Journal of the Korean Society of
Tobacco Science, Vol. 2, No. 2(1980),
Printed in the Republic of Korea.

향끽미종 잎담배 성분조성에 관한 연구
1. 휘발성이 적은 유기성분의 특성조사

김 경 례 · 박 진 우 · 이 윤 철 · 허 일

한국연초연구소 기기분석실

(1980. 10. 4 접수)

Composition Studies on the Aromatic Tobacco Varieties (*Nicotiana tabacum* L.):
1. Characteristics of Less-Volatile Organic Components.

Kyoung Rae Kim, Jeen Woo Park, Un Chul Lee, and Il Heu

Instrumental Analysis Laboratory,
Korea Tobacco Research Institute, Seoul, Korea

(Received Oct. 4, 1980)

초 록

오리엔트산 및 한국산 향끽미종 잎담배의 휘발성이 적은 성분들을 간단한 장치와 간단한 조작을 통해 분리 농축하였다.

각 휘발성이 적은 농축물들은 가시영역에서 흡광도를 측정하고 박층 크로마토그라피(TLC)와 고성능 액체 크로마토그라피(HPLC)에 의해서 상세한 조성분석을 하였다.

각 품종간의 정량적인 차이가 가시광선 흡수 스펙트라, TLC 패턴, HPLC프로파일 상에서 확인되었다.

ABSTRACT

Less-volatile organic components in leaves from aromatic tobaccos of different varieties, both Oriental and Korean types were isolated and concentrated using a simple apparatus with fewer manipulations.

Each less-volatile concentrate was then subjected to spectrophotometric recording in the visible range, to thin-layer chromatographic group separation, and high-performance liquid chromatographic profile analysis.

The methods allow detection of significant quantitative differences in visible absorption spectra, TLC patterns, and high resolution HPLC profiles among varieties.

Introduction

Chemical composition of leaf tobacco varies with genetic makeup, environmental conditions, and every step of production and handling.

Oriental tobaccos grown mainly in the Aegean-Mediterranean-Black Sea regions are produced with limited supplies of nutrients and water. Therefore, the chemical balance is toward carbohydrates and the leaves are highly aromatic. They have been an integral component of the worldwide cigarette blend for their contribution to the acceptability and overall quality of the cigarette.

Greek and Turkish aromatic tobaccos are the typical Oriental type. Extensive investigations on their chemical compositions have been reported (1-6).

Among sun-cured, aromatic tobaccos cultivated in Korea, Hyangcho (7) is known to possess characteristically strong aroma and to contain in smoke higher contents of total phenols and nicotine compared with Oriental tobaccos. Sohyang (8), a new Korean hybrid of aromatic variety, was found to be similar to Greek Basma in its aroma and contents of nicotine, sugar, and ether extract.

Chemical characterization of Korean- and Oriental type aromatic tobaccos was undertaken in our laboratory to provide basic guidelines for improving quality of Korean varieties. The objective of the present work was to survey whether varietal variations exist in the less-volatile fraction from the tobacco complex matrix.

As a preliminary study for the elucidation of the chemical structures of components responsible for varietal differences, we analyzed the concentrates from five varieties using visible absorption spectrophotometry, thin layer chromatography, and high performance liquid chromatography.

Materials and Methods

Tobacco samples: Aromatic varieties (*Nicotiana tobacum* L.) used for this study were Greek Basma (I/III), Turkish İzmir (B/G), Yugoslavian Basma (I/III), Korean Sohyang (Tip), and Korean Hyangcho (Tip) of 1978 crops. Each sun-cured leaf sample was redried at 60° and ground to pass 16 mesh.

The ground tobaccos were then stored in the colored containers at room temperature in the absence of light.

Reagents: Dichloromethane, methanol, and n-hexane (Lichrosolv,® chromatographic grade, E.Merck, Darmstadt, Germany). Toluene (GR grade, Chameleon Analytical Reagent, Osaka, Japan). Ace-

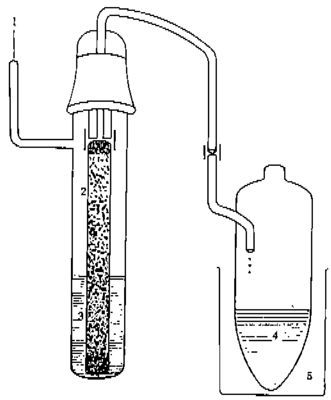

Fig. 1. Sampling system.
1 = purified nitrogen; 2 = column packed with ground tobacco; 3 = dichloromethane; 4 = eluate; 5 = water bath.

tonitrile and propionic acid (GR grade, Hayashi Pure Chemical Industries Ltd., Japan).

