Characterization of Tobacco Products by High-Performance Anion Exchange Chromatography–Pulsed Amperometric Detection

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A simple and reproducible method has been developed for the classification of cigarette versus cigar tobacco. Although the tobacco used for both cigars and cigarettes may be of the same natural origin, tobacco types and processing parameters alter the relative amounts of natural constituents (e.g., carbohydrates). In this method, carbohydrates are obtained by water extraction. The extracts are then analyzed using high-performance anion exchange chromatography followed by pulsed amperometric detection. The relative amounts of glucose, fructose, and sucrose between cigarette and cigar tobaccos are used for their characterization. Complete analysis of a tobacco product is achieved in less than 60 min.

Keywords: *Pulsed amperometric detection; tobacco; chromatography; carbohydrates*

INTRODUCTION

All natural products contain varying amounts of basic biochemical building blocks (e.g., amino acids, carbohydrates, and lipids) and characteristic compounds. Identification of closely related products may be accomplished using a chemical "fingerprint" or profile of these constituents. This approach has been used in the food industry to identify natural and imitation vanilla (Toulemonde et al., 1983; Archer, 1989; Belay and Poole, 1993; Poole and Poole, 1994) and adulterants, such as high-fructose corn syrup to honey (Swallow and Low, 1994). The presence or absence of one or more key compounds allows determination of not only product purity but in some cases also product origin (Patel, 1994). Food adulteration is of importance for health, quality, commercial, and revenue considerations.

The Bureau of Alcohol, Tobacco and Firearms (ATF), an independent agency within the U.S. Department of the Treasury, is responsible for revenue compliance on tobacco products. Classification of a product is crucial to the assignment of the appropriate level of taxation. For instance, the tax on cigarettes is approximately 10-fold greater than the tax on cigars. The current tax rate is $1.125/1000 for small cigars compared to $12.00/1000 for small cigarettes. At present, identification of tobacco products relies on a variety of tests (Table 1). Included are subjective tests such as taste and texture, as well as a lengthy (10 days) sequential extraction procedure (ATF Procedure 76-2).

While the tobacco for cigars and cigarettes may be of the same natural origin, the type of tobacco and processing of the tobacco may lead to reproducible changes in the relative amounts of natural constituents (e.g., carbohydrates). The main differences between cigars and cigarettes are (1) air-cured tobacco is predominantly used in cigars, whereas flue-cured tobacco is the predominant tobacco type used (as part of a blend) in cigarettes, and (2) the plant carbohydrate content varies with species. In cigar manufacturing where air-cured tobacco is predominantly used, the air-drying process allows enzyme degradation of the plant carbohydrates resulting in a tobacco containing a total carbohydrate content of around 3% or less. In addition, the cigar tobacco is put through a fermentation step which further destroys the carbohydrates naturally present in tobacco leaves. In cigarettes, on the other hand, which are filled with predominantly flue-cured tobacco, the flue-curing process employs rather high temperatures to dry the tobacco, and the rapid drying process destroys or inactivates the enzymes, resulting in a tobacco containing ca. 12.5% of free carbohydrates (Jacin et al., 1967) to ca. 23% for total carbohydrate content (Elson et al., 1972). Additionally, manufacturers often add sugars to enhance flavor (Going et al., 1980). Therefore, differentiation of the tobacco products should be possible by a comparison of the carbohydrate profiles of each.

Table 1. List of Official ATF Tests Used To Establish Tobacco Identity

product tests	filler tobaccos	wrapper material
	Physical Criteria	
diameter	composition	color
length	taste	composition
weight/1000		texture
pH of smoke		taste
		fragments of tobacco
		tensile strength
		cellulose fibers
		paper-like qualities
	Chemical Criteria	
	% moisture	
	% nicotine	
	% ash	
	% acid insoluble ash	
	pH of filler	
	sequential differential solvent extraction[a]	

[a] Proposed replacement by carbohydrate profiling.

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Carbohydrates typically lack an inherent chromophore and/or fluorophore. Hence, optical detection methods without the benefit of chemical derivatization often suffer from poor sensitivity. In addition, these compounds do not give a persistent amperometric response for a constant (dc) applied potential at noble metal electrodes due to extensive fouling of the electrode surface. Refractive index (RI) detection, which is a bulk property detector, and colorimetric methods for reducing sugars are not analyte specific and suffer from poor sensitivity.

