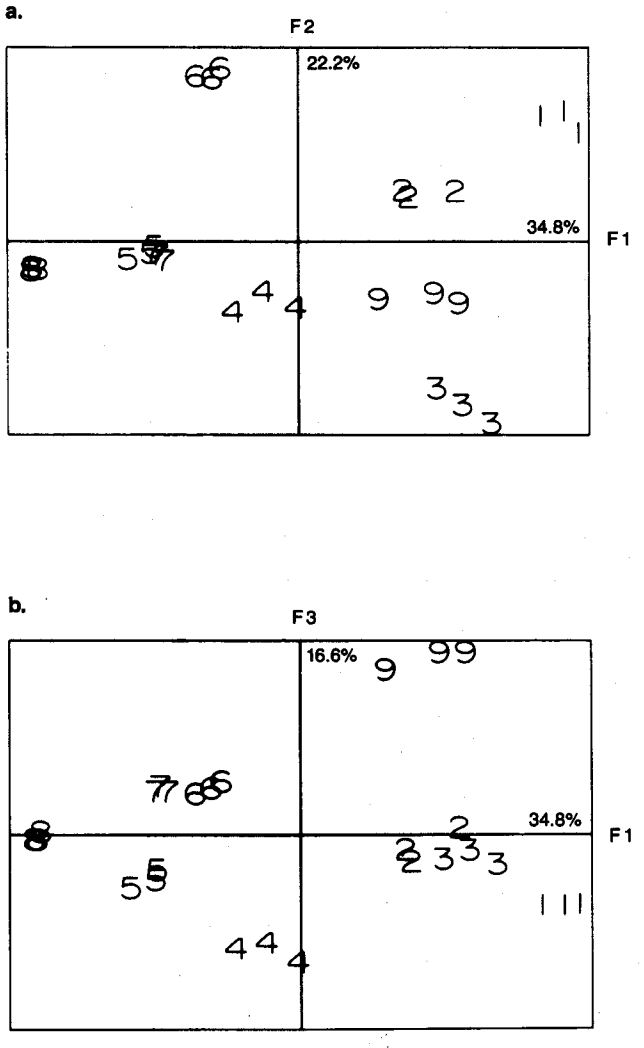


Figure 7.

Factor loadings of the 17 peaks retained for principal component analysis (a) on the principal components F 1 (x-axis) **and F 2** (y-axis) **and (b) on the principal components F 1** (x-axis) **and F 3** (y-axis).

Peak numbering corresponds to numbering in Fig. 3. The closer a number is positioned to the periphery of the circle, the better this peak is represented and the more important it is for interpretation (see text).