Sample preparation: Organic components of low-volatility were isolated and concentrated from tobaccos using a custom-made glass apparatus as shown in Figure 1. A full detail of the procedure was previously given (9). Briefly, a column packed with 1 g of ground tobacco was eluted with a 5 ml portion of dichloromethane under the slow stream of nitrogen gas. The eluate was subsequently evaporated to dryness at an elevated temperature by purging with nitrogen at an increased flow rate. The resulting residue was redissolved in 1 ml of dichloromethane containing 1% methanol, and stored in a freezer.

Spectrophotometry: A Varian Model Cary 17D UV-VIS spectrophotometer was used. Each concentrate was diluted to 0.10 (W/V) % in dichloromethane and scanned at 2 nm/sec from 700 to 325 nm with absorbance range of 0 - 2.0, and cell pathlength of 1 cm.

Thin-layer chromatography (TLC): A 20 x 20 cm plate, precoated with silica gel G (Baker-flex,® J. T. Baker Chemical Co., Phillipsburg, N. J. USA) was dried at 110° for 3h before use. Each of the concentrates was diluted to make 5% (W/V) solution in dichloromethane. 1 μl each was loaded to the TLC plate, followed by one-dimensional development using a solvent system, toluene/acetonitrile/propionic acid (60:12:1, by volume). At the completion of development, it was dried at ambient temperature and exposed to iodine vapor for the visualization of the separated sample bands.

High-performance liquid chromatography (HPLC): A 5 μl aliquot of each concentrate was analyzed using a Waters Associates Model ALC/GPC 200 liquid chromatograph equipped with dual Model 6000A solvent dilivery systems, a Model U6K universal injector, a Model 660 solvent programmer, and a Model 440 UV absorbance detector. Gradient elution was performed on a μ Porasil column (30cm x 0.39cm I.D.; Waters Associates) according to the operating conditions as shown in Table 1.

Table 1. HPLC conditions.

Column	: μ Porasil (30cm×3.9mm I. D.)
Mobile phase	: Gradient elution, linearly from 0% to 100% B (A=n-hexane, B=1% methanol in dichloromethane) in 20 min, and maintained at 100% B for further 20 min.
Flow rate	: 2.0 ml/min.
Injection	: 5.0 μl
Detector	: UV at 254 nm.
Sensitivity	: 0.1 AUFS
Chart speed	: 0.5 cm/min.

Results and Discussion

The sampling devices used for the present study permits isolation and concentration of the less-volatile organic constituents within an hour. The possibility of artifacts formation is minimal since the elution and concentration are carried out in an all-glass unit, under the stream of inert gas. The procedure is expected to enrich relatively less-volatile and less-polar organic groups, such as pigments, terpenes, alkaloids, phenols, solanesol, sterols, hydrocarbons, etc. (3, 10-17).

The dichloromethane extracts of Greek Basma, Turkish Izmir, Yugoslavian Basma, and Korean Sohyang, upon evaporation to dryness, yielded dark-green, waxy, resinous residues in 5.7%, 6.0%, 4.4%, and 4.2% of dry leaf weight, respectively. The residue from Korean Hyangcho, however, was dark-yellow and was not resinous, amounting to 5.5% of dry leaf weight. It is well known that the aroma of Oriental tobacco is furnished by resins and waxes. In this point, Sohyang appears to be closer to Oriental type rather than to Hyangcho.

Characteristic color of the concentrate is provided mainly by chlorophyll and carotenoid pigments. Visible absorption spectrum of each concentrate in Figure 2 gives information about the chemical constituents and their concentrations in the multicomponent system of the concentrate: four absorption bands at 410, 532, 604, and 663 nm indicate the presence of chlorophylls; bands at 435 and 478 nm are attributed to carotenoids (16). Differences in pigment levels among varieties are observed and compared in Table 2: Yugoslavian Basma was most concentrated, then declined in the order of Greek Basma, Turkish Izmir, Sohyang, and Hyangcho was least concentrated, as is anticipated from the color of its residue. Sohyang and Izmir were closest in their visible absorption spectra.