On the other hand, direct, sensitive, and reproducible detection of polar aliphatic compounds, such as carbohydrates, is accomplished by pulsed amperometric detection (PAD) at a gold electrode. The electrocatalytic mechanism of detection is often accompanied by rapid fouling of the electrode surface by oxidation products. In PAD, this situation is remedied by following the detection step with electrochemical (i.e., pulsed potential) cleaning of the electrode surface. Unlike methods for reducing sugars, PAD is applicable to virtually all carbohydrates. High-performance anion exchange chromatography (HPAEC) is used for the separation of carbohydrates in alkaline media. The weakly acidic sugars are present as anions under these conditions and can be eluted according to their pK_a's either isocratically or using an acetate gradient. HPAEC followed by PAD has been applied to the direct detection of sugar alcohols, monosaccharides, oligosaccharides, aminoglycosides, amino alcohols, amino acids, and numerous thio compounds. Reviews of PAD, which is classified as a pulsed electrochemical detection (PED) technique, and its applications have been published (LaCourse, 1993; Johnson and LaCourse, 1992, 1990).

The sole purpose of this paper is to distinguish between cigarette and cigar tobaccos for taxation purposes, and, as a consequence, only the relative (and not the absolute) amounts of selected carbohydrates need be determined. In this paper, the amounts of glucose, fructose, and sucrose (i.e., the three most prominent sugar peaks) in tobacco extracts are determined using high-performance anion exchange chromatography followed by pulsed amperometric detection (HPAEC–PAD). The results are a chemical fingerprint, specifically a carbohydrate profile, of the tobacco product. These profiles are used as a means of differentiating these closely related products. Described here are the development, optimization, and application of this method for the classification of cigarette and cigar tobaccos. Of major significance is that the complete analysis of a tobacco product is achieved in less than 60 min, which is in contrast to over 1 week by the existing procedure (i.e., sequential differential solvent extraction). This method is designated to replace only the sequential differential solvent extraction procedure (Table 1), and carbohydrate profiling by HPAEC–PAD will be used in conjunction with all other official ATF tests to establish tobacco identity.

MATERIALS AND METHODS

Reagents. All solutions were prepared from reagent-grade chemicals, except sodium acetate (NaOAc) solutions, which were prepared from HPLC-grade chemicals (Fisher Scientific, Fair Lawn, NJ). Sodium hydroxide solutions were diluted from 50% (w/w) stock solution (J.T. Baker Inc., Phillipsburg, NJ). All mobile phases were filtered with 0.2 μm nylon-66 filters (Rainin Corp., Woburn, MA) and a solvent filtration apparatus (Microfiltration Systems, Rainin). All mobile phases were deaerated with dispersed N_2. Water was purified using a reverse osmosis system coupled with multitank/ultraviolet ultrafiltration stations (US Filter/IONPURE, Lowell, MA).

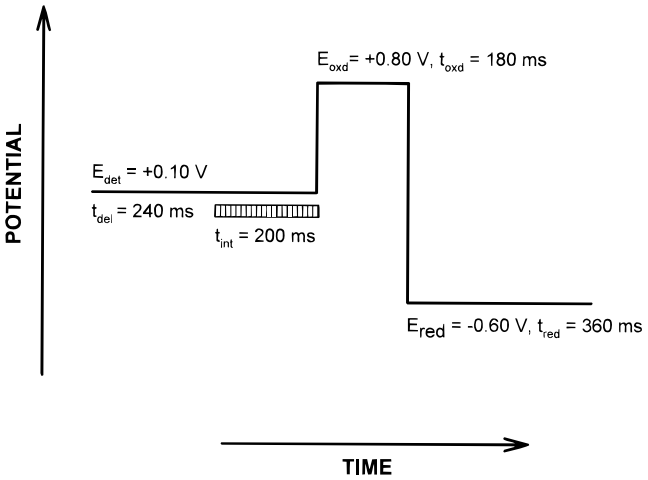

Figure 1. Optimal pulsed amperometric detection waveform.

