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The Quality Estimation of Different Tobacco Types Examined by Headspace Vapor Analysis

by

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SUMMARY

In order to judge the quality of tobacco leaf, it is necessary to conduct sensory smoke evaluations. However, these are subjective and the results are difficult to quantify. Therefore, we have attempted to establish a quantitative method for evaluating tobacco quality by comparing results of headspace analysis. Forty-seven leaf samples of different types (flue-cured, Burley, Oriental) were analyzed.
The first step in this study was to have a panel of experts smoke cigarettes made from the test tobaccos and have them evaluate 10 sensory attributes. The scores were then analyzed by the technique of principal component analysis (PCA). Results showed that the score for the flavor note attribute indicated the type of tobacco and the scores of the other 9 attributes were combined as a total to indicate smoking quality.
Following the sensory study, headspace vapors of the test tobaccos were analyzed with a headspace sampler, gas chromatography, mass spectroscopy system (HS-GC-MS), in which the gas sampling loop and the HS-GC transfer line were deactivated. In order to obtain conditions for good reproducibility, the heating temperature and time of the headspace vials were examined. PCA was performed for the headspace vapor (HSV) analysis results for 31 selected peaks. The first and second principal components could be used to classify tobacco types. The third principal component partially indicated differences of smoking qualities.
Finally, multiple regression analysis was performed on the HSV analysis results in order to estimate the smoking quality scores. The regression model of all samples combined had a low regression coefficient. Then, we separated the results of the three tobacco types, as we considered that the headspace data might reveal information about the classifications themselves. The final outcome was a regression model that could be applied to each type with a higher accuracy. The variables that entered the models were compared. [Beitr. Tabakforsch. Int. 18 (1999) 213–222]

ZUSAMMENFASSUNG

Um die Qualität von Tabak beurteilen zu können, müssen sensorische Rauchbewertungen durchgeführt werden. Diese sind jedoch subjektiv und die Ergebnisse schwer quantifizierbar. Aus diesem Grund haben wir versucht, eine quantitative Methode zur Bestimmung der Qualität von Tabak zu entwickeln, indem Ergebnisse der Headspace-Analyse miteinander verglichen wurden. Siebenundvierzig Proben verschiedener Tabake (Flue-Cured, Burley- und Orienttabake) wurden untersucht.
Im ersten Schritt der Studie rauchte eine Expertengruppe für Aromaevaluierung die Testcigaretten der unterschiedlichen Tabakproben und beurteilte zehn definierte sensorische Attribute. Die erzielten Werte wurden dann mit Hilfe der Hauptkomponentenanalyse [principal component analysis (PCA)] näher untersucht. Die Ergebnisse zeigten, dass die für die Geschmacksnote vergebenen Werte mit der Tabaksorte assoziiert waren;

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die Werte der neun anderen Attribute waren als Ganzes mit der Rauchqualität assoziiert.
Nach der sensorischen Untersuchung wurden die Gasphasenbestandteile der Tabakproben im Headspace-Bereich mit einem gaschromatographisch-massenspektrometrischen Headspace-Analysegerät (HS-GC-MS) untersucht, bei dem die Gasprobenschleife und die Verbindung zwischen Headspace und Gaschromatographen deaktiviert waren. Um Bedingungen für eine gute Reproduzierbarkeit herzustellen, wurden die Erhitzungstemperatur und -zeit der Headspace-Röhrchen untersucht. Die Ergebnisse von 31 ausgewählten Peaks der Headspace-Gasphasenanalyse wurden mit Hilfe der PCA-Methode berechnet. Die ersten und zweiten Hauptkomponenten ermöglichten eine Klassifizierung der verschiedenen Tabakarten. Die dritte Hauptkomponente zeigte teilweise Unterschiede in der Rauchqualität auf.
Schließlich wurden die Ergebnisse der Headspace-Gasphasenuntersuchung in multiplen Regressionsanalysen untersucht, um die Rauchqualität zu modellieren. Das Regressionsmodell aller Tabakproben zusammengenommen wies einen niedrigen Erklärungsgehalt auf. Dann untersuchten wir getrennt die Ergebnisse der drei Tabakarten, da wir annahmen, dass die Headspace-Daten Informationen über die Klassifizierungen selbst liefern könnten. Das endgültige Ergebnis bestand in einem separaten Regressionsmodell, für die einzelnen Tabakarten mit einer jeweils größeren Genauigkeit. Die Variablen, die in die Modelle Eingang fanden, wurden miteinander verglichen. [Beitr. Tabakforsch. Int. 18 (1999) 213–222]

