Characterization of Cigarette Tobacco by Direct Electrospray Ionization–Ion Trap Mass Spectrometry (ESI-ITMS) Analysis of the Aqueous Extract—A Novel and Simple Approach

LAY-KEOW NG,* PIERRE LAFONTAINE, AND MICHELINE VANIER

Laboratory and Scientific Services Directorate, Canada Border Services Agency, 79 Bentley Avenue, Ottawa, Ontario, Canada K1A 0L8

In support of the efforts to combat smuggling, as well as illegal sale and distribution of cigarettes, an analytical approach for the characterization of tobacco has been proposed and evaluated. It involves aqueous extraction of the filler tobaccos followed by direct analysis of the extracts by electrospray ionization–ion trap mass spectrometry (ESI-ITMS) in the negative mode. Typically, the deprotonated ions, $[M - H]^-$, of organic acids (malic, citric, caffeic, quinic acid) and polyphenols (chlorogenic acid, rutin, scopoletin) were detected. MS/MS spectra of the ion at *m/z* 191, which is the $[M - H]^-$ of quinic acid, citric acid, and scopoletin, and a fragment ion of chlorogenic acid were acquired. Significant differences in the MS and MS/MS spectra were observed between counterfeit samples and the corresponding authentic brand name cigarettes. Analysis of 25 commercial cigarettes showed that straight Virginia blends were readily distinguished from the blended products containing different tobacco types (Virginia, burley, and Oriental). The former exhibited consistently higher relative abundances of *m/z* 353 (chlorogenic acid) to *m/z* 133 (malic acid) in the MS spectra (0.9–1.2 vs 0.4–0.6) and higher intensity ratios of *m/z* 176 (scopoletin) to *m/z* 173 (0.4–0.8 vs 0.1–0.3) and of *m/z* 127 (quinic acid) to *m/z* 173 (0.7–1.0 vs 0.3–0.5) in the MS/MS spectra. Evidence is presented to demonstrate that the spectral differences were related not only to the tobacco type (Virginia, burley and Oriental) but also to the tobacco part (stem, lamina) used in the manufacture of the cigarettes.

KEYWORDS: Electrospray ionization–ion trap mass spectrometry; cigarette tobaccos; tobacco type; counterfeit; organic acids; polyphenols

INTRODUCTION

High taxes on cigarettes worldwide have led to a sharp increase in the sales of contraband and counterfeit cigarettes. To support government efforts to combat smuggling, sales, and distribution of illegal cigarettes, it is necessary to develop methods that can readily distinguish authentic brand name cigarettes from the counterfeits. In addition, for investigation purposes, more specific information regarding the blending composition of the cigarette tobacco is often required. A survey of the literature indicated that there were no existing methods that addressed these issues.

Tobacco blend is an important factor in determining the smoke quality of cigarettes. The most preferred product, universally, is the American style blend, which typically contains the three major tobacco types (*1*): Virginia (flue-cured), burley, and Oriental (Turkish). However, in Canada and the United Kingdom, cigarettes made with only Virginia tobacco are preferred (*1*). Because the three tobacco types differ substantially in their chemical composition (*2*, *3*), chemical profiling of tobaccos could, in principle, differentiate between Virginia and

mixed-type blends. Chemical profiling could also be used to detect counterfeits if the authentic brand name cigarettes yield characteristic fingerprints.

Among the many constituents found in cigarette tobaccos, nonvolatile organic acids and polyphenols are the two major chemical groups that show considerable differences in concentration by tobacco type (*2*). In general, burley contains lower polyphenols content but higher acid levels than Virginia and Oriental tobaccos (*3*). The predominant nonvolatile acids are citric, malic, and oxalic. Major polyphenolics found in tobacco include chlorogenic acid, rutin, kaempferol 3-rutinoside, scopoletin, and scopolin. These highly polar acids and polyphenols are usually analyzed by techniques using ion-exchange (*4*) and liquid chromatography (*5*, *6*), respectively. Gas chromatography has also been employed to analyze the acids as silylated derivatives (*7*). In our laboratory, GC-MS profiling of nonvolatile acids has been reported for the characterization of cigar tobacco (*8*). These methods are in general time-consuming because chromatographic separation of components and sample cleanup are involved. Furthermore, the acids and polyphenols have always been analyzed separately by different methods. A fast and simple method involving simultaneous analysis of both

* Author to whom correspondence should be addressed [telephone (613) 954-7974; fax (613) 952-7825; e-mail Rachel.Ng@ccra-adrc.gc.ca].

acids and polyphenols without separation and purification is desired. This requires a relatively clean extract and a highly selective detection technique that can provide characteristic signals for individual molecular species. The use of an organic solvent as extractant is not favored because fat will unavoidably be extracted, which is the major problem in plant analysis. Water has the major advantage of minimizing the extraction of fat (*9*). Previous studies have shown that both polar nonvolatile acids (*8*) and polyphenols (*10*) can be quantitatively extracted by water. As a detection technique, mass spectrometry (MS) is inherently highly sensitive and specific. With the availability of soft ionization techniques, such as electrospray ionization (ESI) and matrix-assisted laser desorption ionization, MS can provide molecular ion or quasi- molecular ion for each ionized analyte, and as a result the mass spectrum obtained from direct analysis without chromatographic separation represents the fingerprint of the molecular species in the sample. Tobacco acids and polyphenols dissociate to different degrees in aqueous solution to form negatively charged carboxylic and phenoxy ions, respectively. This makes ESI-MS, a technique known for analyzing compounds preformed as charged species in the sample solution, the method of choice. Negative ESI-MS has been used as a tool for monitoring the quality of commercial herbal extracts (*11*) based on the MS profiles. In this study, detected ions were assumed to be [M − H]⁻ and assigned to various polyphenols known to be constituents of the herbs, but no effort was directed at providing a systematic evaluation of the method with regard to its suitability for the intended use.