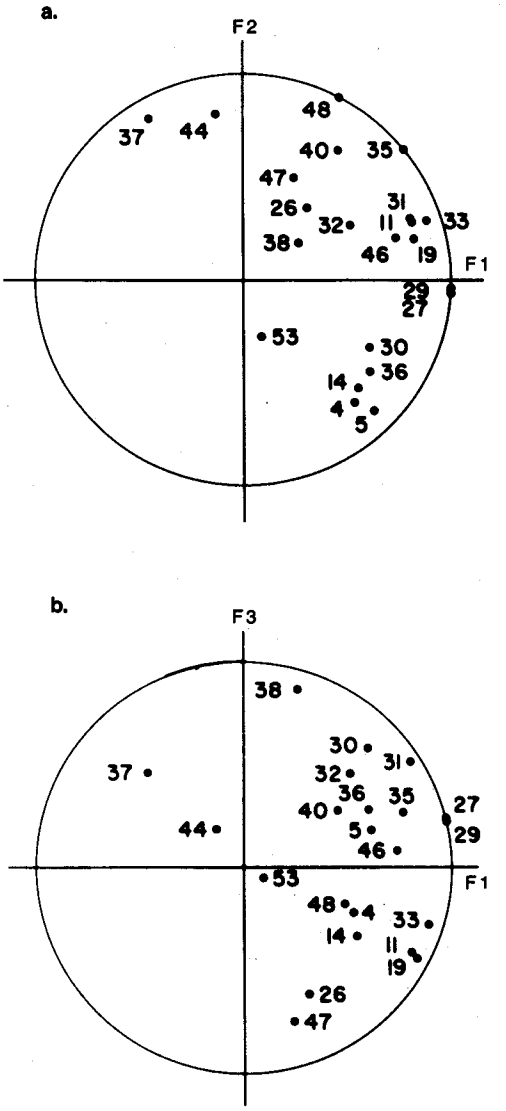


Figure 8.
Results of discriminant analysis plotted on the first and second discriminant function; classification of the nine investigated Virginia flue-cured tobaccos according to the quality groups I (●), II (▲) and III (■) as defined in Table 1.

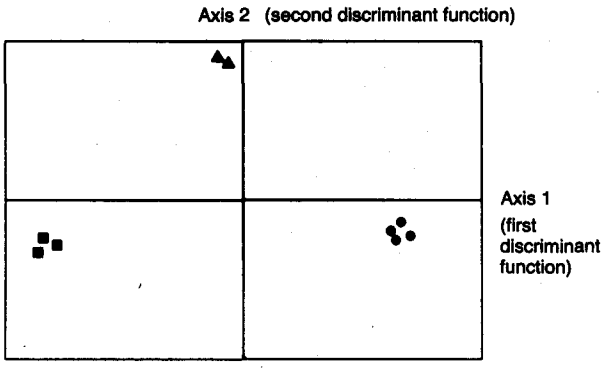
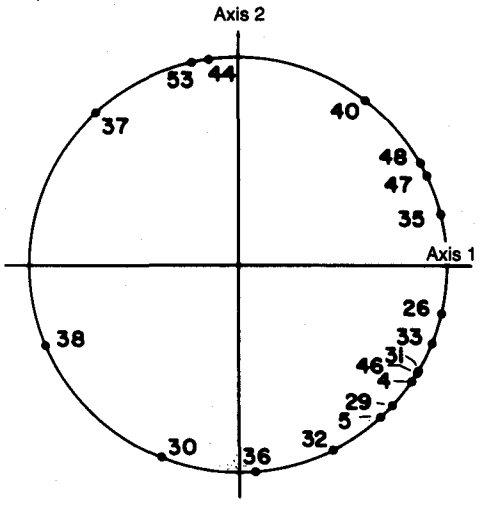


Figure 9.
Discriminant analysis — Factor loadings of 17 GC peaks plotted on the first (axis 1) **and second** (axis 2) **discriminant function** (peak numbering corresponds to numbering in Fig. 3).



based on the discriminant functions 1 and 2); the corresponding factor loadings of the variables (the GC peaks) can be seen in Fig. 9.

DISCUSSION OF THE RESULTS

As already briefly mentioned earlier, principal component analysis attempts to establish *"natural groupings"* of the tobaccos by detecting underlying patterns in the data, whilst reducing the number of variables describing the data set to a few "principal components". Similarly, peaks can be identified which enable the positioning of a given tobacco in the data space. Such an identification can be accomplished in a graphic way by superimposing Fig. 6 and Fig. 7: a tobacco is characterized by the *presence* of peaks in the same "hemisphere" of the plot or by the *absence* of peaks located on the opposite side of the correlation circle.

Inspection of Figures 6a and 6b shows that typical Virginias (group I of Table 1) are in general characterized by high positive ratings on the first principal component F 1.

F 1 is in turn characterized by high factor loadings for most peaks (this confirms the "visual" impression upon inspection of the headspace chromatograms where group I tobaccos show usually rich profiles with a great number of intense peaks). It is interesting that tobacco No. 9 (qualified as untypical Virginia by the panel) ranks amidst tobacco Nos. 1, 2 and 3 on the axis F 1. It is only principal component F 3 which separates tobacco No. 9 clearly from group I tobaccos (see below).