RESUME

Pour juger la qualité du tabac il est nécessaire de réaliser des essais d'évaluations sensorielles de la fumée du tabac. De telles évaluations sont pourtant subjectives et les résultats sont difficiles à quantifier. Pour cette raison nous avons cherché à établir une méthode quantitative qui permet d'évaluer la qualité du tabac en comparant les résultats obtenus par l'analyse des composants du headspace. Quarante-sept échantillons de tabacs de différents types (tabac blond, Burley et Oriental) ont été analysés.
Premièrement un panel d'experts-fumeurs a fumé les cigarettes faites à partir des divers échantillons et a évalué dix attributs sensoriels. Les scores obtenus étaient ensuite soumis à l'analyse en composantes principales (ACP). Les résultats de l'ACP ont montré que le score obtenu pour l'arome indiquait le type de tabac et les scores obtenus pour l'ensemble des autres attributs indiquaient la qualité de la fumée.
Après l'évaluation sensorielle, les composants volatils dans le headspace des échantillons de tabacs ont été analysés par un appareil headspace/gas chromatographie/spectrométrie de masse (HS-GC-MS), dans lequel la boucle d'échantillon de gaz et le transfert HS-GC étaient désactivés. Pour garantir une bonne reproductibilité la température et la durée du chauffage des flacons d'échantillonnage ont été mesurées. Les résultats de 31 pics sélectionnés obtenus par l'analyse des composants volatils du headspace (HSV) ont été soumis à l'ACP. Les premières et les secondes composantes principales servaient à classifier les types de tabacs. La troisième composante principale indiquait partiellement les différences par rapport à la qualité de la fumée.
Finalement les résultats obtenus par l'analyse HSV ont été soumis à l'ACP pour évaluer les scores de la qualité de la fumée. Le coefficient de régression du modèle qui décrit l'ensemble des échantillons était bas. Nous avons ensuite examiné séparément les résultats obtenus pour les trois types de tabacs, parce que nous avons considéré que les donnés recueillis par l'analyse du headspace pourraient révéler des informations sur les classifications elles-mêmes. Le modèle final de régression peut être appliqué à chaque type de tabac avec une plus grande précision. Les variables des différents modèles ont été comparées. [Beitr. Tabakforsch. Int. 18 (1999) 213–222]

INTRODUCTION

It is necessary to conduct smoke sensory evaluations as well as appearance testing in order to judge the smoking quality of tobacco leaf. This procedure is time-consuming because the tobacco needs to be made into cigarettes. In addition, sensory evaluation is a subjective technique and the results are difficult to use in a quantitative manner to estimate quality. On the other hand, chemical analysis is objective. In particular, headspace analysis is a quantitative method that requires little pre-treatment. Headspace analysis has previously been studied (1) as a method to evaluate the smoking quality of tobacco. While static headspace analysis was found to be inappropriate for quantitative analysis when the amount of sample gas was small, dynamic headspace analysis with the proper adsorbent could be used. Recent improvements in the apparatus for static headspace analysis have improved the reproducibility of the method.
In this study, we analyzed the headspace vapors of different tobacco types using a static headspace sampler and examined the relationship between HSV composition and tobacco smoking quality by multivariate analysis. The purpose of this study is to investigate the possibility of headspace analysis as a replacement for the sensory evaluation of tobacco leaves.

EXPERIMENTAL

Material

Table 1 shows samples of aged flue-cured, Burley, and Oriental tobaccos that were used in this study. These laminas were cut into strips of 0.8 mm width. Their moisture content was adjusted in a room kept at 22 °C and 60 % RH for over a week. Any fines from the cut tobacco which passed through a 1.5-mm sieve were discarded. In preparation for the headspace analysis, 1.0 g of each sample was loaded into a 20-mL headspace vial into which a piece of filter paper treated with 10 μL of a water solution of 1-pentanol (0.1 g/50 mL) had been added as an internal standard. For sensory evaluation, the cut tobacco was manufactured into cigarettes of 70 mm length and 25 mm circumference. The pressure drop was roughly controlled for each tobacco type as each cigarette can be drawn properly.

Sensory evaluation of smoking quality

Sensory evaluation of smoking quality for ten attributes was scored on a nine-point scale (a range from 1 to 9) by an expert panel trained to estimate smoking quality quantitatively. The ten attributes estimated were aroma, taste, offensive aroma, offensive taste, irritation, smoothness, smoke volume, smoke expanse, after-taste and flavor note. High scores in nine of the attributes with the exception of flavor note indicate good smoking quality. For example, large quantity was expressed as a high score in the attribute of aroma, taste, smoke volume and smoke expanse. On the contrary, small quantity was expressed as a low score in the attribute of offensive aroma, offensive taste, irritation and after-taste. Smoother smoke had a high score in the smoothness attribute.

Our panel expresses the image of smoke flavor with a color word as a flavor note attribute. The low score was given to the green color image and the high score was given to the brown color image in this study.

