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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Aroma Characteristics of Stored Tobacco Cut Leaves Analyzed by a High Vacuum Distillation and Canister System

MASAHIRO CHIDA,[†,‡] YUKIO SONE,[‡] AND HIROTOSHI TAMURA*[,†]

Department of Biochemistry and Food Science, Kagawa University, 2393 Miki-Cho, Kita-Gun, Kagawa 761-0795, Japan, and Tobacco Science Research Center, Japan Tobacco Inc., 6-2 Umegaoka, Aoba-ku, Yokohama, Kanagawa 227-8512, Japan

An extraction method using a high vacuum distillation extraction apparatus coupled to a canister was newly developed for the analysis and sensory test of tobacco leaf volatiles. We extended the application of the canister that is used in environmental analysis, to the extraction of the aroma components in tobacco leaves. The volatile components with vapor pressures higher than 0.1 mmHg were easily evaporated under decompression and then trapped into the vacuumed canister. After the collection of volatiles, the canister was pressurized by a slow stream of inert gas in order to emit the whole aroma under a controlled flow. Applying a preconcentrator−gas chromatography/mass spectrometry (GC/MS) and sensory test to the headspace gas components, the aroma alteration between 0 and 2 weeks of storage was simultaneously or individually evaluated. As a result, after the storage, alcohols such as 1-hexanol, linalool, and benzyl alcohol decreased significantly. The amount of carotenoid derivatives that have the characteristic tobacco leaf aroma had not changed. Sensory evaluation of the same headspace gas with that used for GC/MS demonstrated the alternation of the aroma quality before and after storage. The main changes were the decrease of greenness and smoothness in aroma and the decrease of ethylbenzene, 2-pentylfuran, 1-hexanol, benzaldehyde, and linalool concentrations.

KEYWORDS: Vacuum distillation; tobacco; canister; sensory test

INTRODUCTION

Numerous studies on volatile components of the tobacco leaf have been carried out, and hundreds of components have been reported to date (*1*, *2*). It is believed that key note chemicals are not present in tobacco flavor. Thus, the volatile constitution itself makes the highly complex flavor (*3*). Sometimes, variation of the volatile composition makes differences in the aroma character (*2*, *4*). A big alteration of tobacco leaf aroma may arise before and after the whole leaves are finely cut because of the aroma releasing. Frankenburg (*3*) referred to the effects of self-oxidation, polymerization, and condensation of chemicals on the aroma changing during extraction, curing, and storage processes. So, several analytical techniques for volatile components in the solid matrices have been developed with simple preparation methods or no sample preparations (*5*−*7*).

Headspace analysis is the most practical technique used on the elucidation and quantification of aroma constituents in various types of tobaccos (*5*, *8*) under different storage conditions. Headspace gas generally reflects the intact aroma more than solvent extracts and other extracts because individual components in the headspace gas would realize the real aroma, which are perceived by the human nose (*6*, *9*, *10*). However, to increase the amount of volatiles collected, a sample vessel is often heated to an appropriate temperature. Consequently, the volatile composition may not be the same as the original. A more mild extraction technique for tobacco flavor has to be developed.

On the other hand, a sensory approach is perhaps indispensable for understanding aroma characters during storage. Especially, aroma samplings from different stages of storage periods are sometimes necessary for understanding the dynamic change of the aroma quality. However, organoleptic judgment of headspace gas samples from different stages of the storage period is less effective if there is a large time interval between trials. Human beings cannot memorize aroma quality and intensity as absolute values. Thus, this means that the evaluation is not reproductive. So, simultaneous performance of sensory tests of some headspace gas samples from different stages of the storage has been much preferred.

The goal of the present study was to simultaneously get accurate sensory and chemical assessments of the aroma quality in stored tobacco cut leaves. Therefore, a newly developed high vacuum distillation system coupled to a canister trap is successfully introduced and applied here.

* To whom correspondence should be addressed. Tel: +81-87-891-3104. Fax: +81-87-891-3021. E-mail: tamura@ag.kagawa-u.ac.jp.
[†] Kagawa University.
[‡] Japan Tobacco Inc..

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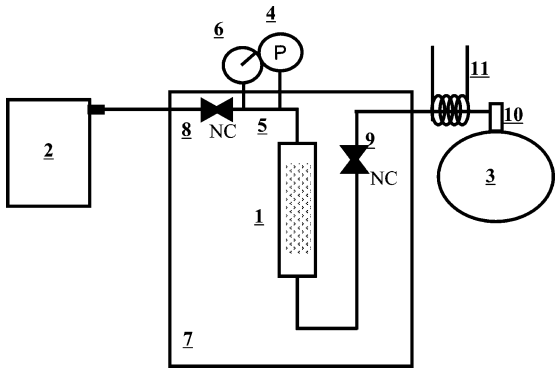

Figure 1. Schematic diagram of HVDA coupled to a canister. Key: 1, sample vessel; 2, nitrogen gas source in four layer aluminum bag; 3, canister; 4, pressure sensor; 5, mass flow controller; 6, mass flow meter; 7, isothermal box; 8, solenoid valve 1 (SV1); 9, solenoid valve 2 (SV2); 10, canister port connect; and 11, heating wire.

