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Analysis of free and bound volatiles by gas chromatography and gas chromatography–mass spectrometry in uncased and cased tobaccos

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Abstract

The free and bound volatiles of tobaccos were analyzed by capillary GC and GC–MS. Bound volatiles were isolated by dichloromethane extraction followed by stream distillation continuous extraction (SDE) at pH 2.5 acid hydrolysis. The bound aromatic compounds were hydrolyzed by acid at pH 2.5, and the bound volatiles were liberated and extracted into dichloromethane by SDE simultaneously. In total, 23 volatiles were identified, with neophytadiene, 2-ethyl hexanol, damascenone, benzene ethanol, palmitic acid, stearic acid, linoleic acid, farnesyl acetone, 3-oxo-ionol, and megastigmatrienone being the major components. They consisted mainly of compounds exhibiting aromatic characteristics. The quality and quantity of free and bound volatiles exhibited different distributions in uncased or cased tobaccos. The volatiles existed in higher amounts in bound form than in free form. Compared with uncased tobaccos, free form volatiles showed a decrease after the casing process, while bound volatiles showed an increase. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Tobacco; Volatiles

1. Introduction

Aromatic volatiles of tobaccos that presented in either free or bound form could contribute to the flavor of cigarettes during smoking. Bound aromatic compounds had been found to be important precursors of aromatic volatiles [1]. Free volatiles were easily volatilized during processing, storing and smoking, which resulted in the change and unevenness of flavor concentration from a cigarette from puff to puff. While the volatiles were in bound form, they were not easily volatilized during processing and storing, but they could be pyrolyzed as aromatic components contributing to the flavor of cigarette during smoking. Both the enzymes (e.g. during maturation and fermentation) and the heat in processing (under normal low pH) could result in the hydrolysis of bound volatiles and the release of aromatic compounds.

Since the 1980s, many studies had demonstrated that aromatic volatiles of fruits such as quince, apricot, pineapple, and tomato presented either in free and bound form, and there were patents about the applications of bound aromatic compounds [2–

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5]. Glycosides of some ionone-related compounds had been isolated from tobacco [6]. The bound aromatic compounds were important to the taste and aroma obtained from a cigarette. Some conjugated forms of benzyl alcohol, acetophenone and geranyl acetone had been used as flavorings in cigarette blends [7]. A review by Hechman pointed out that glycosides were among the most important flavor precursors for tobacco and smoke [8]. However, information about the quantitative determination of free and bound aromatic compounds and their changes during tobacco processing was still lacking. Considering that some of the volatile compounds in tobacco could be released from nonvolatile precursors (i.e. glycosides, etc.) by enzymatic or acid hydrolysis, this study reports for the first time the free volatile composition as well as the occurrence of 20 bound volatiles in tobaccos. Additionally, this paper describes the on-line qualitative and quantitative analysis of the free and bound volatiles of tobaccos by capillary GC and GC–MS. Their changes during the casing process were also investigated.

2. Experimental

2.1. Materials

Cased and uncased flue-cured tobaccos (''SEPTWOLVES®'', Longyan Cigarette Manufactory of China) were collected from processing lines. A 1-kg aliquot of each sample was collected and mixed thoroughly, put into an oven at 40 °C for 8 h to remove moisture, then ground to 40–60 mesh powder.

All solvents employed (including anhydrous dichloromethane, anhydrous ethanol and heptadecane) were of analytical grade quality and were redistilled before use. Standards of 2-ethyl-hexanal, furfural, menthol, phenylethanol, 3-oxo-ionol, benzyl alcohol, acetophenone, damascenone, palmitic acid, stearic acid, geranyl acetone, linoleic acid, and heptadecane were all purchased from Sigma (St Louis, MO) and were all in GC purities. Standard solutions were used to optimize GC–MS and GC conditions. All were refrigerated at 4 °C during storage.