Table 1.
Number of tobacco samples.

Flue-cured		Burley		Oriental	
USA	9	USA	6	Greece	4
Brazil	9	Brazil	3	Turkey	3
China	1	Malawi	3	Yugo	2
		Zimbabwe	3		
		Italy	1		
		Mexico	1		
		Kenya	1		
		Mix	1		
Total	19		19		9

HS-GC-MS analysis

The analysis of tobacco headspace vapor was performed on an HP7694 headspace sampler-HP6890 gas chromatograph-HP5973 mass selective detector system. The gas sampling loop and HS-GC transfer line were deactivated as an option device of the maker. A DB-WAX capillary column (60 m length, 0.25 mm i.d. and 0.5 μm film thickness, J&W Scientific) was used for the gas chromatography. The headspace sampling vial was pressurized by helium carrier gas at 10 psi for 0.2 min. The headspace vapor was sampled with a 3-mL loop and injected onto the column with a split ratio of 15:1. The temperature of the HS-GC transfer line and the injection port was maintained at 200 °C. The temperature of the column oven was programmed from 35 °C (held for 5 min) to 240 °C (held for 10 min) at 4 °C/min. The MS ion source temperature was 230 °C. Peak areas were integrated by total ion chromatogram (TIC) method with a mass range from 29 to 300 amu. Peaks were identified by comparison of their spectra with known data in a WILEY database (the 6th edition of 275,821 spectra).

The HSV peak area ratio to the internal standard peak area was used as the variable of the peak. Analyses were repeated three consecutive times for each sample. The average value of the three analyses was used in the multivariate analyses.

Multivariate analysis

Principal component analysis and multiple regression analysis (MRA) were performed by the JUSE (The Institute of Japanese Union of Scientists & Engineers) Package Software for Windows. PCA was applied to a correlation coefficients matrix of the smoking quality score or the HSV data in order to interpret the results. MRA was performed by the stepwise method with the default F-values (Fin = Fout = 2.0) of the software to obtain a multiple regression model that was used to estimate the smoking quality score from HSV data. The fit of the regression model was evaluated by comparing a double adjusted multiple correlation coefficient (R^{**2}).

Table 2.
Factor loadings of ten attributes.

Attribute	F1	F2
Aroma	0.9783	0.0139
Taste	0.9595	0.0419
Offensive aroma	0.9781	-0.0262
Offensive taste	0.9874	0.0464
Irritation	0.9693	-0.0346
Smoothness	0.9763	-0.0384
Smoke volume	0.9680	-0.0146
Smoke expanse	0.9882	-0.0003
After-taste	0.9750	-0.0084
Flavor note	0.0196	0.9995
Eigenvalue	8.567	1.007
Cumulative variance	0.857	0.957

RESULTS AND DISCUSSION

PCA of smoking quality scores

The smoking quality scores of the ten attributes defined previously were analyzed by PCA. Two principal components, of which the eigenvalues exceeded 1.0, account for 96 % of the total variance. Table 2 shows factor loadings of the ten attributes. Nine attributes had high positive correlation and heavy loadings in the first principal component. Only the flavor note attribute had a heavy loading in the second principal component.

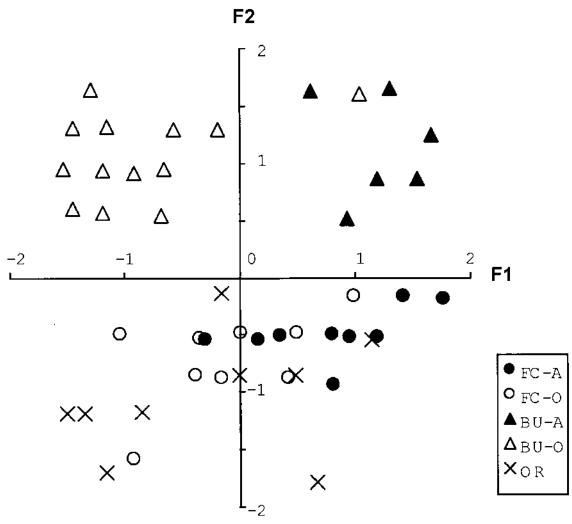

Figure 1.
Scattergram plots for the first and second principal components (PCA of smoke panel sensory attributes against tobacco type).

Each sample was plotted on the scores calculated from the two principal components (Figure 1). Burley tobacco samples were separated from others by the second principal component in which the flavor note had a heavy loading. Samples of flue-cured and Oriental tobaccos were not clearly separated. This may be related to the fact that Burley tobacco leaf has a deeper brown color than flue-cured and Oriental tobaccos. In addition, USA Burley samples seemed to be separated from the other Burley tobacco samples by the first principal component in which the other 9 attributes had heavy loadings. From this result it appears that USA Burley tobacco samples have better smoking quality than the others.