MATERIALS AND METHODS

Authentic Chemicals. All standard chemicals of a series of alkanes were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). A series of alkanols, alkanals, and other chemicals were purchased from Wako Pure Chemical Industries Ltd. (Osaka). β-Damascenone, solanone (5-isopropyl-8-methyl-6,8-nonadien-2-one), and geranylacetone were raw materials supplied by the Japan Tobacco Inc. (Tokyo).

Quantification. 1-Pentanol (Wako Pure Chemical Industries Ltd.) was used as an analytical internal standard. Ten microliters of an aqueous solution of 1-pentanol (2 g/L) was blotted to a 1 cm^2 square of filter paper (paper filter 5C, Advantec Toyo Kaisha Ltd., Tokyo) to simulate the tobacco cut leaf. A blotting paper was placed on the top of the sample in a sample vessel. A solution containing 1 ppm each of hydrocarbons between C8 and C16 was prepared by diluting the stock with ethyl alcohol (Wako Pure Chemical Industries Ltd.). The hydrocarbon solution instead of the sample was also blotted on another filter paper. That is the same size with that of internal standard.

Plant Materials. Typical aged flue-cured tobacco (*Nicotiana tabacum*) laminas from the United States were prepared for these studies. The laminas were cut into strips of 0.8 mm width. Their moisture content was adjusted to 60% relative humidity in an air-conditioned room at 22 °C for over 2 weeks. Any finer pieces from the cut leaves that passed through a 1.5 mm sieve were discarded. For all studies of instrument analysis, 3 g of cut leaves was loaded into each sample vessel containing a piece of filter paper with absorbed 1-pentanol. For the sensory study, the analytical standard was not used.

Design of a High Vacuum Distillation Apparatus (HVDA). A HVDA for the isolation of volatiles in this study is schematically presented in **Figure 1**. The inner pressure of the canister container (6 Liter silonite canister, Entech Instruments Inc., California) was reduced until 0.1 mmHg by using an automated canister cleaner (Entech 3000, Entech Instruments Inc.). The main line between the high vacuum system and the canister container was connected with a 1/8 in. stainless tube. The stainless steel sample vessel (1, the number in parentheses means the part of the instrument illustrated in **Figure 1** manufactured in our laboratory), which was equipped with two stainless meshes at both sides of the vessel, had a 45 mL (approximately) volume of capacity. The assembly was placed in a thermosetting heating oven (7, CO-8020, Tosoh Co., Tokyo). Nitrogen gas was supplied from a four-layer aluminum gasbag (2, 5 L, GL Sciences Co., Tokyo) and was used to regulate the pressure. A mass−flow controller (5, Veriflo SC423, Parker Hannifin Co., California) regulated the nitrogen gas flow under the decompression atmosphere in a canister and maintained an isothermal condition. To avoid condensation of volatiles, the line at the end of the sample line (11) was heated. To control the nitrogen gas flow for extraction and collection, two solenoid valves were inserted at both sides of the sample chamber (8, SV1; 9, SV2; FSD-0408C, Flon Industry Co, Ltd., Tokyo).

Operation Procedure for the HVDA. At the beginning of extraction, both solenoid valves (8, 9) were closed. Three grams of sample was placed in the sample vessel (1), and inert quartz glass wool (GL Science Co.) was put at both ends of the vessel. The sample vessel was warmed to the same temperature with the isothermal box. A canister (inner pressure, 0.1 mmHg) was connected to the extraction apparatus by opening its own valve. Just after opening SV2 (9), the inner atmospheric pressure of the sample vessel (1) was effused. The volatiles were vaporized and released from the cut leaf samples and immediately introduced into the canister. After SV1 (8) was turned on, nitrogen gas was transferred from the gasbag into the canister at 100 mL/min. During the extraction, the flow rate and integrated volume were monitored by a mass flow meter (6, SEF51, STEC Inc., Kyoto). When the total volume reached 1000 mL, the SV1 and SV2 valves were closed. Finally, the nitrogen gas was supplied up to a 2280 mmHg pressure in the canister trap.

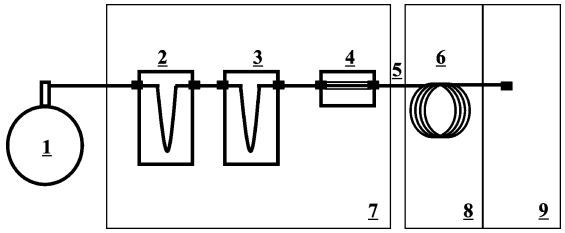

Figure 2. Schematic diagram of preconcentrator–GC/MS system with a canister. Key: 1, canister; 2, module 1 (glass beads); 3, module 2 (Tenax TA); 4, module 3 (fused silica capillary); 5, transfer line; 6, GC column; 7, preconcentrator; 8, GC; and 9, mass spectrometer.

Table 1. Analytical Condition for the Preconcentrator[a]

part	event description	temp (°C)	flow (mL/min)	volume (mL)	duration (min)
module 1 (2)	concentration	−150	100	1000	10
	preheat	20			
	desorption	180			
	bake out	200			
module 2 (3)	module 1	−40	25	100	4
	module 2				
	transfer				
	preheat				
	desorption	180			
	bake out	210			
module 3 (4)	concentration	−150			3.5
	desorption	100			
	injection	100			2
	bake out				3
GC trasfer line (5)		100			

[a] The number in the parentheses means the part of the instrument illustrated in **Figure 2**.