Apparatus. Cyclic voltammetric data were obtained using a Au (ca. 0.20 cm^2) rotating disk electrode (RDE) using a Model AFMSRX analytical rotator and a Model AFRDE4 potentiostat (Pine Instrument Co., Grove City, PA). Cyclic voltammetric data were recorded on a Model 3025 X-Y recorder (Yokogawa Corp. of America, Peachtree City, GA).

Pulsed voltammetric data were obtained at the Au RDE with a computer-controlled potentiostat using a DAS-1600 high-speed A/D-D/A expansion board (MetraByte Corp., Taunton, MA) in an IBM-AT compatible computer. Pulsed voltammetric waveforms were generated with ASYST scientific software (Asyst Software Technologies, Inc., Rochester, NY). LaCourse and Johnson (1993) have described pulsed voltammetry in detail.

HPAEC was performed on an advanced gradient chromatography system (Dionex Corp., Sunnyvale, CA). Separations were done with a CarboPac-PA1 anion exchange analytical column (Dionex), preceded by a CarboPac-PA1 guard column at a flow rate of 1.00 mL/min. Electrochemical detection was performed with a Model PED unit (Dionex) with a thin-layer electrochemical cell. The PAD waveform was applied to a planar Au working electrode (ca. 0.79 mm^2) with a stainless steel auxiliary electrode and a Ag/AgCl reference electrode. All injection volumes were 50 μL.

Samples. Tobacco samples were provided by the National Laboratory Center, Laboratory Service Division of ATF. Filler tobacco was separated from the other components in the case of both cigar and cigarette samples. The filler tobacco samples were ground in a Wiley mill with a 20-mesh screen (Arthur H. Thomas Co., Philadelphia, PA). Values for percent moisture were determined from a representative portion of the sample. Approximately 10.0 g of sample was dried overnight in an oven at 103 ± 2 °C.

Procedure. A sample (ca. 500 mg) of tobacco was shaken for 5 min in 100 mL of water. A C_{18} Extract-Clean cartridge (Alltech, Deerfield, IL) was prepared for use by rinsing with 3.0 mL of methanol and 3.0 mL of water. A 0.2 mL aliquot of the tobacco solution was applied to the column. The carbohydrates were eluted with ca. 1.0 mL of water into a clean 5-mL volumetric flask and diluted to volume with water. The samples were used fresh (within 15 h) or stabilized by filtration through a 0.2 μm filter (Rainin).

Figure 1 shows the PAD waveform used for all the work in this paper unless otherwise noted. In PAD, the electrode is held at a potential appropriate for detection, E_{det}, of the compound of interest. Before sampling the current via integration over the time period, t_{int}, the charging current is allowed to dissipate for a delay period, t_{del}. As a consequence of the detection mechanism, reaction products foul the electrode surface, and the electrode potential is stepped to an oxidation potential, E_{oxd}, and held for a time, t_{oxd}. During the oxidation step, surface oxide is allowed to form and fully cover the surface, which essentially "cleans" the surface of any

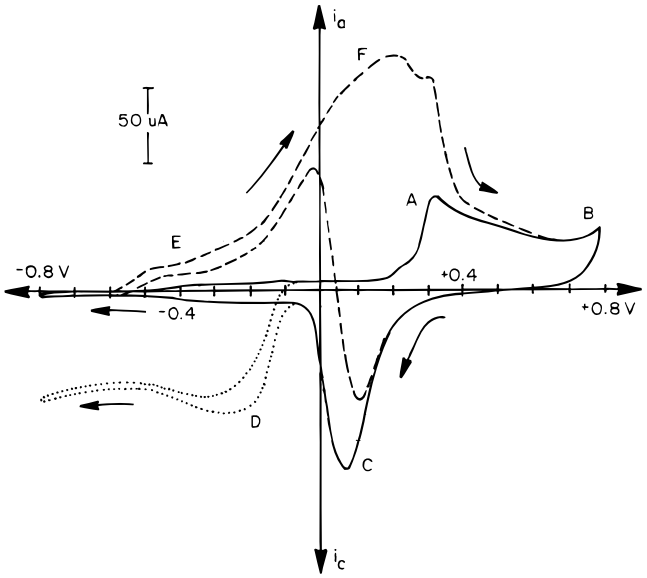

Figure 2. Cyclic voltammogram of glucose at gold RDE in 0.1 M NaOH. Conditions: 900 rpm rotation speed, 200 mV/s scan rate. Solutions: (···) aerated 0.1 M NaOH, (—) deaerated 0.1 M NaOH, and (– – –) 0.4 mM glucose.