The main aim of the present study was to evaluate the applicability of the aqueous extraction/ESI-MS approach to the identification of counterfeit cigarettes and the discrimination between Virginia blend and mixed-type blended products. An ion trap mass spectrometer (ITMS) was employed in this work. Its MS/MS capability provides evidence for the identities of individual molecular ions and additional information essential to the assessment of the blending composition of the tobaccos.

MATERIALS AND METHODS

Chemicals and Materials. Methanol (HPLC grade) was purchased from Fisher Scientific (Ottawa, ON, Canada). Citric acid (>99.5%) and malic acid (99%) were supplied by Aldrich (Oakville, ON, Canada). Chlorogenic acid (∼97%), rutin trihydrate (∼90%), and scopolitin (>98%) were obtained from Fluka (Oakville, ON, Canada). Caffeic and quinic acids were purchased from Sigma (Oakville, ON, Canada). Thirteen straight Virginia blend cigarettes were obtained from three major Canadian cigarette manufacturers. Twelve mixed-type blended cigarettes were supplied by American cigarette manufacturers through the Alcohol and Tobacco Tax and Trade Bureau, U.S. Treasury Department (Ammendale, MD). The research cigarettes, 2R4F and 1R5F, were purchased from the University of Kentucky (Lexington, KY). Cured tobacco leaves of Virginia, burley, Maryland, and Oriental Izmir types, and cut strip, cut stems, extended and reconstituted tobaccos of Virginia type for use in cigarette manufacture were supplied by Imperial Tobacco Canada Ltd. (Montreal, PQ, Canada). Burley and Oriental stems were mid-ribs separated from the burley and the Oriental Izmir leaves. Two cut burley samples and four cut Virginia tobaccos were obtained from the Customs Laboratory of the Czech Republic, Prague, Czech Republic.

Sample Preparation. The filler tobacco from the two test cigarettes, a Canadian cigarette (Virginia blend) and 2R4F (mixed-type blend), was separated from the other components. Each sample (150 g) was ground for 3 s at 5000 rpm using a Retsch laboratory knife mill, Grindomix GM200 (VWR Canlab, Montreal, PQ, Canada). This gave a particle size of ∼12 mesh. Samples of Virginia tobacco stems and the tobacco leaves of Virginia, burley, and Oriental Izmir types (10 g) were also ground before use. Moisture contents of the test samples were determined by using an HR73 moisture analyzer from Mettler

Toledo (VWR Canlab, Montreal, PQ, Canada). Five hundred milligrams of the ground tobacco was extracted with 200 mL of deionized water (18 mohms) in a 250 mL Erlenmeyer flask for 1 h on an orbital shaker at 200 rpm. About 2 mL of the supernatant liquid was introduced into a 2 mL microcentrifuge tube and centrifuged at 13200 rpm for 5 min using an Eppendorf centrifuge model 5415D (VWR Canlab). Then 300 μL of the supernatant solution was pipetted into a 2 mL autosampler vial and diluted with 900 μL of deionized water. For unground cigarette tobacco, the same extraction procedure was followed, using the tobacco from one cigarette. Because of the large tobacco strip size, it was not necessary to centrifuge the extraction mixture before dilution was carried out. Unless otherwise stated, all cigarette samples were extracted in duplicate.

ESI-MS Analysis. All tobacco extracts were analyzed using a Finnigan LCQ Duo equipped with an electrospray interface (ESI), a Spectra-System P4000 gradient pump, and a Spectra-System A3000 autosampler (all from Thermo Finnigan). The system was controlled by the Finnigan Xcalibur data system revision 1.2. The extracts were directly injected into the MS detector without chromatographic separation. The autosampler injector valve was connected to the inlet valve of the ESI via a red PEEK tube (0.005 in. i.d. × ¹/₁₆ in. × 30 in.). The mobile phase was 10% MeOH/90% H_2O; it was introduced isocratically into the ESI interface at a flow rate of 200 μL/min. The ESI probe was set at position 3. Typically 5 and 10 μL of each sample were injected for MS and MS/MS analyses, respectively. The MS/MS analysis was designed to fragment the *m/z* 191 ion using a normalized collision energy of 30%. The system was optimized for ion *m/z* 353 in the negative mode, using a typical Canadian tobacco extract solution as a calibration substance. The LCQ Duo was scanned from 50 to 1000 amu in the negative mode, at a scan rate of 5555 amu/s. The acquisition time was set at 1.5 min, plus a delay of 0.5 min after the acquisition period. The final mass spectral profile of the extract was obtained by averaging all of the spectra across the broad sample peak in the total ion chromatogram. The MS and MS/MS spectra of the freshly prepared extracts of the two test tobaccos were acquired before and after each sequence of analysis. The spectra should be comparable with those in **Figure 1**, indicating that the method procedure had been properly followed and the instrument response was stable throughout the analysis of the sequence.

Microscopy Examination. Tobacco samples were examined using a Leica MZ 125 microscope (Leica Microsystems Ltd., Heerbrugg, Switzerland) equipped with a JVC digital camera KY-F70B (JVC Canada, Scarborough, ON, Canada). The system is linked to a computer, and the images were captured using the application software VSC 200/ HR v. 4.4 (Foster and Freeman, Evesham, U.K.).

RESULTS AND DISCUSSION

The method includes (1) aqueous extraction of tobacco and (2) direct ESI-MS and MS/MS analysis of the extract. The tobaccos from a commercial Canadian cigarette of Virginia blend and 2R4F containing a mixture of Virginia, burley, and Oriental tobaccos were ground and used as test samples for establishing the method conditions. Both cigarette tobaccos have moisture contents of ∼12%.