The principal component F 2 separates tobacco No. 4 clearly from the others; inspection of the corresponding peak factor loadings (Fig. 7a) shows that the important peak Nos. 37 and 44 (whose nature is unknown) are among the important factors for the special classification of this tobacco. In contrast to F 2 which does not group all the tobaccos in the same order as defined by the panel results, the principal component F 3 seems to be very important in this respect (see Fig. 6b showing the F 1 / F 3 plane). Rather *negative* ratings on F 3 seem to be typical for class I Virginias whereas positive ratings are negatively correlated with typical Virginia taste and are thus the rule for Virginias of group III (example: No. 9!). Inspection of Fig. 7b shows that probably peak No. 38 (not identified) contributes to the extreme grouping of tobacco No. 9 and thus eventually to its low panel ratings. On the other hand, peak Nos. 26 (nicotine) and 47 (not identified) are well correlated with class I tobaccos.

Following the principal component analysis, a discriminant analysis was performed. Discriminant analysis is a supervised learning technique where a group membership of the objects (tobaccos) is defined from the beginning and the variables are combined in such a way as to give factors which separate the defined groups most efficiently. In our case we defined three quality (and origin) groups I, II and III corresponding to the subjective scores obtained by our panel (see Table 1).

Fig. 8 shows that the first discriminant function obtained (axis 1) is capable of nicely separating the three tobacco groups, whilst the second discriminant function (axis 2) does not separate groups I and III but does distinctly separate group II from the other two. Thus, in the two-dimensional plane of axes 1 and 2 all the tobaccos are well classified according to their group as defined in Table 1. Closer inspection of the factor loadings (see Fig. 9) shows that group I (typical Virginia) can be discriminated from the other two groups by the fact that a multitude of peaks have to be present (predominantly Nos. 35, 26, 33, 31) *and* by the absence of peak 38. This reconfirms that rich chromatograms are typical of group I tobaccos.

A first attempt to classify a series of six additional Virginia tobaccos, whose organoleptic scores were unknown, based on the same principal components and discriminant functions did not yield an easily interpretable result: the scatterplot of the principal component analysis as well as of the discriminant analysis showed the six unknown tobaccos way outside the formerly found quality or origin groups.

By analogy of the "geographical" position, a tentative classification could nevertheless be made. The reasons for this incompatibility with the first series are probably twofold: first the investigated number of tobaccos in the basis classification (only nine) is too small to allow the establishment of generally valid classification functions based on peak surfaces; secondly a closer inspection of the capillary column used for GC analysis showed that its performance had slightly deteriorated in the several months between the first and second analytical series. Due to these slightly modified retention characteristics, the integrator probably did not correctly "recognize" certain peaks (especially unresolved ones) in these complex chromatograms and therefore the peak areas — essential for principal component analysis and discriminant analysis — were partially incorrect. It is obvious though that a correct peak area is necessary to obtain a reproducible and interpretable result in pattern recognition techniques.

Interestingly enough the column in question had not been used for any analytical work other than analyses of tobacco headspace. As mentioned in the introduction our use of a headspace technique was intended to preserve the capillary columns and guarantee a long column life since by definition only volatile compounds are analyzed. All the same, the immobilized columns were irreversibly damaged, probably by volatile fatty acids present in large amounts in tobacco headspace. For future work we intend to use non-immobilized Carbowax columns on $BaCO_3$ (18) which had exhibited a practically indefinite lifetime in routine gas phase analysis of cigarette smoke in our laboratories. Moreover, the split/splitless injection technique will be used again since the on-column injection procedure applied in this work might also be responsible for the shortened column life. By these means we hope to obtain a more solid analytical tool and to continue our work to improve the described new method to judge organoleptic scores of tobacco in a more objective way — a new method which we feel is very promising indeed.

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