Gas Chromatography (GC)/Mass Spectrometry (MS) Analysis of Volatiles from Canisters. The analysis of canister samples was accomplished with a GC/MS instrument (Agilent 6890N gas chromatography/Agilent 5973N mass spectrometry, Agilent Technologies, California), equipped with a preconcentrator (Entech 7000, Entech Instruments Inc.) as an interface (*11*). A schematic diagram of the preconcentrator is shown in **Figure 2**. The detailed parameters for preconcentration are described in **Table 1**. These parameters were referred to the EPA compendium TO-15 method that was used to evaluate ambient air for hazardous organic compounds (*12*). After the sample (1000 mL) was preconcentrated on the first trap (2, module 1), the trap (2) was heated and the sample volatiles were thermally desorbed and recondensed on the next trap (3, module 2). Finally, the sample volatile was concentrated in the module 3 (4). Thus, the sample gas passed through three different adsorbents, which were glass beads, Tenax TA, and fused silica in the modules 1, 2, and 3 (2−4, respectively). The trapped chemicals in the module 3 (4) were heated at 100 °C, and the volatile components were thermally desorbed onto the head of the capillary column. The temperature of the transfer line (5) was maintained at 120 °C. The temperature of the column oven was programmed to rise from 40 (held for 5 min) to 240 °C (held for

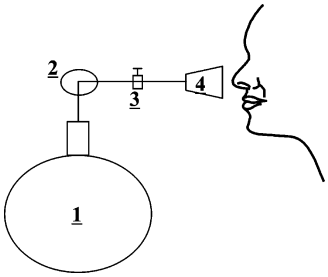

Figure 3. Sensory test using a canister. Key: 1, sample canister; 2, passive flow controller; 3, stop valve; and 4, sniffing port.

10 min) at 4 °C/min. A DB-Wax capillary column (60 m length, 0.25 mm i.d., and 0.5 μm film thickness, Agilent Technologies) was used for GC/MS. The MS ion source was heated to 230 °C, and the quadrupole was kept at 160 °C. Their collision energy for MS fragmentation was at 70 eV.

The volatile chemicals detected were identified by comparison of the retention indices and their MS spectra with known data in a Wiley MS-spectral database (7th edition) and a spectra data library of authentic compounds in our laboratory. Extracted ion chromatograms (EIC) with setting proper target ions (TIs) and qualifier ions (QIs) in a mass range from 27 to 300 amu (1−2 Hz) (*13*) were applied for quantitative analysis of each volatile. Thus, the peak area of a given chemical was integrated by the TI peak and then the TI peak area was adjusted by proper QIs. As TI chromatography may sometimes form a mixed shape of ion peaks with chemicals closely eluted, a QI that has a pure and symmetrical shape of the ion peak has to be selected as an adjusting fragment ion because the relative ratio of TI to QI in a pure chemical is always constant. Peak area ratios of individual components to the internal standard peak (1-pentanol: TI, 42 *m*/*z*; QI, 55 *m*/*z*) area were used as the variable of the peak (*5*, *13*, *14*). Analyses were carried out with five repetitions for each sample. The mean value of the five analyses was used in the studies.

Static Headspace GC/MS. Static headspace vapor (HSV) analysis was carried out along with Hasebe's method (*5*). Three grams of sample was loaded into a sample vessel together with the analytical standard. Headspace sampling was carried out using an HP-7694 headspace injector (Agilent Technologies) and a gas chromatograph HP-6890 (Agilent Technologies) equipped with an HP-5973 (Agilent Technologies) mass spectrometer. A DB-Wax capillary column (60 m length, 0.25 mm i.d., and 0.5 μm film thickness, Agilent Technologies) was used for the gas chromatography. Headspace gas was collected in a glass vial (20 mL, nominally) fitted with a PTFF-lined silicone−rubber septum. Liquid standard samples were blotted on 1 cm^2 of a filter paper (ADVANTEC 5C filter paper, Advantec Toyo Kaisha, Ltd.). The sample vial was preheated at 90 °C for 30 min and then, the headspace sampling vial was pressurized by helium gas at 10 psi for 0.2 min. The HSV was sampled with a 3 mL sample loop and injected into the column with a split ratio of 10:1. The temperature of the transfer line and injection port was maintained at 200 °C. The column oven temperature was programmed to increase from 35 (held or 5 min) to 240 °C (held for 10 min) at 4 °C/min. The MS ion source temperature was 230 °C, and the quadrupole was 160 °C. Volatile compounds were identified by comparison of their spectra with known data in a Wiley MS-spectral database (6th edition). Peaks of EIC with a proper TI and QI, in a mass range from 29 to 300 amu (1−2 Hz), were integrated. A 78 amu ion was used as a TI of alkanes. The HSV peak area ratio against the internal standard peak area was used as the variable of the peak. Headspace analyses were carried out five consecutive times for each sample. The mean value of the five analyses was used in the studies.