Aqueous Extraction. Tobacco is known to be highly complex and contains hundreds of compounds. To minimize the coextraction of other matrix components, aqueous extraction of acids and polyphenols was carried out at room temperature. All analytes are known to be readily soluble in water except rutin and scopoletin. A large water-to-sample ratio (200 mL to 0.5 g of cigarette tobacco) was used to improve the extraction of the less soluble polyphenols. For profiling purposes, exhaustive extraction of analytes is not required as long as the resulting profile is reproducible. The intensities of the major MS ions at *m/z* 133, 191, 353, and 609, relative to the base peak, were used to characterize the profile (**Figure 1**). They were monitored after 15, 30, 60, and 90 min of extraction and found to level
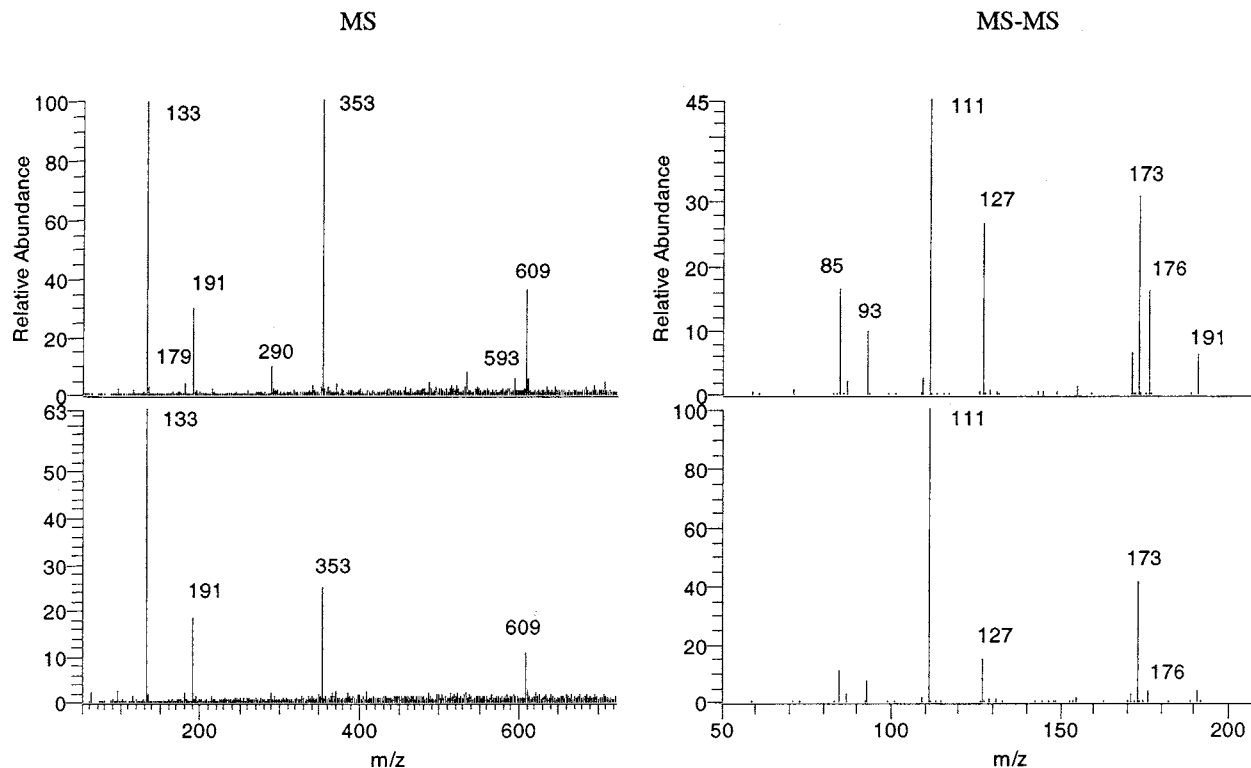
Characterization of Cigarette Tobacco by ESI-MS

J. Agric. Food Chem., Vol. 52, No. 24, 2004 **7253**

Figure 1. Typical cigarette tobacco profiles: (top) Virginia blend (test sample); (bottom) Virginia/burley/Oriental blend (Research Cigarette 2R4F test sample).

off after 30 min. All samples were extracted for 1 h throughout this study to ensure negligible effect of time on the overall profile.

Varying the tobacco weight from 0.5 to 0.7 and 0.9 g while using the same amount of water (200 mL) for extraction did not significantly alter the MS profiles. Unground cigarette tobacco also gave the same profile as the corresponding ground sample. The negligible effects of sample weight and particle size on the mass spectral profile suggest that the tobacco from a single cigarette, which typically weighs in the range of 0.55–0.8 g with ~12% moisture content, can be used directly for extraction without grinding and weight adjustment. Analysis of the tobaccos of five brand name cigarettes showed that five different cigarettes from the same pack gave very similar MS profiles. This was expected because tobaccos are normally well blended and homogenized before being manufactured into the final products.

The relative abundances of MS ions at m/z 133, 191, 353, and 609 remained stable over the test period of 6 h at room temperature for both test tobaccos. However, significant changes in the profiles were observed overnight. Ion m/z 133 degraded notably to a much larger extent than the other components. Refrigeration prolonged the shelf life of the extracts to ~24 h, whereas freezing to −20 °C preserved the extracts for as long as 2 months. In view of these observations, it is recommended that all extracts be analyzed within 6 h after preparation. Otherwise, they should be frozen until use.