HSV analysis conditions and reproducibility

As a measure of the reproducibility of the conditions used for headspace analysis, four heating temperatures and times for vials were examined. To evaluate the reproducibility of each condition, the coefficients of variation (CV) of selected peaks were calculated for three consecutive analyses of one sample selected from each type. Results are shown in Table 3. These show the trend that the CV of all types decrease with higher temperatures and longer heating times. Additionally, some of the peaks begin to decrease at 100 °C (Figure 2a). This change may be due to chemical reactions accelerated by the heat. Moreover, as different peaks had different optimal equilibrium times (Figure 2b), the balance of compounds might change over longer heating times. Upon consideration of these results, we decided to use analysis conditions of 90 °C and 30 min duration heating time because at these conditions the CV of all peaks was less than 10 %.

The GC profiles of HSV of the three kinds of tobacco

Figure 3 shows typical chromatograms of the head space vapors of the flue-cured, Burley and Oriental tobacco samples analyzed under the conditions of the study. In total, 70 compounds including alcohols, aldehydes, ketones, organic acids, furans were identified in these chromatograms. Peaks for acetaldehyde (#1), methanol (#11), acetic acid (#45) and nicotine (#64) were common major peaks on the chromatograms. The relative amounts were characteristic of the tobacco type. On the chromatogram for flue-cured tobacco, the methanol peak was largest followed by acetic acid and acetaldehyde. On the Burley tobacco chromatogram, nicotine was the most abundant compound. The peak area of nicotine represented about 60 % of the total peak area. On the Oriental

Table 3.
Comparison of reproducibility under different conditions ((CV (%) = standard deviation / average × 100).

Test for heating temperature (°C) (heating time: 40 min const.)												
	Burley				Flue-cured				Oriental			
Peak#	70	80	90	100	70	80	90	100	70	80	90	100
4	6	1	6	8	4	4	1	2	6	5	3	3
11	14	7	5	0	6	6	4	4	2	9	5	4
45	20	2	8	2	20	3	1	7	18	6	4	4
50	2	1	2	2	5	4	1	5	25	7	4	6
58	6	3	1	3	3	1	2	2	4	5	1	4
65	23	12	1	2	15	2	4	4	26	20	2	2
avg.	12	5	4	3	9	3	2	4	14	8	3	4

Test for heating time (minutes) (heating temp. : 90 °C const.)												
	Burley				Flue-cured				Oriental			
Peak#	10	20	30	40	10	20	30	40	10	20	30	40
4	3	4	7	2	6	4	6	1	3	13	6	3
11	8	5	5	3	3	3	7	4	11	1	4	5
45	9	2	5	1	16	2	2	1	14	1	2	4
50	4	2	5	2	2	1	5	1	9	5	2	4
58	4	1	6	2	8	2	2	2	21	9	4	1
65	20	6	5	12	20	1	4	4	48	32	5	2
avg.	8	3	5	4	9	2	4	2	18	10	4	3