Sensory Test for the Canister-Trapped Volatile Samples. Ten panel members (three females, seven males, average age of 32 ± 7 years) participated in sensory tests. All subjects were volunteers who signed consent forms. We instructed them not to eat, drink, chew gum, or smoke for at least 1 h prior to testing. Odor stimuli were presented from the canister as shown in **Figure 3**. The odor flow rate was controlled at 200 mL/min using a passive flow controller (2 in **Figure 3**). Each panelist scored in sensory scales, which were classified by "greenness", "fruity", "smoothing", "terpeny", and "hay-like" odors. The sensory scale was 15 cm long, and both sides had anchors with "strong" and "weak" odors for perceived intensity of odor sense. Each sample was presented three times for each panel member, randomly.

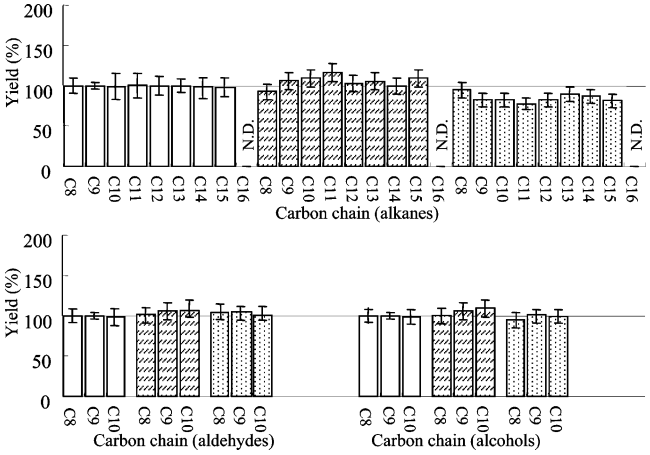

Figure 4. Yield of hydrocarbons, alcohols, and aldehydes using HVDA. The peak areas calculated under the different vaporization temperatures (70, 80, and 100 °C) were normalized by the ion peak at 57 *m*/*z* of octane, octanol, and octanals.

RESULTS AND DISCUSSION

Trapping Capability of a HVDA. Figure 4 shows the recovery yields of standard chemicals by using a HVDA. A silonite canister that is inert to chemical components was used to trap the headspace gas. Recovery tests were performed at different temperatures such as 40, 60, and 80 °C, and the results showed that temperatures lower than 80 °C did not affect the recovery efficiency. Therefore, recovery yields were calculated at the extraction temperature of 40 °C and then standardized by the TI area of octane (C8, TI 57 *m*/z). Series of alkanes, alkanols, and alkanals analyzed under the same condition had almost complete recovery (100%) with coefficients of variation of less than 13%. This means that HVDA does not require any sample heating for vaporization, although typical static headspace analyses have been affected by heating conditions. This high yield may be caused by the low atmospheric pressure (0.1 mmHg) of the HVDA and the sweeping of headspace volatiles with a nitrogen stream. On the other hand, the HVDA could not extract several chemicals such as larger molecules of alcohols and aldehydes (>C16). This could be partially due to a vapor pressure's (VP) constant from Antoine coefficients (*15*). Antoine's equation at 40 °C showed that theoretical VPs of C14, C15, C16, and C17 alkanes are 0.043, 0.014, 0.004, and 0.001 mmHg, respectively. Under the decompressed atmosphere (0.1 mmHg) and ambient temperature, there was a detectable limitation in the recovery of less than C15 alkanes (0.014 mmHg) by the limitation of vaporization.

Identification of Volatile Components in Flue-Cured Tobacco. The total ion chromatograms of cut leaf volatiles extracted with HVDA and static headspace methods are comparatively illustrated in **Figures 5** and **6**. The components identified are listed in **Table 2**. Sixty tobacco aroma volatiles were determined by the HVDA method.

The theoretical VP of each volatile in tobacco cut leaves extracted by the HVDA method was also calculated and shown in **Table 3** as the same manner of that of alkanes. Acetaldehyde, which has a low boiling point (bp 20.1 °C), showed 1504 mmHg of VP at 40 °C. VPs at 40 °C and boiling points of some

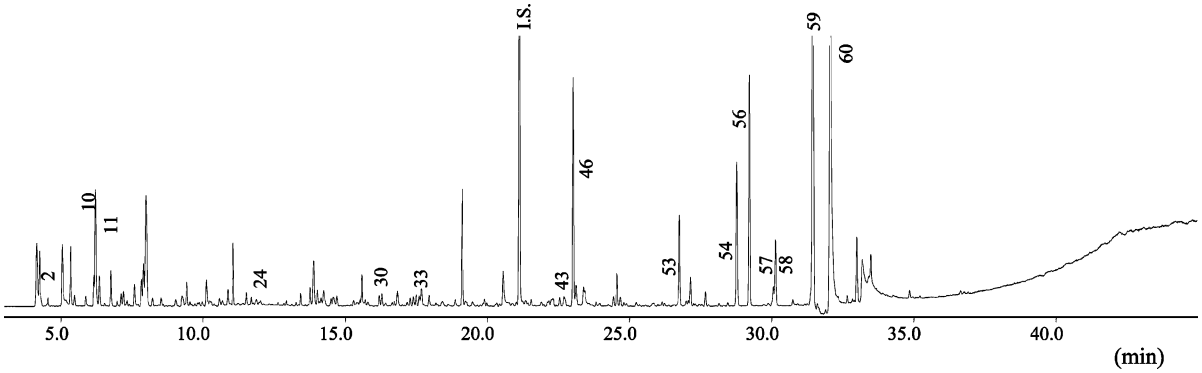

Figure 5. Total ion chromatogram of aroma components obtained from flue-cured tobacco cut leaf by HVDA. Each peak number corresponds to those in **Table 2**. IS, 1-pentanol as an internal standard.