Mass Spectrometric Characteristics. The chemical standards of major tobacco acids and polyphenols were analyzed separately. All of them were found to yield $[M - H]^-$ ions of different masses, except citric acid, scopoletin, and quinic acid, which gave the same deprotonated molecular ion at m/z 191 (**Table 1**). These three chemicals (**Figure 2**) were readily differentiated by their MS/MS spectra. Scopoletin yielded a single peak at m/z 176, which was due to the loss of a methyl

Table 1. MS and MS/MS Data of Acids and Polyphenols Standards

compound	MS ion, $[M - H]^-$, m/z	product ions of m/z 191, m/z
malic acid	133	115
citric acid	191	111, 173
quinic acid	191	111, 173, 85, 93, 127
scopoletin	191	176
chlorogenic acid[a]	353	191
rutin	609	300, 301

[a] Chlorogenic acid also gave a fragment ion at m/z 191, which yielded a product ion spectrum the same as quinic acid.

group from the $[M - H]^-$ ion. The MS/MS spectra of the two hydroxylated acids, citric and quinic acids, shared the same ions at m/z 111 and at m/z 173, which was the dehydrated form of m/z 191. However, the abundance ratio of m/z 111 to m/z 173 was much higher in citric acid than in quinic acid (3 vs 0.5). The presence of additional ions (m/z 85, 93, and 127) observed in the MS/MS spectrum of quinic acid, with ion m/z 127 as the base peak, also helps differentiate the two acids. Chlorogenic acid also yielded a fragment ion at m/z 191, which produced a MS/MS spectrum the same as that of quinic acid. Therefore, m/z 191 is likely due to quinic acid ion resulting from cleavage of the C–O bond of the ester linkage.

In the aqueous tobacco extracts, nicotine and sugars were coextracted with the acids and the polyphenols. Nicotine was found in the positive mode as $[M + H]^+$, whereas sugars were not detected in either positive or negative ESI. As **Figure 1** shows, the major negative ions in the MS spectra were m/z 133 (malic acid), m/z 353 (chlorogenic acid), m/z 609 (rutin), m/z 191 (citric acid, scopoletin, quinic acid, and chlorogenic acid), and m/z 290. The identity of the ion m/z 290 remains unknown; it cannot be assigned to any known acids or polyphenols in tobacco. Oxalic acid, which is one of the major tobacco acids, was not detected. It might have undergone decarboxylation to
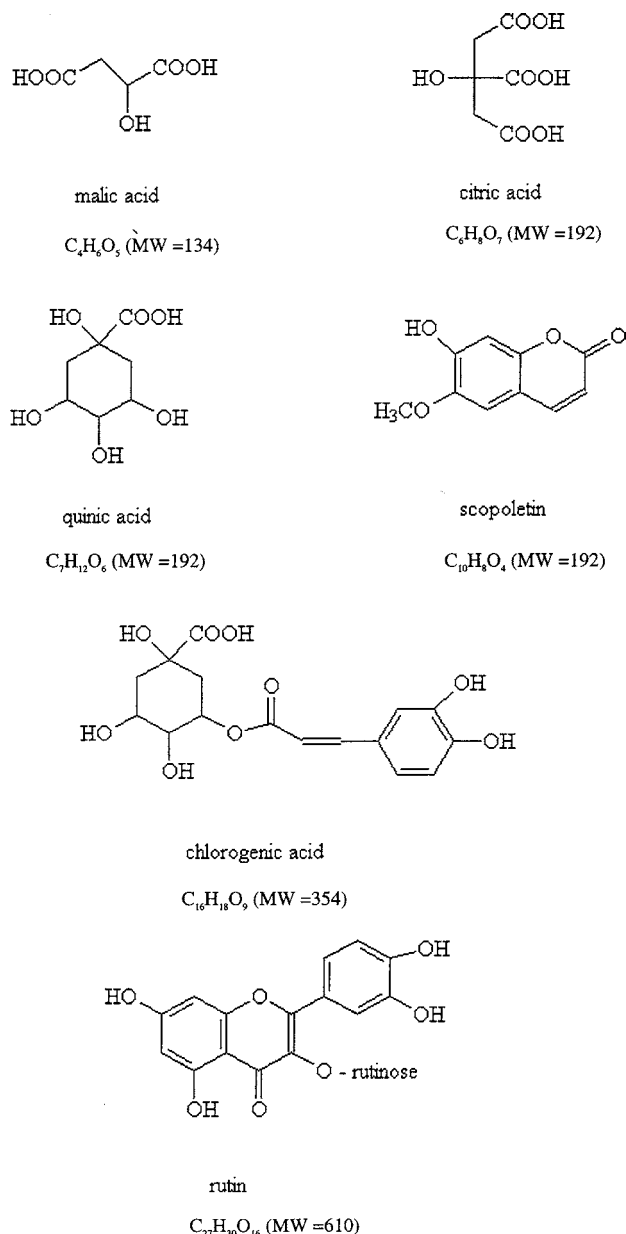
7254 *J. Agric. Food Chem.,* Vol. 52, No. 24, 2004

Ng et al.

malic acid

$C_4H_6O_5$ (MW =134)

citric acid

$C_6H_8O_7$ (MW =192)

quinic acid

$C_7H_{12}O_6$ (MW =192)

scopoletin

$C_{10}H_8O_4$ (MW =192)

chlorogenic acid

$C_{16}H_{18}O_9$ (MW =354)

rutin

$C_{27}H_{30}O_{16}$ (MW =610)

Figure 2. Structures of major acids and phenolics.

form a low-mass ion that is below the manufacturer's specified mass range of the mass spectrometer. Caffeic acid appeared as a minor peak at m/z 179; another weak ion at m/z 593 could be the $[M - H]^-$ of kaempferol 3-rutinoside, a key flavone known to exist in tobacco. The identity of this peak could not be confirmed due to the lack of a standard. Scopolin, a mono-β-glucopyranoside of scopoletin without any ionizable phenolic or carboxylic group, was not detected by ESI-MS.

The relative abundances of the major ions at m/z 133, 191, 353, and 609 were used to determine the reproducibility of the MS profile. They were repeatable from run to run with typically 5% RSD (N = 5). The day-to-day variations, determined from the eight MS profiles obtained on eight different days over a period of two months, generally doubled the run-to-run variations. In the MS/MS spectra, the abundance ratios of m/z 176 (scopoletin) to m/z 173 (citric and quinic acids) and m/z 127 (quinic acid) to m/z 173, referred to as the S and Q ratios, respectively, were reproducible from day to day with 3−10% RSD, which is comparable to run-to-run precisions. Larger variation (25% RSD) was observed with the S ratio in 2R4F,

which was mainly caused by the uncertainty associated with measuring the weak signal at m/z 176 (**Figure 1**).