2.2. Isolation of free volatiles

A 10.0-g tobacco sample was extracted with 250 ml of dichloromethane in a Soxhlet apparatus (Velp, Scientifica, Italy) [9], the extract was concentrated to near dryness in a rotary evaporator (Laborata 4001, Heidolph, Germany) at 40 °C, then added to 250 ml of 0.2 mol l^{-1} phosphate buffer (pH 5.5). Using a Likens-Nickerson stream distillation continuous extraction (SDE) head [10] with dichloromethane as extraction solvent, the mixture was extracted and refluxed for 2.5 h at atmospheric pressure. The resulting dichloromethane extract was then added to 100 μl of internal standard solution (10 μg ml^{-1} heptadecane) and dried over anhydrous sodium sulfate. Prior to GC analysis, the diethyl ether extract was transferred into a K.D. concentrator and concentrated to 1 ml; 1 μl was used for GC or GC–MS analysis.

2.3. Isolation of bound aromatic compounds

A 10.0-g tobacco sample was added to 150 ml ethanol, the mixture was shaken overnight and then filtered, and the tobacco residue was re-extracted with 150 ml methanol. The combined methanol extract was concentrated to dryness using a rotary evaporator at 50 °C under reduced pressure, the dry extract was then dissolved in 100 ml deionized water, remaining free volatiles were removed by extraction with 1:1 hexane/ether (50 ml×3) three times. The water-soluble bound fraction was obtained.

2.4. Hydrolysis of bound fraction

To obtained the free form of bound aromatic compounds, the bound fraction was subjected to SDE in 250 ml 0.2 mol l^{-1} phosphate buffer (pH 2.5, 4.1 and 5.5, respectively, adjusted with 3 mol l^{-1} H_3PO_4). The bound aromatic compounds were hydrolyzed and extracted with dichloromethane. The conditions of SDE, dryness, concentration and GC–MS were similar to analysis of free aromatic compounds. According to the method of Voirin et al.

[11], 100 μl heptadecane (10 μg ml^{-1}) was used as internal standard.

2.5. Capillary GC and GC–MS analysis

Capillary GC analysis was performed with a Hewlett-Packard 6890 Series Gas Chromatograph with FID detector. For separation of free and bound aromatic constituents, a (30 m×0.25 mm I.D., d_f= 0.25 μm) fused-silica column (HP-5, Hewlett-Packard) was used. The injector and detector temperatures were 250 and 280 °C, respectively. Split injection 30:1 was used. Hydrogen was used as carrier gas with column head pressure at 15 p.s.i. The column oven was programmed from 40 °C (after 2 min) to 270 °C at 2.5 °C/min and the final temperature was held for 10 min. Chromatographic data acquisition and processing were carried out with a HP Chemstation Rev. A 06. 01.

Quantitative data for free and bound aromatic compounds hydrolyzed released were obtained by the internal standard method using heptadecane as internal standard or standards as reference substances, respectively, without considering calibration factors (i.e. F=1.00 for all compounds).

GC–MS analysis of free and bound aromatic compounds was carried out on a Hewlett-Packard 5973 mass selective detector directly coupled to a HP 5890 gas chromatograph. The same type of column and temperature conditions as mentioned above for capillary GC analysis was used. Helium was used as carrier gas with column head pressure at 12.5 p.s.i. The temperature of the GC–MS transfer line was 280 °C. The mass spectrum was operated at 170 °C in the electron impact mode (70 eV), scanning from m/z 33 to 350 in one scan. The voltage of the electronic multiplier tube (EMT) was 230 V above tuning. The mass spectral identification of the free and bound aromatic compounds were carried out by comparing to the NIST98 (National Institute of Standards and Technology, Gaithersburg, MD) mass spectral library as well as to the Wiley 6.0 (Wiley, New York) mass spectral library. Qualitative analysis (mass spectral data) was verified by comparing the retention indices and mass spectra of identified compounds with those of authentic reference substances.

3. Results and discussion

3.1. Analysis of free volatiles

The identity of free and bound volatiles by capillary GC and GC–MS in tobaccos is shown in Table 1. Each compound was qualified by authentic reference substances or mass spectra. As shown in Table 2, 22 free volatiles (including four isomers of megastigmatrienone) were identified with 10 aromatic ketones (62.44%), neophytadiene (23.08%), aliphatic acids (5.49%), three aromatic alcohols (3.81%), and two aromatic aldehydes (1.07%) being the major components in uncased flue-cured tobaccos. Twenty-one free volatiles were identified with 11 aromatic ketones (63.21%), neophytadiene (19.73%), three aromatic alcohols (5.75%), three aliphatic acids (5.66%), and two aromatic aldehydes (2.38%) being the major components in cased flue-cured tobaccos. In uncased tobaccos, aromatic ketones and neophytadiene (85.52%) predominated in the tobacco free volatile profile followed by aliphatic acids, aromatic alcohols and aromatic aldehydes. In cased tobaccos, aromatic ketones and neophytadiene (82.94%) predominated in the tobacco free volatile profile followed by aromatic alcohols, aliphatic acids, and aromatic aldehydes. Neophytadiene was the most abundant free volatile in various cased or uncased tobaccos. Benzyl alcohol and phenylethanol had been found previously in free form in fruits by Williams et al. [12].