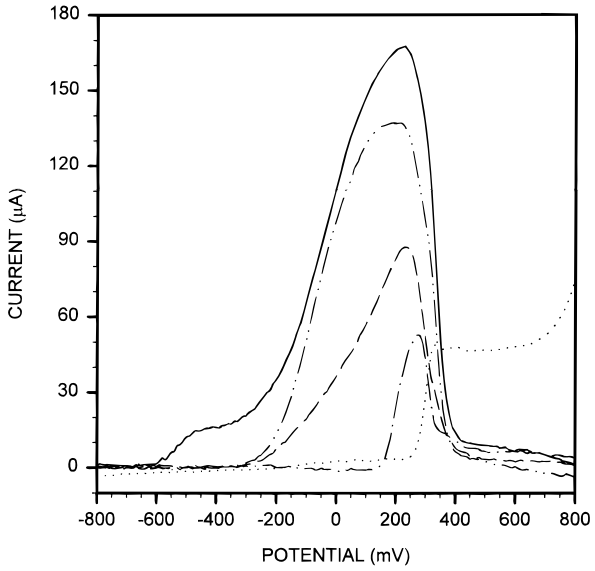

Figure 3. Pulsed voltammetric response of (—) 0.2 mM glucose, (– ··–··–) 0.2 mM fructose, (– – –) 0.2 mM sucrose, and (– · – · –) 0.2 mM glycine at Au RDE in 0.1 M NaOH, background subtracted. Background is shown for reference (···). Conditions: 900 rpm rotation speed, waveform as in Figure 1 with incremental change in E_{det}.

fouling species from the detection process. The electrode is now inert, and a negative potential step, E_{red}, is applied to the working electrode for a time, t_{red}, which cathodically dissolves the surface oxide and reactivates the electrode.

RESULTS AND DISCUSSION

Voltammetry. Figure 2 shows the cyclic voltammetric response (I vs E) of a Au RDE in 0.1 M NaOH with (—) and without (···) deaeration. The residual response for the supporting electrolyte (—) exhibits an anodic wave at ca. 0.2−0.8 V (wave A) on the positive scan due to the formation of surface gold oxide. The anodic breakdown of water commences at ca. 0.7 V (wave B), which results in the evolution of O_2. On the reverse scan, the dissolution of the surface gold oxide results in a cathodic peak at ca. 0.3 to ca. −0.05 V (wave C). Before deaeration, a cathodic wave for the reduction of dissolved O_2 is observed at potentials < −0.1 V (wave D) on both positive and negative scans.

With the addition of glucose (– – –), the positive scan shows clearly a two-step anodic response beginning at ca. −0.6 V. The first step corresponds to oxidation of the aldehyde group of glucose to produce the corresponding carboxylate anion (Larew, 1989). This process (wave E) commences at ca. −0.6 V, reaches a plateau at ca. −0.5 V, and proceeds until the onset of surface oxide formation. The second step, which peaks at ca. 0.2 V (wave F), corresponds to the combined responses from the oxidation of the aldehyde and alcohol groups of glucose (Johnson and LaCourse, 1990; Larew, 1989). At the onset of gold oxide formation (>0.2 V), the signal is sharply attenuated, which is characteristic of an oxide-free-catalyzed detection. This is confirmed on the reverse scan as activity returns to the electrode once the oxide is cathodically dissolved, as is evidenced by a difference between the residual response and response with the presence of glucose.

Although cyclic voltammetry (CV) is useful as a guide in the selection of PAD waveform potentials (LaCourse and Johnson, 1991), it has been determined that the selection and optimization of all PAD waveform parameters are best accomplished using pulsed voltammetry (PV) at a hydrodynamic electrode (LaCourse and Johnson, 1993). In PV, the PAD waveform is applied to a Au RDE with small incremental changes in one of the PAD parameters for each cycle of the multistep PV waveform. The resulting I vs E or I vs t plots reflect the effect on the PAD response over a specified range for a selected parameter.