Effects of Tobacco Type on the Profiles. Three Virginia tobacco leaves, one burley tobacco leaf, one Maryland tobacco leaf, and one Oriental Izmir tobacco leaf for use in the manufacture of cigarettes were analyzed. Virginia samples were labeled as top, middle, and bottom, referring to the stalk position of the plant from which the tobaccos were derived. All Virginia samples were characterized by the presence of a strong m/z 353 ion in the MS spectra (**Figure 3a**). It appeared either as the base peak or with intensity comparable to that of the base peak at m/z 133. They also gave a moderately strong signal at m/z 609, with a relative abundance of >30%. Similar profiles were obtained from four cut Virginia tobacco samples obtained from a different source. By contrast, the polyphenolic ions at m/z 353 and 609 (**Figure 3b**) were not discernible in the spectra of burley and Maryland tobaccos. Two other burley samples received from another source in the form of cut strips also gave the same profiles. The differences observed between burley and Virginia tobacco profiles have also been verified independently by Ondrousek using a single-quadrupole ESI-MS (*12*). These results are consistent with the previous findings that polyphenols content is lower in air-cured burley than in flue-cured Virginia tobacco (*2, 3*). The Oriental Izmir sample showed a similar MS pattern as the Virginia samples, except with a stronger m/z 609 ion (**Figure 3c**). The two tobacco types were readily differentiated by their MS/MS spectra, in which all Virginia samples were characterized by the strong presence of scopoletin ion (m/z 176), which was hardly discernible in the Oriental and burley samples. The characteristics of the three tobacco types, as defined by C (relative intensities of m/z 353 to 133), Q, and S ratios, are summarized in **Table 2**.

The profile of a Virginia sample changed in an expected manner when the tobacco was blended with an equal amount of a burley tobacco. The relative abundances of the polyphenolic ions, m/z 353 and 609, were attenuated because burley was almost void of polyphenols (**Figure 3d**). In agreement with the relatively high polyphenols content in Oriental tobacco, part of the lost intensities was restored when the Oriental sample was added to the mixture to give a 2:2:1 Virginia/burley/Oriental blend (**Figure 3e**). The change in the MS profile is summarized in **Table 2**, as indicated by the C ratio that is the relative abundance of m/z 353 to m/z 133. Blending Virginia with burley and Oriental tobaccos, which are scopoletin deficient, drastically affected the S ratio in the MS/MS spectra.

Effects of Tobacco Part on the Profiles. In the manufacture of Virginia blend cigarettes, tobacco stems, expanded tobacco and/or reconstituted tobacco of Virginia type could be used to blend with tobacco lamina (*1*). Expanded tobacco is obtained from a process applied to cut tobacco leaves to increase irreversibly their bulk volume and thus filling capacity, whereas reconstituted tobacco is formed in sheets from tobacco scraps and reinforcing adhesives.

Unlike Virginia lamina, no chlorogenic acid (m/z 353) and rutin (m/z 609) were detected in the Virginia cut stems (**Figure 4b**). Burley stems shared the same MS characteristics, whereas only low levels of the two polyphenols were detected in the Oriental stems. However, unlike the Virginia stems, both the burley and Oriental stems did not yield scopoletin (m/z 176) peak in their MS/MS spectra. The observations made on the leaves and stems of different tobacco types justify the use of scopoletin as an indicator of Virginia tobaccos.

The feedstock for reconstituted Virginia tobacco normally contains a fair amount of stems mixed with lamina fines. This
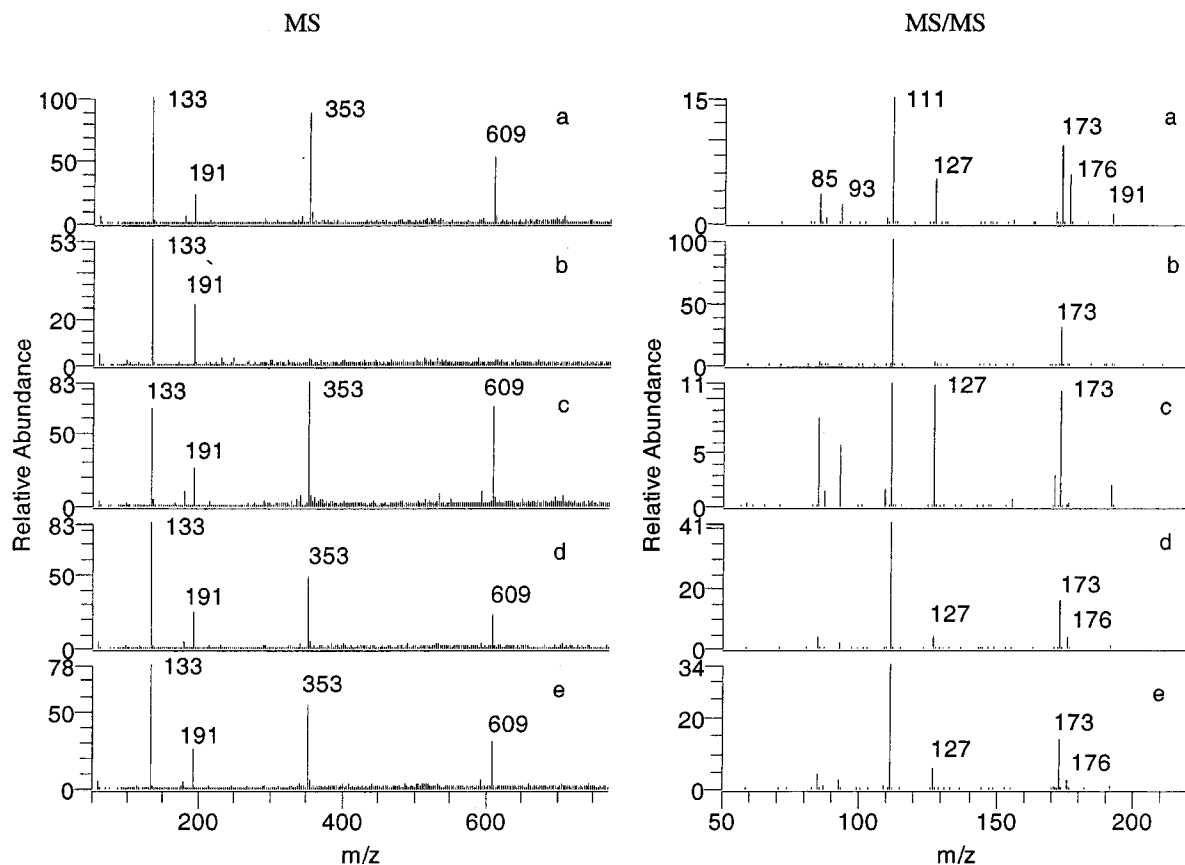
Characterization of Cigarette Tobacco by ESI-MS