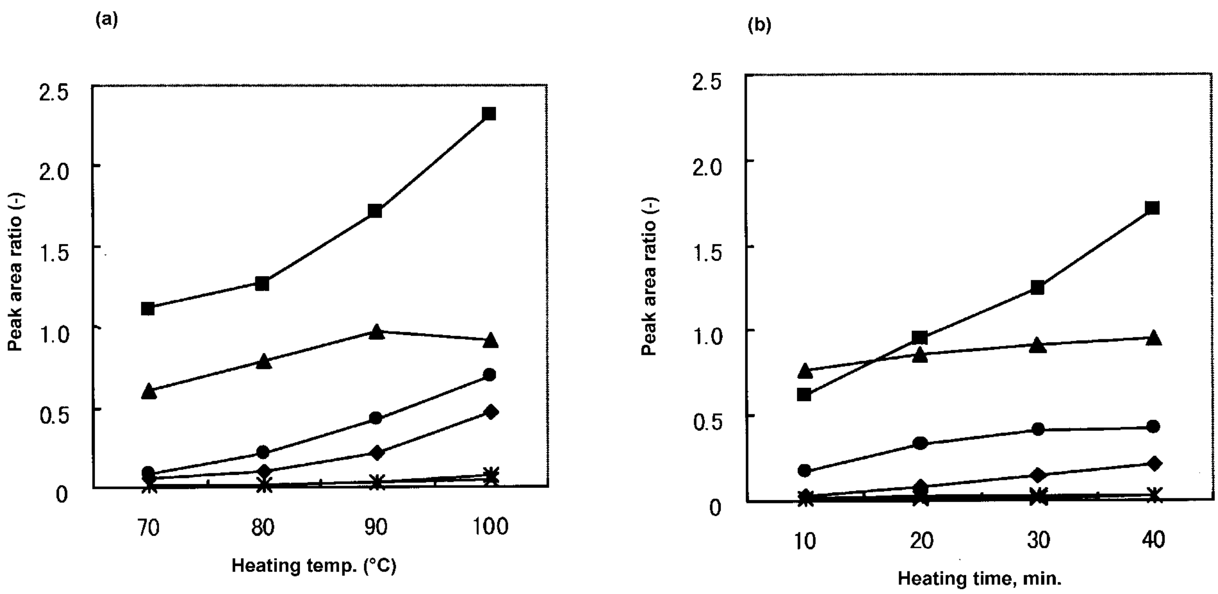

Figure 2.
Tests for heating temperature (a) and heating time (b) of a flue-cured sample.

tobacco chromatogram, the most abundant compound was acetic acid, and the acetone peak (#5) was larger than for other types.

Because the nicotine content of all the tobacco samples was in a narrow range (1–4 %), the clear difference in HSV nicotine among the three types appears to be caused by other properties of the tobacco leaf. Burley tobacco is known to be more alkaline than the other tobaccos and this may be reflected in the size of its nicotine peak.

Principal component analysis of headspace vapor

PCAwas performed with values from 31 peaks that were selected according to their reproducibility. Through principal component analysis of the HSV data of the three tobacco types, five principal components with eigenvalues exceeding 1.0 were extracted. The cumulative variance up to the fifth principal component was 84 %, therefore, 84 % of the total variance in the 31 peaks could be condensed into these five principal components. Table 4 shows the selected peaks and their calculated factor loadings.

Each sample was plotted on the principal component scores calculated from the first and second principal components (Figure 4a). The first principal component separated Orient, flue-cured and Burley groups in that order. The second principal component separated the flue-cured samples from other samples. Because the tobacco samples analyzed in this study could be separated