3.2. Analysis of bound volatiles

The results of volatiles liberated from bound aromatic compounds revealed the presence of 23 bound volatiles in uncased or cased tobaccos (Table 2). In uncased tobaccos, the quantified bound aromatic compounds consisted mainly of aromatic ketones (77.62%), carboxylic acids (7.41%), aromatic aldehydes (5.31%) and aromatic alcohols (3.38%). In cased tobaccos, the identified bound aromatic compounds consisted mainly of aromatic ketones (76.62%), carboxylic acids (7.91%), aromatic alcohols (6.41%), and aromatic aldehydes (2.68%). The bound volatiles were dominated by 2-ethyl-hexanol, stearic acid, and megastigma-

Table 1
Free and bound volatiles identified by capillary GC and GC–MS in tobaccos

No.	Compound	Retention time (min)	Identified by MS or standards
1	5-Methyl-furanone	9.56	MS
2	Furfural	10.46	S
3	2-Ethyl-hexanol	11.24	S
4	5-Methyl-furfural	13.33	MS
5	Menthol	15.13	S
6	Damascenone	19.77	S
7	Geranyl acetone	20.82	S
8	Acetophenone	21.05	S
9	Benzyl alcohol	21.41	S
10	Ethyl maltol	21.95	MS
11	4-Vinyl guaiacol	22.03	MS
12	Phenylethanol	22.22	S
13	9,12,15-Octadecatrienal	22.71	MS
14	Megastigmatrienone(1)	27.77	MS
15	Megastigmatrienone(2)	28.75	MS
16	Megastigmatrienone(3)	30.22	MS
17	Megastigmatrienone(4)	30.91	MS
18	Farnesyl acetone	32.79	MS
19	3-Oxo-ionol	35.99	S
20	Palmitic acid	43.51	S
21	Stearic acid	47.01	S
22	Linoleic acid	48.67	S
23	Neophytadiene	50.35	MS

S, compound identified by authentic standards; MS, compound identified by comparing mass spectra data to NIST or Wiley mass spectral library.

trienone. The major bound volatiles might differ in other tobaccos. The bound volatiles were an important flavor precursor of cigarette that should be studied in more detail.

3.3. Effectiveness of sampling conditions on analysis

The quality was accomplished by comparing GC retention time and mass spectra data with that of either authentic reference substances or NIST and Wiley mass spectral library [13,14]. The relative quantity of each compound was determined using authentic standards or heptadecane as the internal standards, without considering recovery of aromatic compounds and GC–FID response factors.

Stream distillation continuous extraction in pH 5.5 buffer solution to isolate free volatiles in tobacco was used in this investigation. At this pH, only a small amount of nicotine was extracted. Consequently, a small nicotine peak appeared in the GC chromatogram. The column overload problem could be resolved. As a result, the denicotinized step before GC analysis was no longer needed. In addition, pH 5.5 approached the natural pH of flue-cured tobaccos.

The acid hydrolysis of bound aromatic compounds had been tested in buffer solutions with pH 2.5, 4.1 and 5.5, respectively. By using the SDE method, the mixtures were boiled for 2.5 h at 100 °C. Bound aromatic compounds (e.g. glycosides) are released and extracted into dichloromethane. Corresponding gas chromatograms are shown in Fig. 1A, B and C, respectively. At pH 5.5 and 4.1, only a small amount of bound aromatic compounds were obtained, while pH 2.5 condition seemed more effective for hydrolysis of tobacco bound aromatic compounds. So pH 2.5 buffer was chosen for hydrolysis of bound aromatic compounds in this investigation.