Figure 3 shows the PV response of E_{det} for equimolar concentrations of glucose (—), fructose (– ·· –·· –), sucrose (– – –), and glycine (– · –· –) at a Au RDE in 0.1 M NaOH. All responses have been background-corrected for the residual response (···), which is also shown. All carbohydrates show anodic peaks from ca. −0.2 to 0.4 V with maxima at ca. 0.2 V. As expected from the CV data, the oxidation of the aldehyde group of glucose commences at ca. −0.6 V. Glycine exhibits an anodic peak from ca. 0.15 to 0.45 V with a maximum at ca. 0.25 V. At the optimal detection potential (ca. 0.2 V) for all carbohydrates, glycine and all other amino acids are also detected. Enhanced selectivity of carbohydrates over amino acids and peptides, which are present in the tobacco extracts, can be accomplished by setting E_{det} to a lower value (< ca. 0.15 V). Hence, the detection potential was chosen to be 0.10 V. Since preadsorption of the analyte of interest occurs predominantly during E_{red}, further selectivity for carbohydrates over amine-based compounds is afforded via the choice of $E_{red} = -0.6$ V, which is not optimal for amine-based compounds (Dobberpuhl and Johnson, 1995). All other PAD waveform parameters have been determined previously to be optimum for carbohydrate detection (LaCourse and Johnson, 1991, 1993). The waveform used in this method is shown in Figure 1.

HPAEC. A requirement for PAD of carbohydrates at a gold electrode is that the supporting electrolyte be alkaline. Under these conditions, weakly acidic carbohydrates are present as anions, amenable to separation by anion exchange chromatography. Elution of monosaccharides is directly correlated with the pK_a of the carbohydrate (Paskach et al., 1991). Acidic carbohydrates, oligosaccharides, amino acids, and peptides can also be eluted by using an acetate gradient.

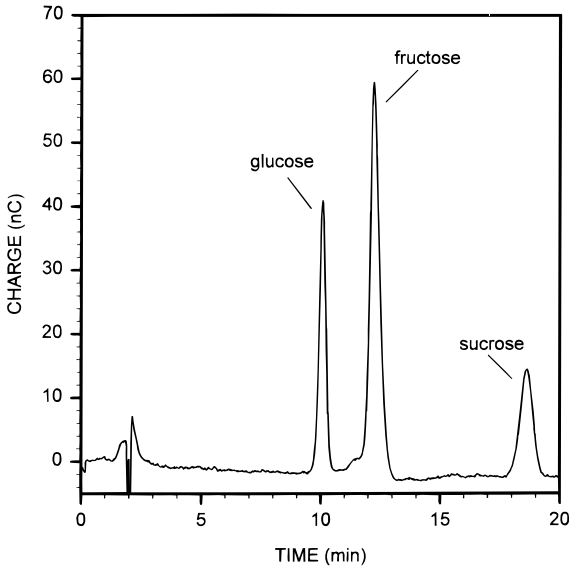

Figure 4. Isocratic separation of glucose (0.010 mM), fructose (0.025 mM), and sucrose (0.010 mM). Conditions: 50 mM NaOH, 1 mL/min, CarboPac-PA1 column, detection as in Figure 1.

Table 2. Step Gradient Program Used for Isocratic Separation of Natural Carbohydrates and Column Wash

time (min)	1 (100 mM NaOH)	2 (water)	3 (600 mM NaOAc)
00.0	50	50	0
18.0	50	50	0
20.0	50	0	50
35.0	50	0	50
37.0	50	50	0
60.0	50	50	0

Figure 4 shows the separation of glucose, fructose, and sucrose, under isocratic conditions of 50 mM NaOH. Note that the separation of these monosaccharides and disaccharide is accomplished in less than 20 min. For reproducible chromatography, it is important to remove any carbonate, which builds up on the column under low NaOH concentrations (i.e., <50 mM) after each chromatographic run. The carbonate originates from the NaOH eluant, which is capable of adsorbing CO_2 from the atmosphere to form the carbonate ion (Dionex, 1989). Removal of carbonate is accomplished by ramping the acetate concentration of the mobile phase to 300 mM and holding for 15 min. The wash step also insures that any other highly retained matrix components extracted from the tobacco products are eluted from the column. After the wash step, the mobile phase is stepped back to 50 mM NaOH, and the column is allowed to equilibrate for 25 min. The gradient program is shown in Table 2. Figure 5 shows the separation of a typical cigarette extract, which includes isocratic separation, column wash, and column equilibration. The peak for carbonate elutes at ca. 24 min.