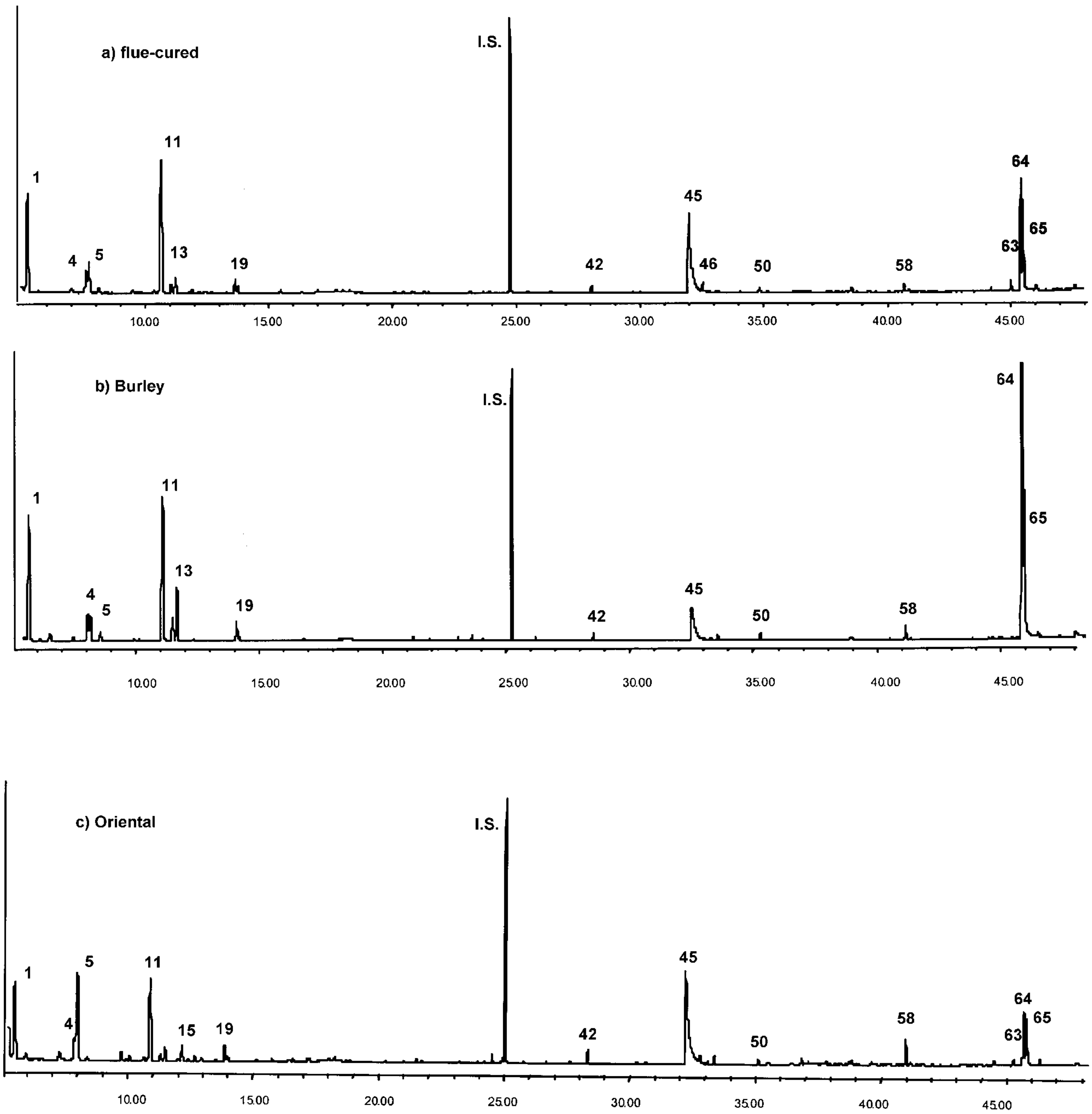

Figure 3.
Chromatograms of the flue-cured (a), Burley (b), and Oriental (c) tobaccos HSV.

Table 4.
Selected compounds for PCA and result of factor loadings.

Peak #	Compound	F1	F2	F3	F4	F5
1	Acetaldehyde	-0.076	0.217	0.790	-0.024	-0.228
2	Propanal	0.879	-0.377	0.031	-0.020	0.133
4	2-Methylpropanal	0.282	0.340	0.688	-0.219	0.282
5	Acetone	0.877	-0.356	0.148	-0.106	0.004
6	Methyl acetate	0.068	0.802	0.383	0.286	0.152
7	2-Methylfuran	0.931	-0.285	0.095	-0.038	0.069
8	Butanal	0.482	-0.792	0.115	0.153	-0.039
9	2-Methyl-2-propenal	0.803	-0.554	0.045	-0.008	-0.042
10	3-Methylfuran	0.882	-0.037	0.276	-0.014	0.082
11	Methanol	0.070	0.739	0.480	0.298	0.223
12	2-Methylbutanal	-0.446	-0.493	0.630	-0.075	0.045
13	3-Methylbutanal	-0.295	-0.634	0.661	-0.079	-0.053
15	2-Propanol	0.904	-0.321	0.029	-0.107	-0.074
16	Ethanol	0.417	0.190	0.045	0.598	-0.268
17	3-Buten-2-one	0.887	-0.158	-0.135	-0.052	0.118
18	2-Ethylfuran	0.946	-0.209	-0.028	-0.066	0.099
19	Diacetyl	0.070	-0.597	0.404	0.237	0.215
20	Pentanal	0.709	-0.091	-0.005	0.488	-0.005
24	Hexanal	0.668	-0.534	-0.329	0.252	-0.008
26	Butanol	-0.675	-0.448	-0.038	0.466	-0.032
27	1-Penten-3-ol	0.896	0.180	-0.013	0.088	0.175
33	2-Pentylfuran	0.018	-0.173	-0.130	0.818	-0.136
45	Acetic acid	0.712	0.448	0.038	-0.199	-0.142
46	Furfural	0.627	0.647	-0.088	-0.024	0.075
47	2-Ethylhexanol	-0.154	-0.523	-0.118	0.092	0.671
50	Benzaldehyde	0.517	-0.122	0.532	0.067	-0.120
58	Solanone	0.522	-0.438	0.129	-0.313	-0.404
63	Benzyl alcohol	0.610	0.664	0.049	0.225	-0.074
64	Nicotine	-0.633	-0.584	0.351	0.079	-0.197
65	Neophytadiene	-0.756	-0.106	0.480	0.152	0.117
66	Benzeneethanol	0.608	0.588	0.346	0.044	-0.141
Eigenvalue		12.506	6.641	3.542	2.127	1.171
Cumulative variance		0.403	0.618	0.732	0.801	0.838

into each type on the plane made of the first and second principal components, these two principal components might be used to show the differences in tobacco type.
Next, each sample was plotted on the plane calculated from the first and third principal components (Figure 4b). The third principal component tended to separate USA Burleys, which were shown to have higher smoking quality than other Burleys based upon PCA of smoking panel scores. These results indicate that HSV can give partial information about smoking quality.
We could not extract any meaningful information from the fourth and fifth principal components.