J. Agric. Food Chem., Vol. 52, No. 24, 2004 **7255**

Figure 3. Different tobacco types (leaves) and their mixtures: (**a**) Virginia type; (**b**) burley and Maryland types; (**c**) Oriental type; (**d**) blend of Virginia/burley (1:1); (**e**) blend of Virginia/burley/Oriental (2:2:1).

Table 2. *C*, *S*, and *Q* Ratios in Different Tobacco Samples and Their Blends

	C ratio[a]	*S* ratio[b]	*Q* ratio[c]
blend of tobacco leaves of different tobacco types			
Virginia	0.9	0.7	0.6
burley	0.03	0.004	0.07
Oriental Izmir	1.3	0.001	1.0
Virginia/burley (1:1)	0.6	0.3	0.3
Virginia/burley/Oriental Izmir (1:1:0.5)	0.7	0.2	0.4
blend of different components of Virginia type			
cut stem	0.05	0.3	1.2
cut strip	1.1	0.5	0.7
expanded tobacco	0.9	0.5	0.5
reconstituted tobacco	0.6	0.7	1.0
cut strip/cut stem/expanded/ reconstituted (10:8:1:1)	0.6	0.4	0.9
stems of burley and Oriental types			
burley	0.00	0.00	0.04
Oriental Izmir	0.23	0.00	1.5
Virginia and mixed-type blends			
Virginia blends (Can. cigarettes)	0.9–1.2	0.4–0.8	0.7–1.0
mixed-type blends (Am. cigarettes)	0.4–0.6	0.1–0.3	0.3–0.5
2R4F (Virginia/burley/Oriental ∼ 3:2:1)	0.4	0.1	0.4
1R5F (Virginia/burley/Oriental ∼ 4:8:1)	0.2	0.05	0.3

[a] Relative intensities of *m/z* 353 (chlorogenic acid) to *m/z* 133 (malic acid) in the MS spectra. [b] Relative intensities of *m/z* 176 (scopoletin) to *m/z* 173 (citric and quinic acids) in the MS/MS spectra of ion *m/z* 191. [c] Relative intensities of *m/z* 127 (quinic acid) to *m/z* 173 (citric and quinic acids) in the MS/MS spectra of ion *m/z* 191.

explains why polyphenolic ions at *m/z* 353 and 609 (**Figure 4d**) are weaker in the stems than in the cut strip and the expanded tobacco, which are mainly derived from tobacco lamina. Blending the four different tobacco components in different proportions should give very different MS profiles. As shown in **Figure 4e**, a Virginia blend of 40% stem, 50%

lamina, and 10% of other processed tobacco generated a MS profile very similar to that of a Virginia/burley (1:1) blend (**Figure 3d**) or a Virginia/burley/Oriental (2:2:1) blend (**Figure 3e**). However, the Virginia blend distinguished itself by relatively high *S* and *Q* ratios.