Sample Preparation. The tobacco used for method development was removed from its wrapper and ground. Extraction was accomplished with water in order to minimize extracting lipophilic compounds while extracting hydrophilic carbohydrates. Absolute amount of tobacco to be used was determined by preparing the tobacco in triplicate and plotting the relative standard deviation (RSD) vs the amount of tobacco used. Figure 6 shows that the resulting graph yielded a minimum at 500 mg of tobacco. As the sample size is increased, a more homogeneous sampling, which excludes the

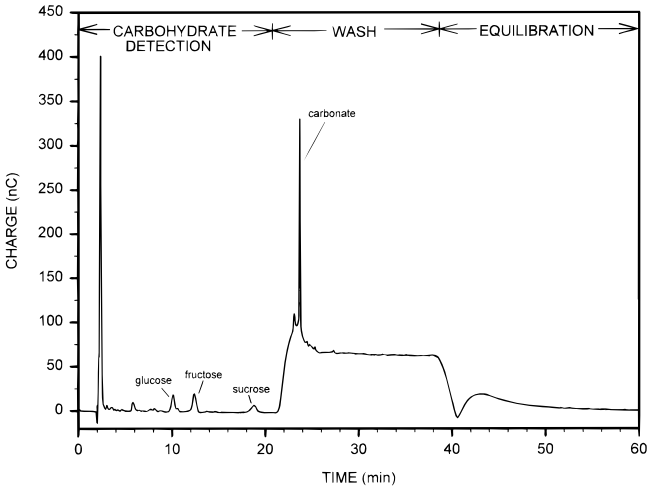

Figure 5. Separation of tobacco extract. Conditions: CarboPac-PA1 column, 1.0 mL/min, gradient as in Table 2, detection as in Figure 1.

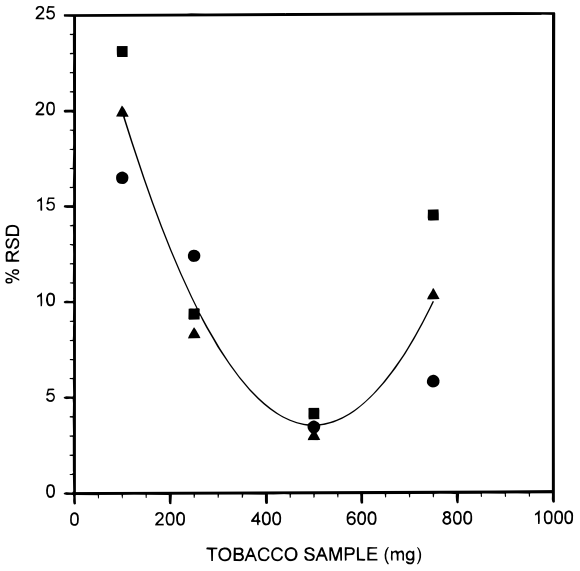

Figure 6. Plot of % RSD for glucose vs mg of tobacco for optimization of sample size. Each symbol represents an individual sample.

biased sampling of small particles, results in lower RSD's for each of the sugars. This trend may be adversely affected for samples greater than 500 mg by improper wetting of the tobacco sample for the extraction volume used, and, as a consequence, the RSD's for each sugar increase.

In order to insure efficient extraction, the tobacco/water mixture is shaken for ca. 5 min. Although additional shaking did not improve the extraction of carbohydrates, the amount of matrix components did increase. The use of ultrasonic extraction proved to be less reproducible due to inadequate wetting of the sample, which resulted from sample floating to the surface of the container. At this point, the extract contains a solution of carbohydrates, amino acids, and other compounds characteristic of the tobacco leaves. Injection of this solution yields a chromatogram with numerous peaks, some of which interfere with the quantitation of the peaks of interest. Further cleanup of the sample was accomplished by passing the extract through a C_{18} Extract-Clean cartridge. The cartridge retained lipophilic and aromatic compounds from the extract while allowing carbohydrates to pass through.