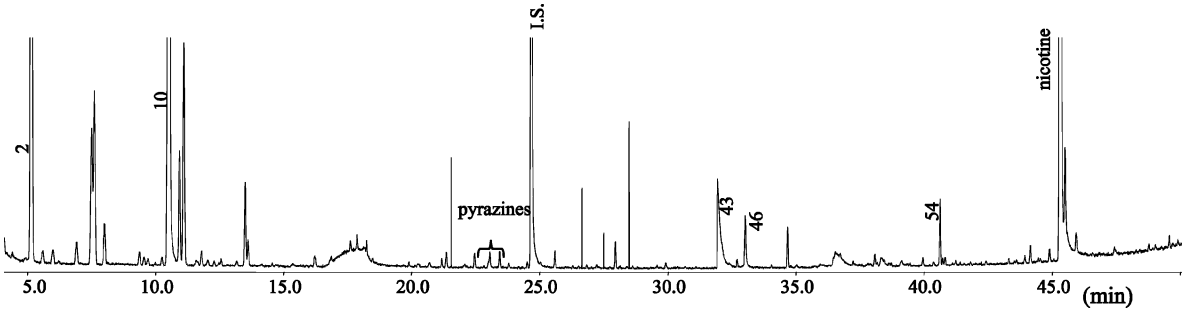

Figure 6. Total ion chromatogram of aroma components obtained from flue-cured tobacco cut leaf by HSV. The sample was preheated at 90 °C for 30 min and then pressurized by helium carrier gas at 10 psi for 0.2 min. Each peak number corresponds to those in **Table 2**. IS, 1-pentanol as an internal standard. Pyrazines, 2-ethylpyrazine (RI, 1310) and 2,3-dimethylpyrazine (RI, 1318).

compounds such as acetone, methyl acetate, ethanol, hexanal, 1-butanol, and acetic acid were 424.156 mmHg and 56 °C, 405.614 mmHg and 57.5 °C, 134.284 mmHg and 78 °C, 25.466 mmHg and 131 °C, 18.295 mmHg and 117.7 °C, and 34.935 mmHg and 117−118 °C, respectively. These were the major volatile components detected by the static headspace method. The HVDA method could detect lower volatile components such as n-valeraldehyde (VP, 0.652 mmHg at 40 °C), tetradecane (VP, 0.043 mmHg at 40 °C), and 1-phenylethanone (VP, 0.942 mmHg) as fairly big peaks. Hiatt isolated volatile chemicals in a fish tissue (*16*) under a vacuum distillation system. He determined the limitation of vacuum pressure to be 0.78 mmHg in his system and concluded that chemicals having VP greater than 0.78 mmHg can be recovered. This supports our results, which showed that in the canister system, volatile compounds having VPs greater than 0.01 mmHg could be trapped. In other words, the limitation of the decompression capability of a canister would be around 0.04−0.014 mmHg (for C15 alkane). Hence, the volatile component listed in **Table 2** had a VP mostly over 0.014 mmHg.

Noteworthy, carotenoid derivatives such as β-damascenone, 2,6,6-trimethyl-2-cyclohexen-1,4-dione, geranylacetone, and other volatiles such as 2-acetyl-5-methylfuran, linalool, and 6-methyl-5-hepten-2-one, which have a characteristic aroma and very low odor threshold, were also identified as larger peaks. Pfannkoch (*7*) comparatively demonstrated the GC sensitivity of several volatile extracts from direct static headspace gas, solid phase microextraction, and direct thermal desorption on solid food matrices. They showed that static headspace analysis provided information only on the major components, which have high volatilities. The low volatile components, carotenoid degradation components, and some other aroma components could not be measured quantitatively with the static headspace measurement. The HVDA system introduced here was found to be able to have a wider analytical range in volatility, for tobacco cut leaf volatiles. Moreover, some pyrazines such as 2-ethylpyrazine [retention index (RI) at DB-Wax, 1310] and 2,3-dimethylpyrazine (RI, 1318) as well as nicotine (bp 247 °C; RI, 1843) found in static headspace volatiles (**Figure 6**), were not detected by the canister−HVDA system. It is well-known that these pyrazines are the thermally degraded products (*17*) and undesirable chemicals for the aroma character.