ESI-MS Profiles of Typical Virginia Blends and Mixed-type Blends. Thirteen Canadian brand name cigarettes of straight Virginia blends and 12 American cigarettes of mixed-type blends were analyzed. The differences in blending composition were reflected in their MS and MS/MS profiles. Compared to the American brand name cigarettes, all Canadian brands yielded higher abundance ratios of ion *m/z* 353 (chlorogenic acid) to ion *m/z* 133 (malic acid) (0.9−1.2 vs 0.4−0.6) in the mass spectra. In the MS/MS spectra of the ion *m/z* 191, the *S* and *Q* ratios were also characteristically higher in the Virginia blend cigarettes (**Table 2**). As expected, the Kentucky reference cigarettes, 1R5F and 2R4F, which consist of the three tobacco types in known proportions, yielded spectral characteristics similar to the American cigarettes. Compared to 1R5F, 2R4F showed higher *C*, *Q*, and *S* ratios due to its higher Virginia content (**Table 2**). In view of possible natural variation within each tobacco type and in blending composition, not all Virginia blends are expected to yield profiles similar to the Canadian samples. This is exemplified by the concocted stem-rich Virginia blend (**Figure 4e**), which has a MS profile similar to those of mixed-type blends, whereas its MS/MS spectrum resembles that of a typical Canadian blend. Also, it may be difficult to ascertain if a sample is 100% Virginia or has been blended with a small amount of other tobacco types. Nonetheless, this study has identified the diagnostic features that allow the differentiation of the two major commercial cigarette blends.
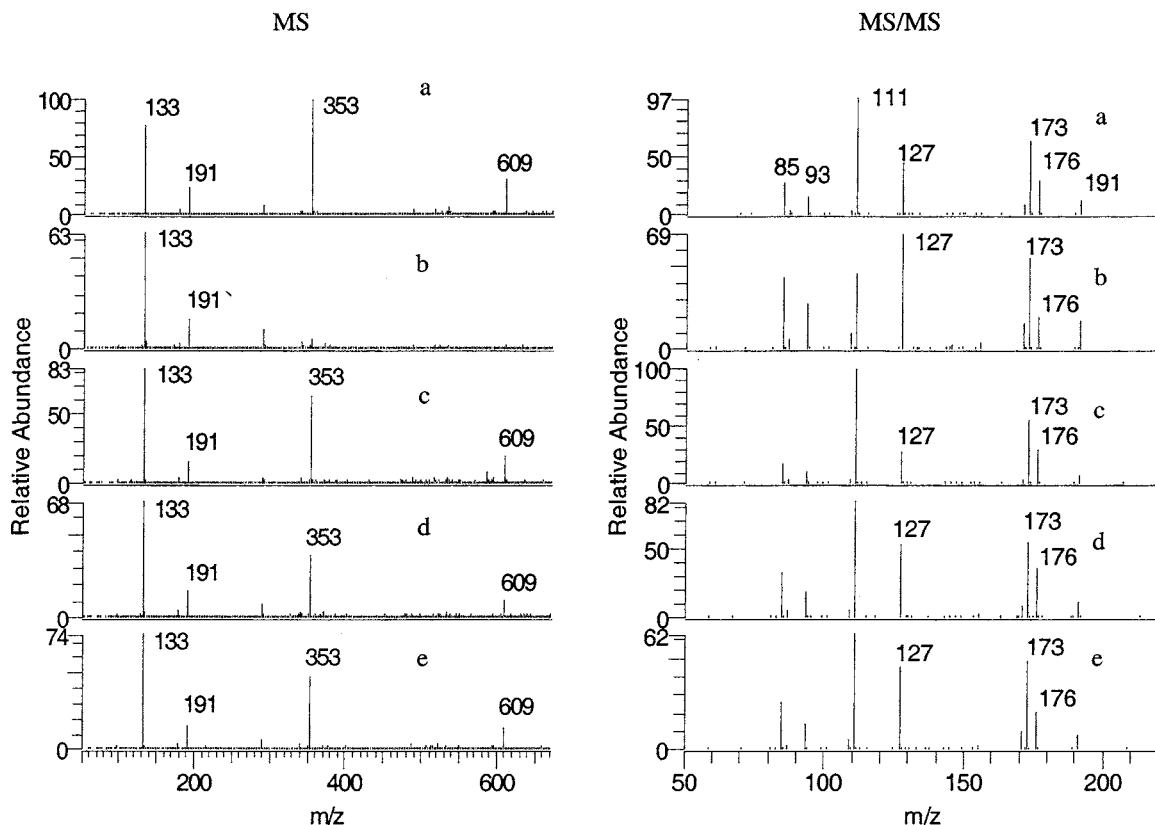
7256 *J. Agric. Food Chem.,* Vol. 52, No. 24, 2004

Ng et al.

MS

MS/MS

Figure 4. Different Virginia components: (**a**) cut lamina; (**b**) cut stem; (**c**) expanded tobacco; (**d**) reconstituted tobacco; (**e**) straight Virginia blend of lamina/stem/expanded/reconstituted (50:40:5:5).
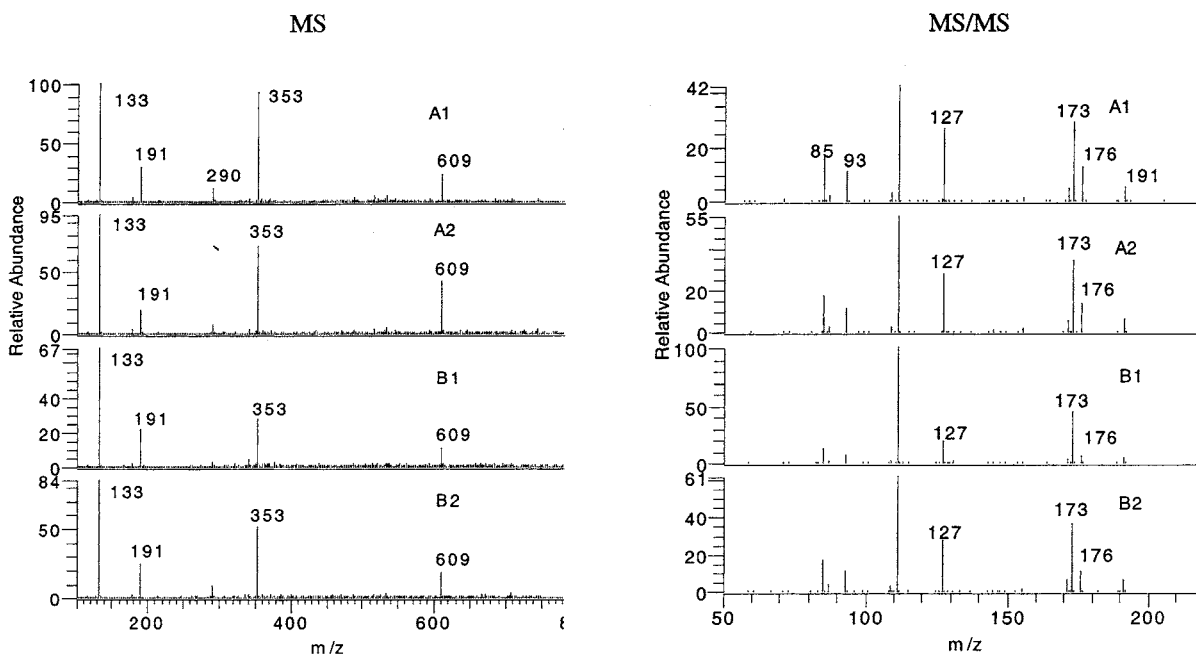
MS

MS/MS

Figure 5. Comparison of authentic and counterfeit brand name cigarettes: A1 and B1 are profiles for authentic brands A and B, respectively; A2 and B2 are profiles for the corresponding counterfeits.