Table 3. Quantitation Parameters of Selected Carbohydrates Using HPAEC–PAD

	limit of detection[a] (pmol, ppb, μM)	linear dynamic range[b] $nC = a(\mu M) + b$		% RSD at LOQ[c] (μM inj, $n = 6$)
		a	b	
glucose	10, 40, 0.2	4.331	0.278	0.9 (1.0)
fructose	20, 70, 0.4	2.211	0.184	2.6 (2.5)
sucrose	25, 170, 0.5	1.923	0.260	1.6, (5.0)

[a] $S/N = 3$. [b] Linear dynamic range = $10N$ − departure from linearity. [c] Limit of quantitation (LOQ = $10N$).

Inclusion of this preparatory step did not alter the elution profile of carbohydrates and amino acids. The use of the acetate wash after the separation served to elute oligosaccharides as well as any other late-eluting compounds. Under these conditions, hundreds of samples were injected without any noticeable loss in chromatographic efficiency.

Quantitative Parameters. Analytical figures of merit for the method can be found in Table 3. For the analytes of interest, the calibration plots were linear for glucose and sucrose from 1.25 nmol injected to their limit of detection (LOD), and fructose was linear from 2.50 nmol injected to its LOD. Since the samples tested fell well within the linear dynamic range determined here, linearity of these compounds beyond these values was not tested. Reproducibility was determined daily by repetitive injections of a standard. On any given day, the RSD for peak heights of standards ranged from 0.9% to 2.6%. Over the course of 24 h, injection of the standard yielded deviations of less than 10%. Method repeatability is reflected in the preparation of three determinations of the same sample, and the average RSD's for glucose, fructose, and sucrose of all samples tested (i.e., cigarette and cigar tobaccos) are 5.5%, 4.0%, and 4.4%, respectively. Only results from samples at or above the limit of quantitation were included.

HPAEC–PAD of Tobacco. Figure 7 shows the elution profile of typical (A) cigarette and (B) cigar tobacco extracts. The carbohydrate peaks were identified by spiking a sample with standard solutions. Note that the relative amounts of free carbohydrates in the cigarette and cigar extracts are dramatically different. This difference is attributable to the processing of the tobacco. Cigar tobacco undergoes a fermentation step in its processing, thereby destroying some of the carbohydrates naturally present, while cigarette tobacco undergoes no such treatment. This divergence in processing is reflected in the carbohydrate profile of each of these products. A dramatic difference is that sucrose is virtually never present in cigar tobacco extracts, which is in agreement with the findings of Hsu et al. (1980). Glycerol, an additive to cigarette tobacco, was determined to elute at/near the solvent front. Nicotine, which is present in both cigarette and cigar tobacco extracts, also eluted in the solvent front. Many of the additional peaks in the cigarette and cigar chromatograms are conjectured to be free amino acids.

For purposes of quantitation and comparison, three marker peaks were chosen, glucose, fructose, and sucrose. Concentration was calculated from the peak height of each of these sugars, and a standard was run before and after each sample for the calculation of the concentration of each sugar. The concentration of carbohydrates was then used to calculate the gram percentage of each sugar in the dried tobacco sample.

In order to formulate a method to characterize tobacco as either cigarette or cigar, known samples were used