Effect of the Canister System on Sensory Test of Volatiles of Flue-Cured Tobacco. Aroma changes by cutting stored leaves seriously influence the quality and price of cigarette products. To control and manage the cut leaf aroma quality, reliable data for sensory evaluation and quantitative analysis of the dynamical change are needed. However, direct comparison of aroma quality of headspace gases during storage was impossible so far. Using a canister system, aromas from two stages of storage period were simultaneously compared to understand the dynamic changing of the aroma quality of tobacco cut leaves. To compare the odor changes during 2 weeks of storage, we checked the odor stability of some tobacco volatiles in a canister for more than 2 weeks by sensory test. There were no significant differences among them. Cut leaf samples were stored in an isothermal box at 25 °C for 2 weeks. The perceived intensities of odor attributes of 2 weeks of stored leaves and 0 weeks of stored leaves (as a control sample) are recorded in **Figure 7**. Sensory panel members evaluated the character of both extracted volatiles through the canister. As the diagram indicates, the lack of greenness and smoothing of the aroma (5% level of significance, $p < 0.05$) were commonly pointed out on their comments (greenness, $p = 0.042$; fruity, $p = 0.297$; smoothing, $p = 0.031$; terpeny, $p = 0.913$; hay-like, $p = 0.582$; DF = 29; paired *t*-test, two-tail).

Chemical Changes of Tobacco Volatile Components in a 2-Week Storage. During 2 weeks of storage, we could

Table 2. Identified Volatile Components in Flue-Cured Tobacco by HVDA

peak no.	compd	selected fragment ions[a] TI	QI	RI (DB-Wax)	mol weight
1	hexane	57	86	600	86
2	acetaldehyde	44	43	703	44
3	propanal	58		798	58
4	acetone	58	43	825	58
5	methyl acetate	74	43	832	74
6	dichloromethane	84	49	849	56
7	dimethyl disulfide	44	75	853	94
8	2-methylfuran	53	82	877	82
9	nonane	85	57, 43	900	128
10	methanol	32	-	907	32
11	ethanol	45	-	942	46
12	benzene	78	52	955	86
13	n-valeraldehyde	44	58	991	86
14	hexanal	56	44	1024	100
15	2-pentanone	43	86	1025	86
16	1-propanol	42		1037	60
17	α-pinene	93	121, 91	1042	136
18	toluene	91	65	1065	92
19	butyl acetate	43	56	1092	116
20	undecane	57	43, 71	1099	156
21	1-butanol	56	43	1147	74
22	2-methylpropanal	43	72	1154	72
23	1-penten-3-ol	57	–	1158	86
24	ethylbenzene	91	106	1161	106
25	heptanal	70	56, 43	1178	114
26	decanone	57	85,170	1209	170
27	limonene	136	93, 68	1220	136
28	p-cymene	119	134, 91	1250	134
29	2-pentylfuran	138	81	1261	138
30	α-terpinolene	121	136, 93	1323	136
31	isoterpinolene	121	93, 79	1331	136
32	3-methyl-2-cyclopentenone	96	67, 81	1336	96
33	6-methy-5-hepten-2-one	69	55, 93	1375	126
34	1,3,5-trimethylbenzene	120	105	1383	120
35	1-hexanol	56	43	1384	102
36	2,6-dimethyl-5-heptenal	82	67, 41	1389	140
37	tetradecane	57	81	1400	198
38	2,6-dimethyl-2,4,6-octatriene	121	105, 136	1402	136
39	nonanal	57	98	1417	142
40	*cis*-3-hexenol	67	82, 41	1418	100
41	2-butoxyethanol	57	87	1441	118
42	3-(4-methyl-3-pentenyl)furan	69	81, 150	1450	150
43	acetic acid	44	–	1457	60
44	furfural	96	39	1472	96
45	benzaldehyde	106	77	1497	106
46	2-ethyl-1-hexanol	57	83, 70	1512	130
47	menthol	71	138, 95	1514	156
48	linalool	93	71, 121	1565	154
49	carvone	82	108, 54	1575	150
50	octanal	56	84	1578	130
51	2,3-butanediol	45	57	1580	90
52	1,3-butanediol	45	–	1600	90
53	2-acetyl-5-methylfuran	109	124	1658	124
54	solanone	93	121, 136	1661	194
55	β-damascenone	69	121, 90	1679	109
56	acetophenone	105	77, 120	1700	120
57	2,6,6-trimethyl-2-cyclohexen-1,4-dione	68	152	1742	152
58	geranylacetone	69	43	1785	194
59	benzyl alcohol	108	79	1865	108
60	2-phenylethanol	122	91	1870	122

[a] TIs and QIs were used for peak area calculation.

observe some changes of unique odor characters using HVDA. Afterward, we looked for volatile components with the same or similar descriptive terms in the list of identified compounds in **Table 4**. Finally, as for the characteristic flue-cured tobacco volatile, the relative peak ratios were calculated from individual TI areas and then changes of the volatile chemicals were compared by the HVDA procedure before and after 2 weeks of storage with statistical significance. Each TI peak area was integrated on the basis of QIs as the reference (*14*).

Table 3. Theoretical VP of Typical Volatile Components Found in Flue-Cured Tobacco at 40 °C

peak no.	compd	RI (DB-Wax)	VP[a] at 40 °C (mmHg)
1	hexane	600	351.80
2	acetaldehyde	703	1504.92
4	acetone	825	424.16
5	methyl acetate	832	405.61
6	dichloromethane	849	766.74
9	nonane	900	10.51
10	methanol	907	265.77
11	ethanol	942	134.28
12	benzene	955	295.52
13	n-valeraldehyde	991	0.65
14	hexanal	1024	25.47
15	2-pentanone	1025	74.13
16	1-propanol	1037	52.16
20	undecane	1099	1.20
21	1-butanol	1147	18.29
24	ethylbenzene	1161	21.49
25	heptanal	1178	8.77
37	tetradecane	1400	0.04
43	acetic acid	1457	34.93
50	octanal	1578	3.30
56	acetophenone	1700	0.94

[a] Theoretical VP was calculated at 40 °C using Antoine's coefficients (*15*).