Table 2
Free and bound aromatic compounds in uncased and cased tobaccos

No.	Compound	Uncased tobacco		Cased tobacco	
		Free state	Bound state	Free state	Bound state
1	5-Methyl-furanone	1.6	2.4	0.8	2.9
2	Furfural	1.1	2.8	1.92	1.34
3	2-Ethyl-hexanol	35.1	10.4	31.8	16.7
4	5-Methyl-furfural	0.2	0.5	0.6	0.8
5	Menthol	0.7	0.6	1.3	1.4
6	Damascenone	5.6	1.2	8.5	2.9
7	Geranyl acetone	Tr	0.3	0.5	1.5
8	Acetophenone	0.9	2.1	0.2	2.8
9	Benzyl alcohol	3.7	1.1	4.8	2.8
10	Ethyl maltol	0.3	0.7	0.2	0.5
11	4-Vinyl guaiacol	0.2	0.2	Tr	0.4
12	Phenylethanol	0.2	0.5	Tr	0.9
13	Farnesyl acetone	16.1	7.3	10.9	10.5
14	3-Oxo-ionol	3.1	10.2	1.3	11
15	Palmitic acid	2	1.4	1.6	2
16	Stearic acid	1.1	1.7	1.1	2.3
17	Linoleic acid	3.6	1.5	3.3	2
18	9,12,15-Octadecatrienal	3.7	0.7	3	1.4
19	Megastigmatrienone(1)	4.5	1.9	3.2	2.2
20	Megastigmatrienone(2)	1.4	1.8	1.4	1.8
21	Megastigmatrienone(3)	4.2	6.1	4.2	5.8
22	Megastigmatrienone(4)	4.7	6.6	4.4	5.8
23	Neophytadiene	28.2	Tr	20.9	Tr

Values are μg/g, based on dry mass; Tr, trace amount.

3.4. Comparing free volatiles with bound volatiles

Figs. 2 and 3 show chromatograms of free and bound aromatic compounds in cased and uncased tobacco samples. Table 2 shows free and bound aromatic compounds determined in cased and uncased tobacco samples. The results show that, for most of the 20 aromatic compounds, they exist at higher amounts in bound form than in free form after the casing process. Compared with uncased tobaccos, free aromatic constituents showed a decrease, while bound aromatic constituents showed an increase. The results showed that bound volatiles, as well as the free volatiles were important to acquire a complete picture of tobacco flavor. Amounts of components of sensory significance might be released from the odorless bound aromatic compounds at high temperature during cigarette smoking [15,16]. So the bound aromatic compounds are important flavor precursors.

3.5. Changes in free and bound volatiles during the casing process

In a comparison of cased with uncased flue-cured tobacco samples, the free form of most constituents showed a decrease: 15 of the volatiles decreased in our study. Some free form volatiles could be volatilized at high temperature during the casing process such as 5-methylfuranone, furfural, 2-ethyl-hexanol, fanesyl acetone, 3-oxo-ionol, neophytadiene, megastigamatrienone. Neophytadiene and magastigamatrienone could not react with sugars added during the casing process, as shown in Table 2, their bound form and total amount decreased or almost maintained the same level. The total amount of farnesyl acetone and 3-oxo-ionol decreased, and their bound form increased. During the casing process, although they could have volatilized, these compounds might have reacted with some sugars and part of the free form been converted into bound form.