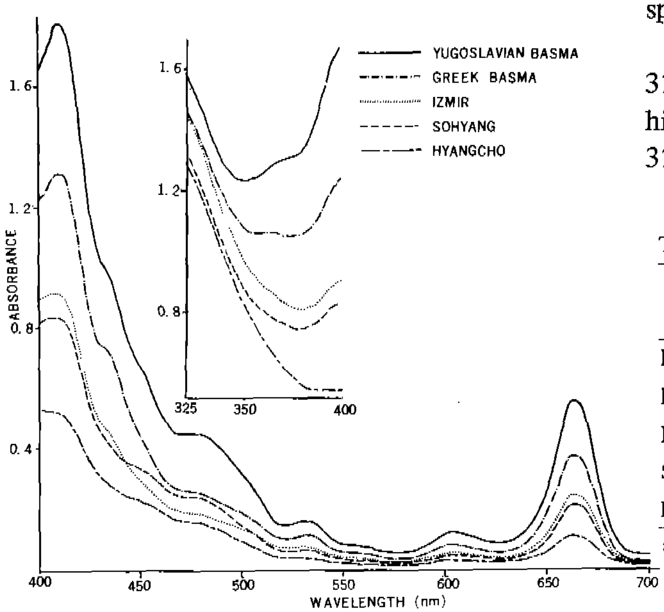

Fig. 2. Visible absorption spectra of dichloromethane concentrates obtained from Yugoslavian Basma, Greek Basma, Turkish Izmir, Korean Sohyang and Korean Hyangcho
Scanning speed, 2nm/sec; chart speed, 30nm/inch; absorbance range, 0-2.0; sample concentration, 0.1% (W/V) in dichloromethane.

Absorbances in the range from 350 to 325 nm are ascribed to phenols. The high ratio of absorbance of Hyangcho at 325 nm to that at 410 nm (Table 2) suggests that it has higher level of phenols compared with its chlorophyll content.

Table 2. Absorbances of the concentrates

Verieties	A325 nm	A410 nm	A663 nm	A325/A410 nm
Basma (Yugoslavian)	1.58	1.82	0.55	0.9
Basma (Greek)	1.46	1.32	0.37	1.1
Izmir (Turkish)	1.44	0.92	0.24	1.6
Sohyang (Korean)	1.32	0.83	0.22	1.6
Hyangcho (Korean)	1.28	0.53	0.11	2.4

* The concentration of each concentrate was 0.1 W/V % in dichloromethane.

As the rapid survey of the concentrates, group separation was made by TLC, to see their overall complexity in organic classes. 7 spots at R_f = 0.75, 0.53, 0.37,

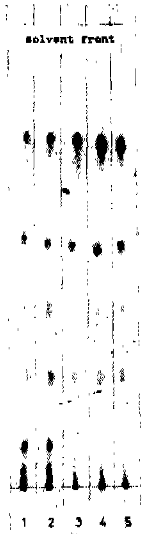

Fig. 3. TLC patterns of concentrates.
1=Hyangcho; 2=Sohyang; 3=Turkish Izmir; 4=Greek Basma; 5=Yugoslavian Basma.
Conditions : TLC plate, silica gel G precoated plate; sample, 5% dichloromethane solution; loaded sample amount, 1 μl; developing solvent system, toluene/acetonitrile/propionic acid (60:12:1, V/V/V %).

0.25, 0.09, 0.02, and 0.00 were seen in Figure 3. Nonpolar compound groups have larger R_f values. Spots at the origin, R_f = 0.02 and 0.09 are of more polar or basic compounds. Oriental type contains apparently higher levels of nonpolar, less levels of polar compounds as compared with Korean type.