Multiple regression analysis

The results of PCA indicated that HSV data can be used to separate the types and possibly give information about smoking quality. Therefore, the possibility of estimating smoking quality by MRA was examined. As the scores of the nine smoking panel attributes have a high correlation, we used their average value as the smoking quality score. At first, MRA was performed on the smoking quality scores with the HSV data of all the 47 samples. Table 5 shows the results of MRA. While the regression model

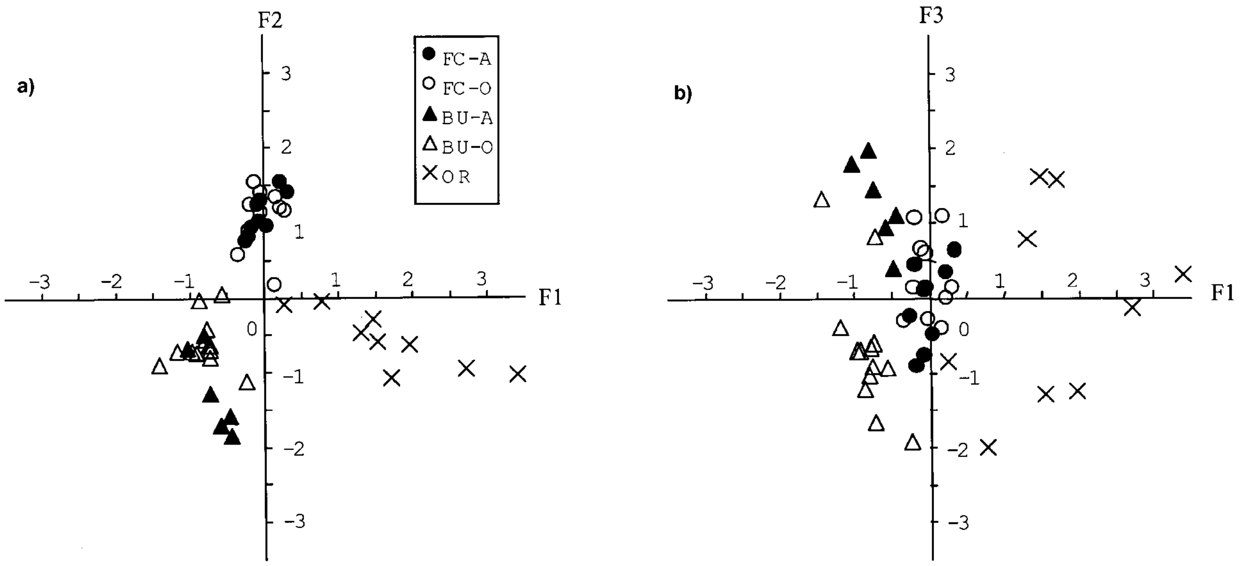

Figure 4.
Scattergrams of samples plotted for the first and second principal components (a) and for the first and third principal components (b) (PCA of headspace analysis components against tobacco type)**.**

Table 5.
Results of multiple regression analysis.

Attribute		R^2	R^{*2}	Se	Variables appearing +	Variables appearing −
Flavor note	(Total)	0.925	0.899	0.782	Butanal Nicotine Benzyl alcohol	Hexanal Furfural 2-Methylpropanal 1-Penten-3-ol
Smoking quality	(Total)	0.761	0.677	0.770	Furfural 3-Buten-2-one Benzyl alcohol Diacetyl Acetaldehyde 2-Methylbutanal	1-Penten-3-ol
	(Flue-cured)	0.929	0.843	0.377	Pentanal Acetaldehyde Acetone Solanone	2-Methylfuran 2-Methylpropanal 1-Penten-3-ol
	(Burley)	0.927	0.858	0.555	Diacetyl 2-Methylbutanal Benzaldehyde 2-Methyl-2-propenal	2-Ethylfuran 1-Penten-3-ol
	(Orient)	0.977	0.952	0.267	Acetaldehyde Benzyl alcohol	Methyl acetate

for flavor note score has a high correlation coefficient (R^{**2} = 0.90), the regression model for the smoking quality score has a lower correlation coefficient (R^{**2} = 0.68).

Because we have shown that classification of tobacco type can easily be derived from the HSV data, we attempted another MRA on the three tobacco types separately. Table 5 also shows these results. We obtained regression models with higher correlation coefficients (R^{**2} = 0.84, 0.86, 0.95) for flue-cured, Burley and Oriental tobaccos, respectively, than for the total case. Figure 5 shows scattergrams plotted for the smoking quality score and estimated value calculated from each model.

Some compounds appearing in the total model also appeared in the models for each of the individual tobaccos. For example, acetaldehyde appears with a plus sign in flue-cured and Oriental tobaccos, but is absent in the Burley model. 1-Penten-3-ol appears in both the flue-cured and Burley models with a minus sign, but is not a factor in the Oriental tobacco model. Thus, each of the three tobacco types has independently different compounds indicating its smoking quality. This finding means that a regression model for each tobacco type may be an effective means of estimating smoking quality.