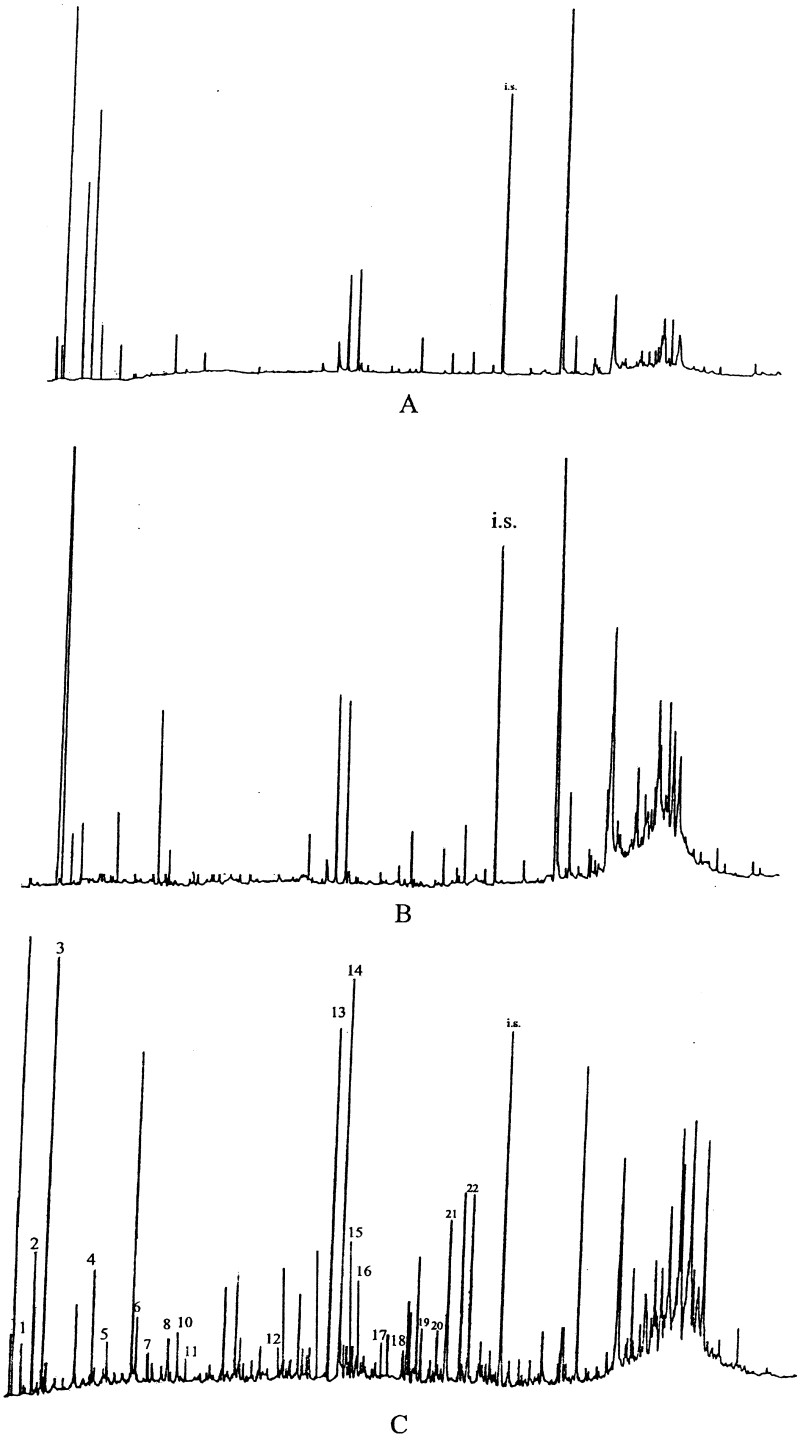

Fig. 1. Chromatogram of bound aromatic constituents hydrolyzed at pH 5.59 (A), pH 4.10 (B) and pH 2.50 (C). For identified peak numbers, see Table 1.