HPLC yielded high resolution chromatograms of UV absorbing components present in the concentrates of tobaccos. Gradient elution was employed for the better resolution of the multicomponent complex mixture. Information with regard to the varietal variations could be traced by comparing the chromatographic profiles. Turkish Izmir, Korean Sohyang and Hyangcho were compared to Greek Basma as demonstrated in Figure 4, 5, and 6, respectively. Significant differences in the concentrates of several components among varieties were observed: Izmir shows increased concentrations of components at peaks 10, 13, and 31 as compared with Greek Basma (Figure 4); Sohyang is more concentrated in components at peaks 7, 12, 28 and 31, but less concentrated in components at peak 2 and 35 than Greek Basma (Figure 5); Hyangcho contains higher concentrations of components at peaks 7, 12, 24, 27, 28, and 36, however lower in peaks 35 than Greek Basma (Figure 6).

We are presently interested in the elucidation of the chemical structures of peaks which make contribution to the significant differences.

Fig. 4.

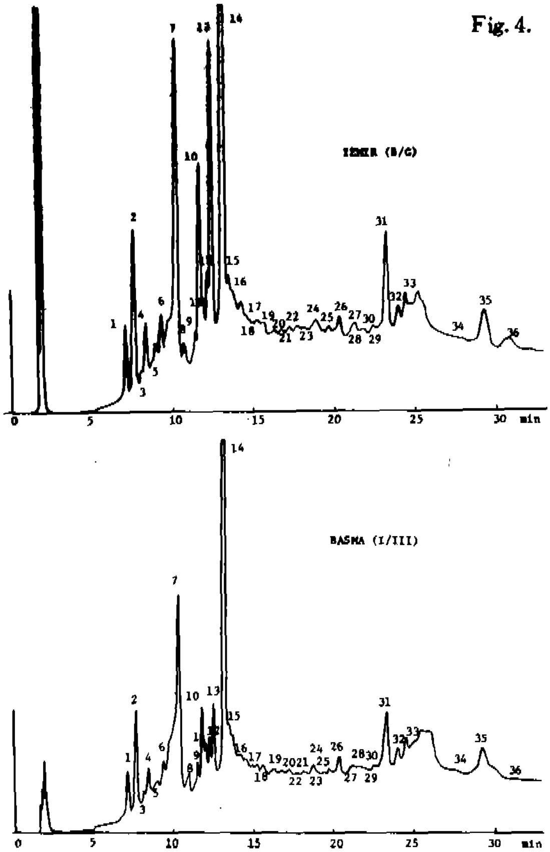

Fig. 4. HPLC profiles of Greek Basma vs. Turkish Izmir. Conditions in text.

Fig. 5.

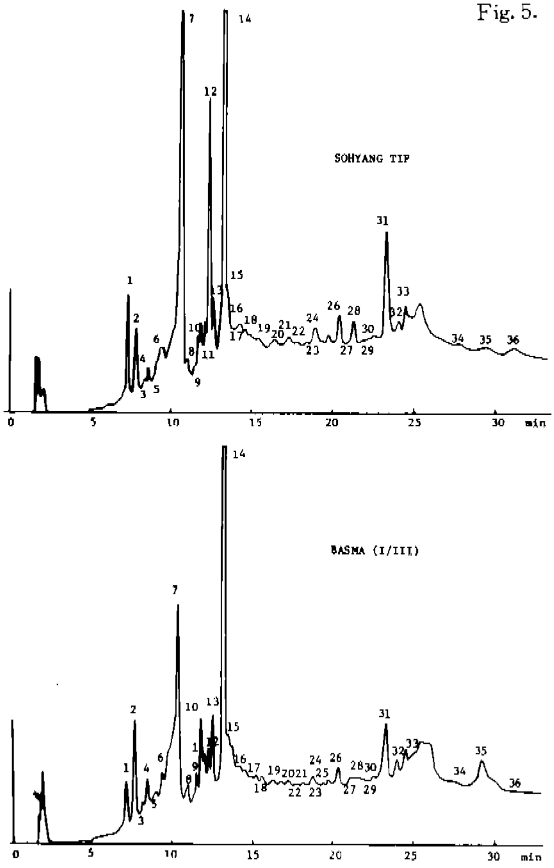

Fig. 5. HPLC profiles of Greek Basma vs. Korean Sohyang.
Conditions in text.

Fig. 6.