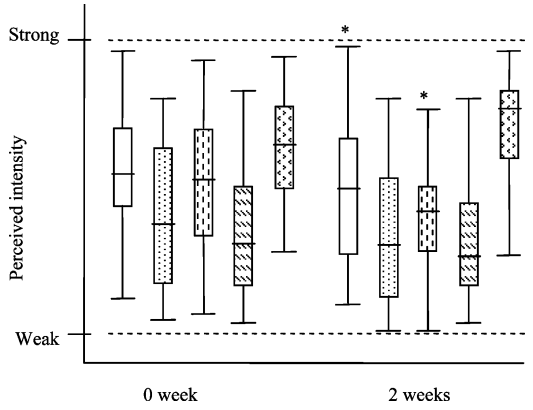

Figure 7. Change of aroma characteristics during 2 weeks of storage. Cut leaves were stored in an isothermal box (at 25 °C) for 2 weeks. *, significant differences at $p < 0.05$, two-side pair *t*-test.

Most volatile components had kept their quantities during 2 weeks. Area percent values of individual TI were very reproducible, with a small variation (less than 10%). A recent study reports good recovery yields of some monoterpenes such as alcohols, aldehydes from a canister for 2 weeks (*18*). This means that the canister−HVDA system at 25 °C can significantly reserve the volatile chemicals from the cut leaves for 2 weeks. Thirteen components such as 2,6,6-trimethyl-2-cyclohexen-1,4-dione [musty, woody, sweet tea, and sweet tobacco leaf odor (*19*)], 2-acetyl-5-methylfuran [strong nutty, hay, and coumarin odor (*20*)], β-damascenone [rose odor (*21*)], carvone [warm-herbaceous, bread-like, penetrating, and diffusive odor (*19*)], geranylacetone [fresh floral, light but rather penetrating, sweet rosy, slightly green, and magnolia-like odor (*19*)], 2,6-dimethyl-5-heptanal [very powerful, oily green, and vegetable-like odor (*19*)], acetophenone [sweet pungent, hawthorn mimosa, almond, and chemical odor (*19*)], heptanal [very powerful and diffusive, oil fatty, and rancid odor (*19*)], hexanal [very powerful, penetrating, fatty green, and grassy odor (*19*)], *cis*-3-hexenol [powerful and intensely green and grassy odor (*19*)], 1-penten-3-ol [powerful, grassy green and very diffusive odor (*19*)],

Table 4. Volatile Components' Change of Flue-Cured Tobacco during 2 Weeks of Storage