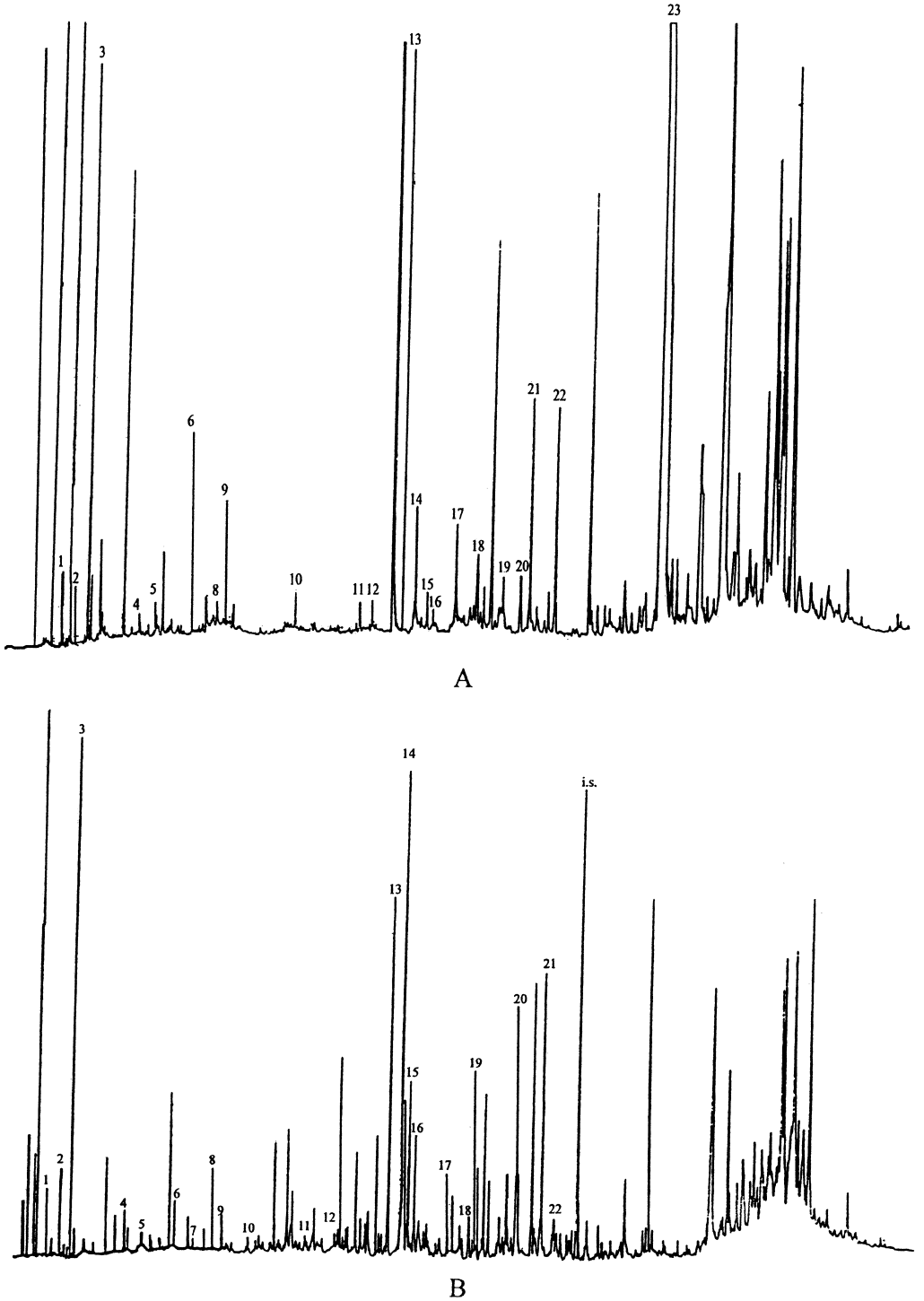

Fig. 2. Chromatogram of free (A) and bound (B) aromatic constituents of uncased tobacco. For identified peak numbers, see Table 1.