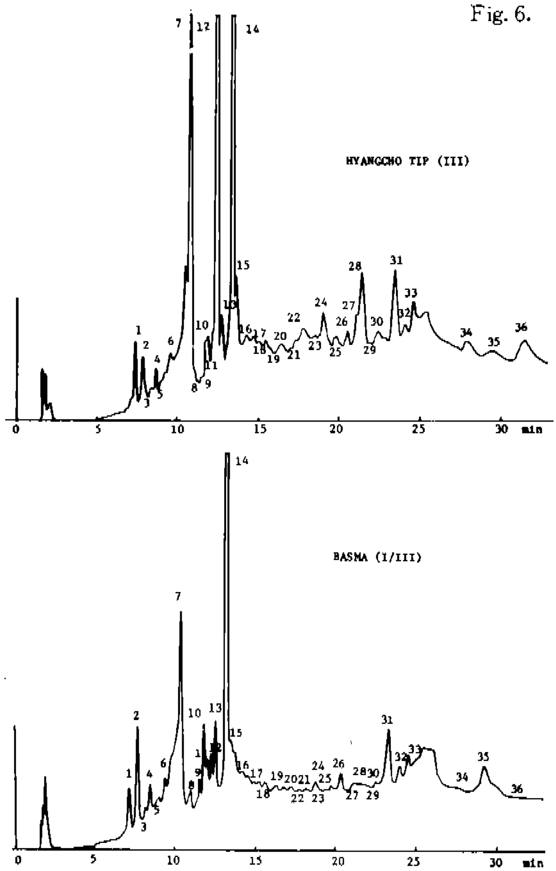

Fig. 6. HPLC profiles of Greek Basma vs. Korean Hyangcho.
Conditions in text.

Conclusion

The results of the present study have shown that Sohyang and Hyangcho, Korean aromatic tobacco varieties, have some differences in their TLC and HPLC profiles of less-volatile components compared with those of the Oriental varieties. This preliminary study prompts us to pursue the characterization of the chemical entities which make up the differences among them.

References

1. Fukuzumi, T., Bull. Okayama Tob. Exp. Stn, 30 : 133 (1971).
2. Austin, D. J., Roeraade, J., Kimland, B., Aasen, A. J., and Enzell, C., Beit. Tobackforsch., 7 : 8 (1973).
3. Schumacher, J. N. and Vestal, L., Tobacco Sci., 18 : 43 (1974).
4. Aasen, A. J., Pilotti, A., Enzell, C. R., Berg, J. E., and Pilotti, A. M., Acta. Chem. Scand., 30 : 999 (1976).

5. Chuman, T. and Noguchi, M., Agric. Biol. Chem., 41 : 1021 (1977).
6. Demole, E. and Enggist, P., Helv. Chim. Acta., 61 : 2318 (1978).
7. Kim, Y. T., Proc. Sixth Inter. Tob. Sci. Congress, Tokyo, Nov., p. 192 (1976).
8. Heu, I., Proc. Sixth Inter. Tob. Sci. Congress, Tokyo, Nov., p. 141 (1976).
9. Kim, K. R., J. Kor. Chem. Soc., (1980) submitted.
10. Woolen, B. H., Irvine, W. J., Brown, P. W., and Jones, D. H., Tobacco Sci., 16 : 98 (1972).
11. Roberts, D. L. and Rohde, W. A., Tobacco Sci., 16 : 98 (1972).
12. LIoyd, R. A., Miller, C. W., Roberts, D. L., Giles, J. A., Dickerson, J. P., Nelson, N. H., Rix, C. E., and Ayers, P. H., Tobacco Sci., 20 : 43 (1976).
13. Court, W. A., J. Chromatogr., 130 : 287 (1977).
14. Severson, R. F., Ellington, J. J., Schlotzhauser P. F., Arrendale, R. F., and Schepartz, A. I., J. Chromatogr., 139 : 269 (1977).
15. Fujimori, Kasuga, R., Kaneko, H., and Noguchi, M., Beit. Tabakforsch., 9 : 317 (1978).
16. Schlotzhauser, W. S., Tobacco Sci., 22 : 44 (1978).
17. Higman, H. C., Seversons, R. F., Chortyk, O. T., and Arrendale, R. F., Beit. Tabakforsch., 10 : 65 (1979).