peak no.	compd[a]	RI (DB-Wax)	changes in storage[b] quantity ratio	*t*-test	odor description[c]
1	hexane	600	99		mild gasoline-like odor[2]
2	acetaldehyde	703	102		pungent, ethereal nauseating odor[1]
3	propanal	798	100		very diffusive, penetrating, suffocating odor[1]
4	acetone	825	95		pleasant odor in dilution[1]
5	methyl acetate	832	120	*	sweet and extremely diffusive, ethereal fruity odor of very poor tenacity[1]
6	dichloromethane	849	95		chloroform-like odor[2]
7	dimethyl disulfide	853	75	**	extremely diffusive, repulsive odor[1]
8	2-methylfuran	877	101		
9	nonane	900	102		odorless
10	methanol	907	98		pungent[1]
11	ethanol	942	110		sweet ethereal[1]
12	benzene	955	106		paint thinner-like odor[2]
13	n-valeraldehyde	991	98		very powerful and diffusive, penetrating, acid pungent odor[1]
14	hexanal	1024	110		in extreme dilution more reminiscent of freshly cut grass and unripe fruits (apple and plum)[1]
15	2-pentanone	1025	97		characteristic ketone odor[2]
16	1-propanol	1037	103		mild, nonresidual, alcoholic odor[2]
17	α-pinene	1042	54	*	warm resinous, refreshing pine-like[1]
18	toluene	1065	88		
19	butyl acetate	1092	79		very diffusive, etheral fruity, pungent odor[1]
20	undecane	1099	101		
21	1-butanol	1147	96		mild fusel-like odor[1]
22	2-methylpropanal	1154	85		fruity, banana-like, pleasant odor[1]
23	1-penten-3-ol	1158	96		powerful, gassy green, and very diffusive odor[1]
24	ethylbenzene	1161	60	**	sweet, gassy odor[1]
25	heptanal	1178	99		very powerful and diffusive, oil fatty, rancid odor[1]
26	decanone	1209	100		
27	limonene	1220	103		fresh, light, sweet citrusy odor[1]
28	p-cymene	1250	130	*	gassy, kerosene-like odor[1]
29	2-pentylfuran	1261	59	*	fruity green earthy beany vegetable metallic[1]
30	α-terpinolene	1323	98		
31	isoterpinolene	1331	97		
32	3-methyl-2-cyclopentenone	1336	103		
33	6-methy-5-hepten-2-one	1375	89		pungent[3]
34	1,3,5-trimethylbenzene	1383	89		
35	1-haxanol	1384	57	*	very powerful, penetrating, fatty green, grassy odor[1]
36	2,6-dimethyl-5-heptenal	1389	88		very powerful, oily green, vegetable-like odor[1]
37	tetradecane	1400	100		
38	2,6-dimethyl-2,4,6-octatriene	1402	100		
39	nonanal	1417	100		in proper dilution, fatty notes become more pleasant, floral waxy, more rosy and sweet, fresh as Neroli[1]
40	*cis*-3-hexenol	1418	100		powerful and intesely green, grassy odor[1]
41	2-butoxyethanol	1441	97		
42	3-(4-methyl-3-pentenyl)furan	1450	105		
43	acetic acid	1457	103		pungent, stinging sour odor[1]
44	furfural	1472	88		pungent, but sweet, bread-like, caramellic cinnamon almond-like odor[1]
45	benzaldehyde	1497	45	**	powerful sweet odor[1]
46	2-ethylhexanol	1512	63	**	comparatively mild, oily, slightly floral rosy odor[1]
47	menthol	1514	96		refreshing, light, diffusive odor with a sweet pungency[1]
48	linalool	1565	48	**	light and refreshing, floral woody odor with a faintly citrusy note[1]
49	carvone	1575	96		warm-herbaceous, bread-like, penetrating, diffusive odor[1]
50	octanal	1578	100		powerful, and in undiluted state harsh fatty, penetrating odor[1]
51	2,3-butanediol	1580	95		fruity[3]
52	1,3-butanediol	1600	100		
53	2-acetyl-5-methylfuran	1658	99		strong nutty hay coumarin[2]
54	solanone	1661	102		
55	β-damascenone	1679	100		rose[3]
56	acetophenone	1700	96		sweet pungent hawthorn mimosa almond chemical[1]
57	2,6,6-trimethyl-2-cyclo-hexen-1,4-dione	1742	102		musty, woody, sweet tea, sweet tobacco leaf[1]
58	geranylacetone	1785	132		fresh floral, light, but rather penetrating, sweet rosy, slightly green, magnolia-like odor[1]
59	benzyl alcohol	1865	75	**	faint, nondescript odor[1]
60	2-phenylethanol	1870	75	*	mild and warm, rose honey-like odor[1]

[a] The compounds were identified by comparing them with the reference compounds on the basis of GC-MS fragment patterns and retention indices. [b] The ratio of peak area was calculated as the following equation: ratio = peak area at 2 weeks of storage/peak area at 0 weeks of storage. *,**, significant differences at $p < 0.05$ or $p < 0.01$ (*t*-test). [c] Odor descriptions were cited in refs (1) *19*, (2) *20*, and (3) *21*.

n-valeraldehyde [very powerful and diffusive, penetrating, and acid pungent odor (*19*)], and 2-methylpropanal (fruity, banana-like, and pleasant odor (*19*)] were not significantly changed during the 2 week storage period. These chemicals are involved in some denoted tobacco leaf odor characteristics such as arytenoids derivatives (*22*).

On the other hand, the amounts of 10 volatile components decreased significantly during the 2 weeks of storage. These were dimethyl sulfide [extremely diffusive and repulsive odor (*19*)], 1-hexanol [very powerful, penetrating, fatty green, and grassy odor (*19*)], benzaldehyde [powerful sweet odor (*19*)], ethylbenzene [sweet gassy odor (*19*)], benzyl alcohol [faint and nondescript odor (*19*)], 2-phenylethanol [mild and warm, rose honey-like odor (*19*)], 2-ethyl-1-hexanol [comparatively mild, oily, and slightly floral rosy odor (*19*)], α-pinene [warm resinous and refreshing pine-like odor (*19*)], 2-pentylfuran [fruity, green, earthy, beany, vegetable, and metallic odor (*19*)], and linalool [light and refreshing, floral woody odor with a faintly citrusy note (*19*)]. The greatest decrease in amount was observed in benzaldehyde (45%). Two chemicals such as methyl acetate [sweet and extremely diffusive, ethereal fruity odor of very poor tenacity (*19*)] and *p*-cymene [gassy kerosene-like odor (*19*)] were significantly increased.

It is well-known that many carotenoid derivatives in tobacco leaves are formed as important aroma substances after harvesting and during the curing, while the amount of carotenoid pigments decrease (*17*, *22*−*25*). During storage, undesirable aroma formation due to the amount of some esters and alcohols was remarkably low in cut leaf storage in a canister trap. This canister−HVDA system led us to know that some unstable volatile components such as α-terpinolene, benzaldehyde, and linalool could be detected by a mild extraction technique without heating. Furthermore, by the simultaneous analyses on the basis of chemical and sensory aspects of the aroma, the decrease of greenness and smoothness in organoleptic test was related to the decrease ($p < 0.05$) of ethylbenzene, 2-pentylfuran, 1-hexanol, benzaldehyde, and linalool having the related descriptive terms to sweet and green aroma.

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