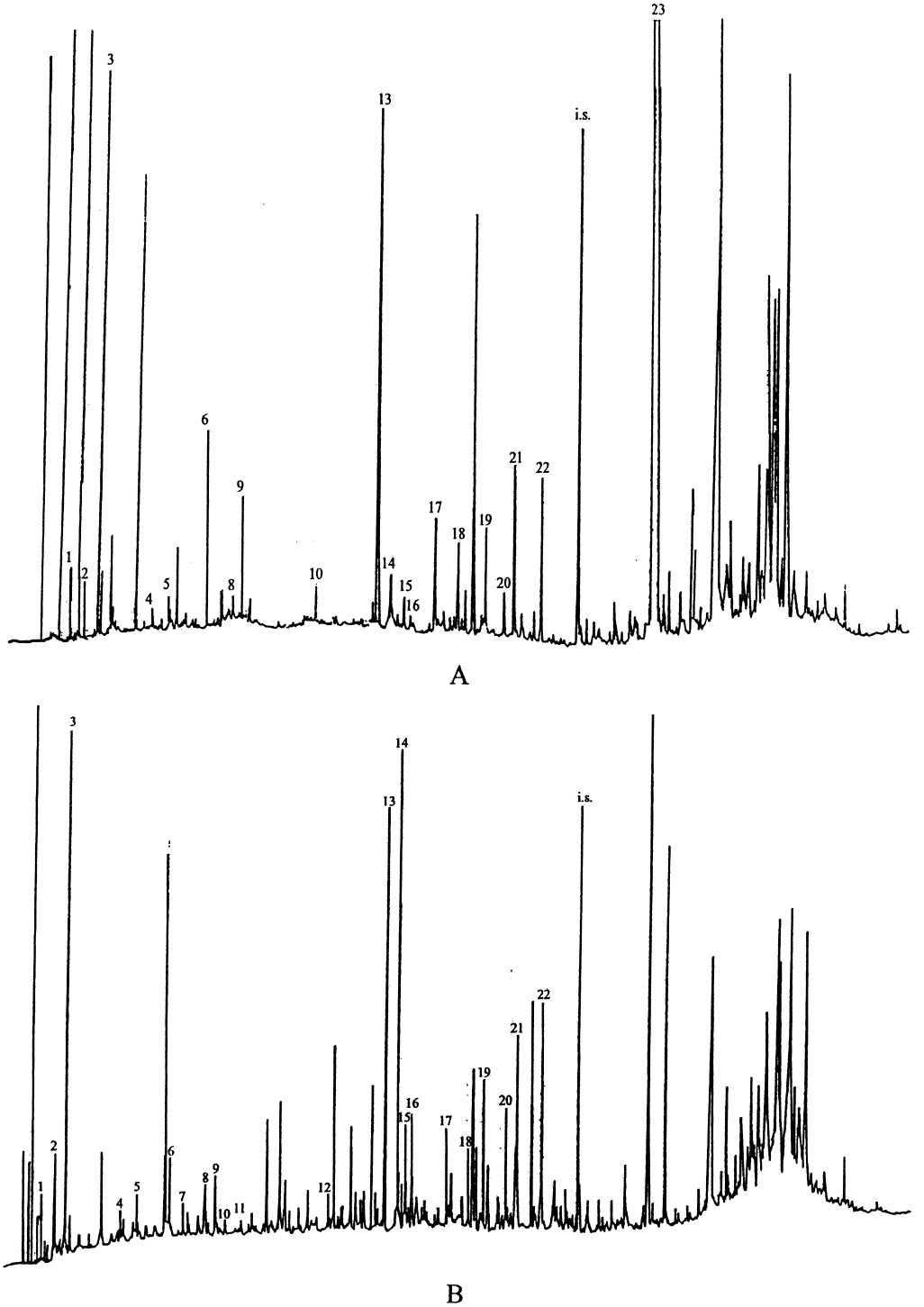

Fig. 3. Chromatogram of free (A) and bound (B) aromatic constituents of cased tobacco. For identified peak numbers, see Table 1.

As for bound volatiles, most showed increased levels during the casing process: 17 volatiles increased, and almost no bound volatiles decreased. During the casing process, the casing solution which contained sugars (e.g. sucrose, glucose, fructose, some organic acid, etc.) was added. Hydroxylic and carbonylic compounds such as 5-methylfuranone, furfural, 5-ethyl-hexanol, menthol, damascenone, geranyl acetone, benzyl alcohol, phenylethanol, farnesyl acetone, 3-oxo-ionol, palmitic acid, stearic acid, linoleic acid, can bind to these compounds in bound form.

The total amount of furfural, 2-ethyl-hexanol, 5-methyl-furfural, menthol, damascone, geranyl acetone, benzylalcohol, phenylethanol, palmitic acid, stearic acid, linoleic acid was increased: this might be due to other compounds converting, or some bound form of volatiles added in the casing solution. This might need further investigation.

The total amount of 9,12,15-octadecatrienal and linoleic acid remained constant, and the decrease in free form equaled the increase in bound form, so that the bound form might be transformed from the free form.

As a result, this distribution of the free and bound volatile forms might be beneficial to the tobacco leaf blends and the flavor of cigarette as a whole.

4. Conclusion

A simple and rapid method for the quantitative determination of free and bound volatiles in tobaccos has been investigated in this study. In tobacco, volatiles could exist in higher, lower or equal levels in both free and bound form. The analysis of bound compounds in tobaccos is a very important aspect of tobacco chemistry. During the tobacco casing process, for most volatiles identified in this investigation, free aromatic compounds decreased, while bound aromatic compounds increased. The distribution of the form of volatiles might be beneficial to the flavor of the cigarette.

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