Application to the Analysis of Counterfeit Cigarettes. Four different batches of a Canadian brand (brand A) and a well-known American brand (brand B) were analyzed in this study. Consistent MS and MS/MS spectra were obtained from batch to batch within each brand. These results were expected because a successful cigarette brand provides consistent taste and flavor, which are in part affected by the composition of the nonvolatile acids and polyphenols (*2, 3*). Deviation from the characteristic

profile of the authentic samples was taken as an indication of counterfeit. Although the *C*, *Q*, and *S* ratios were used for ease of discussion of the spectral profiles, to properly assess the nature of a sample, the overall profiles instead of just the three ratios should be examined. In our study, ESI-MS and ESI-MS/MS analyses were applied to the samples from two different seized shipments, which were suspected of being imitation products of brands A and B after physical examination of the

Characterization of Cigarette Tobacco by ESI-MS

J. Agric. Food Chem., Vol. 52, No. 24, 2004 **7257**

packaging and labeling of the cigarette packs. The spectra are shown in **Figure 5**. For brand A, the abundances of *m/z* 191 relative to that of *m/z* 133 were lower in the counterfeit cigarettes (0.2) than in the four different batches of authentic cigarettes (0.3−0.4), and the reverse was true for the relative intensities of *m/z* 609 (0.4 vs 0.2−0.3). The relatively high *C*, *Q*, and *S* ratios, which were 0.7, 0.8, and 0.4, respectively, indicated that the counterfeit was likely a Virginia-rich blend. For brand B, the seized sample yielded a stronger ion at *m/z* 353 and higher *S* (0.3 vs 0.05−0.2) and *Q* (0.8 vs 0.3−0.4) ratios. The characteristic features of this seizure resembled those of a Virginia blend relatively rich in stems (**Figure 4e** and **Table 2**). The stems showed very unique morphological patterns and were characterized by typical woody structures with clearly defined grains not found in the other three tobacco components. With the aid of a microscope, the stems were separated from the counterfeit sample and found to yield MS and MS/MS spectra very similar to those of Virginia stems shown in **Figure 4b**. Thus, combined microscopic examination and careful evaluation of the spectral fingerprints, with due consideration given to the effects of different tobacco types and parts used in formulating the cigarette tobacco, allows the differentiation between Virginia-rich blend and typical mixed-type blend.

In summary, this paper demonstrates that aqueous extraction coupled with direct ESI-ITMS analysis of acids and polyphenols provides a simple and fast strategy for the characterization of cigarette tobaccos. The MS and MS/MS profiles have been shown to be related to the composition of tobacco with respect to types (Virginia, burley, and Oriental) and components (stems, lamina, etc.), allowing ready differentiation between typical commercial Virginia and mixed-type blends. With respect to the complex problem of identification of counterfeit cigarettes, which relies on comparison with authentic samples, this method provides a rapid means to confirm the finding obtained from physical evaluation of the packaging or to warrant further investigation by other techniques. To our knowledge, it is the first method that allows the simultaneous analysis of tobacco polyphenols and nonvolatile acids. Although it has been applied only to cigarette tobaccos, the simplicity of the sample preparation and the speed of the ESI-ITMS analysis (4 min) lend themselves readily to applications other than tobacco products.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Fisher, P. Chapter 11. Cigarette Manufacture. In *Tobacco, Production, Chemistry and Technology*; Davis, D. L., Nielsen, M. T., Eds.; Blackwell Science: London, U.K., 1999; p 346.

(2) Leffingwell J. C. Chapter 8. Basic Chemical Constituents of Tobacco Leaf and Differences among Tobacco Types. In *Tobacco, Production, Chemistry and Technology*; Davis, D. L., Nielsen, M. T., Eds.; Blackwell Science: London, U.K., 1999; p 265.

(3) Tso, T. C. Phenolics. In *Physiology and Biochemistry of Tobacco Plants*; Tso, T. C., Ed.; Dowden, Hutchinson and Ross: Stroudsburg, PA, 1972; p 259.

(4) Qiu, J.; Jin, X. Development and optimization of organic acid analysis in tobacco with ion chromatography and suppressed conductivity detection. *J. Chromatogr. A* **2002**, *950* (1−2), 81−88.

(5) Li, Z.; Wang, L.; Yang, G.; Shi, H.; Jiang, C.; Zhang, Y. Study on the determination of polyphenols in tobacco by HPLC coupled with ESI-MS after solid-phase extraction. *J. Chromatogr. Sci.* **2003**, *41*, 36−40.

(6) Snook, M. E. An improved HPLC method for tobacco polyphenols. *Tob. Sci.* **1982**, *26*, 26−29.

(7) Court, W. A.; Hendel, J. G. Capillary gas chromatography of non-volatile organic acids, fatty acids, and certain carbohydrates in flue-cured tobacco. *Tob. Sci.* **1986**, *30*, 56−59.

(8) Ng, L.-K.; Hupe, M.; Vanier, M.; Moccia, D. Characterization of cigar tobaccos by gas chromatographic/mass spectrometric analysis of nonvolatile organic acids: application to the authentication of cuban cigars. *J. Agric. Food Chem.* **2001**, *49*, 1132−1138.

(9) Wang, L.; Fang, R.; Li, Z.; Jiang, C.; Yang, G. Determination of polyphenols in tobacco by solid-phase extraction and high performance liquid chromatography. *Sepu* **2001**, *19*, 564−566.

(10) Snook, M. E.; Chortyk, O. T. An improved extraction-HPLC method for tobacco polyphenols. *Tob. Int.* **1982**, *184*, 111−115.

(11) Mauri, P.; Pietta, P. Electrospray characterization of selected medicinal plant extracts. *J. Pharm. Biomed. Anal.* **2000**, *23*, 61−68.

(12) Ondrousek, S., Czech Customs Laboratory, unpublished results.