CONCLUSIONS

We are able to analyze the headspace vapors of different tobacco types with good reproducibility using an HS-GC-MS system. Important factors are appropriate headspace heating temperature and time. From the results of PCA, the HSV data were shown to possess both information on tobacco type classification and smoking quality. When applied to individual tobacco types, the regression

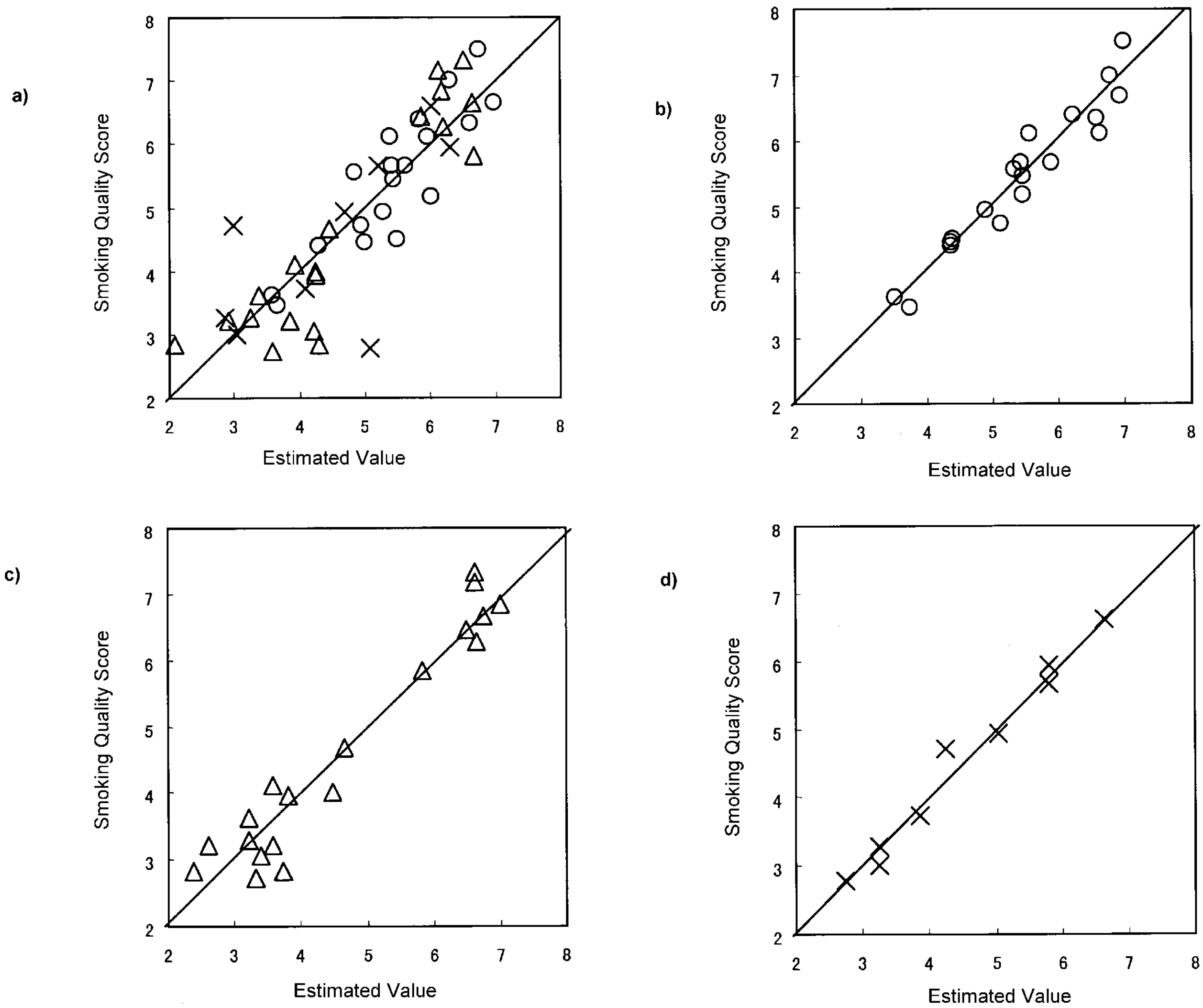

Figure 5.
Smoking quality score vs. estimated value calculated by each model: (a) total samples; (b) flue-cured; (c) Burley; and (d) Oriental.

model could be used to estimate smoking quality with a high correlation coefficient. Thus, the possibility of estimating tobacco quality by headspace analysis appears to be feasible. However, we need to examine more samples and compounds as variables in order to achieve higher accuracy.

Future headspace studies might investigate the estimation of tobacco quality employing an 'aroma sensor' as the analytical device.

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