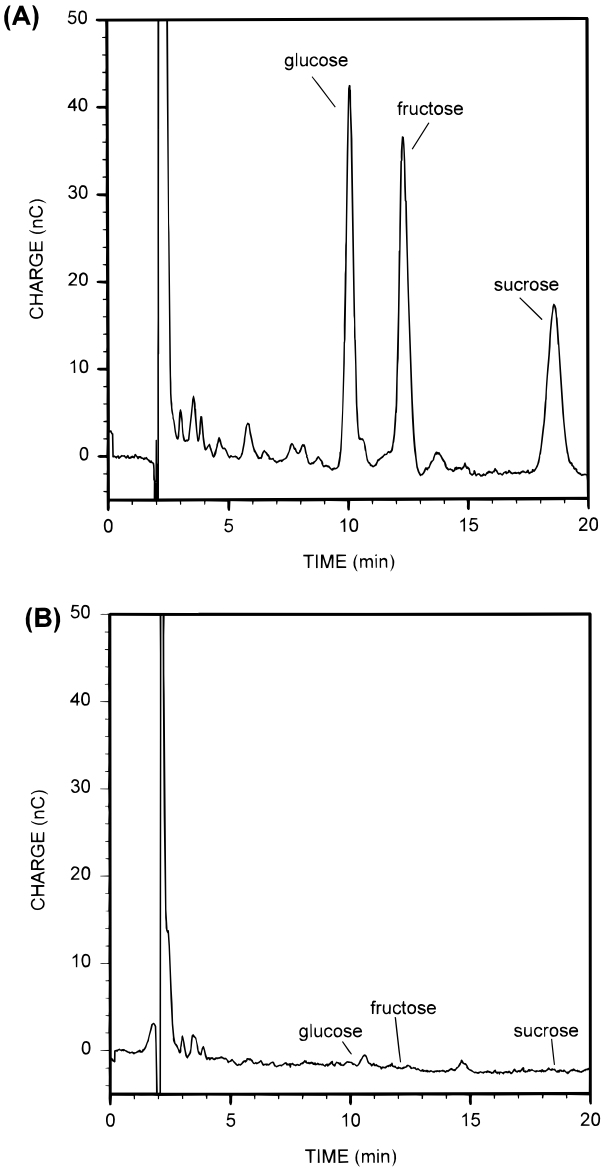

Figure 7. Separation of extracts from cigarette (A) and cigar (B). The wash and equilibration portion of the chromatograms are not shown. Conditions were as in Figure 5.

to develop a base profile. Each tobacco sample was described by three values: % glucose, % fructose, and % sucrose, shown in Table 4. Figure 8 shows the three-dimensional plot (% glucose, the x-axis; % fructose, the y-axis; % sucrose, the z-axis) of these values. Note that all the cigarettes lie above the $x-y$ plane (denoted by drop lines). An alternate method of displaying the data was adopted to simplify interpretation of the results. Each value was treated as a vector along an axis. When these vectors are added, the resultant vector describes the total amount of carbohydrates present in the tobacco sample. This vector's length, along with the value for the angle that the vector makes as measured from the $x-y$ plane, can be used to describe the tobacco sample. In general, it is found that the vector for a cigarette is longer than the vector for a cigar. This is as would be expected if a portion of the carbohydrates was destroyed in the fermentation of cigar tobacco. In addition the angle that the vector makes with the $x-y$ plane, which is essentially a measure of the amount of sucrose present, is seen to be very small for cigar tobacco (all values approach zero), while cigarettes exhibit a relatively large percentage of sucrose. These results can

Table 4. Summary of Tested Tobaccos

tobacco			sugar in dried sample (%)		
sample	no.	moisture (%)	glucose	fructose	sucrose
cigarette	1	11.42	0.35 ± 0.01	0.87 ± 0.00	2.16 ± 0.02
	2	10.45	0.38 ± 0.06	1.38 ± 0.07	2.02 ± 0.03
	3	10.67	0.77± 0.01	1.37 ± 0.04	0.69 ± 0.02
	4	13.13	0.29 ± 0.00	0.50 ± 0.00	2.50 ± 0.03
	5	8.70	0.40 ± 0.01	0.84 ± 0.01	1.33 ± 0.04
	6	12.94	0.87 ± 0.01	1.53 ± 0.03	0.73 ± 0.04
	7	12.98	0.75 ± 0.01	1.34 ± 0.01	0.35 ± 0.02
	8	12.89	0.38 ± 0.04	0.70 ± 0.06	1.37 ± 0.09
	9	12.26	0.28 ± 0.00	0.56 ± 0.01	1.43 ± 0.05
	10	13.50	0.43 ± 0.01	0.82 ± 0.02	0.55 ± 0.01
	11	12.46	0.71 ± 0.05	1.25 ± 0.11	0.72 ± 0.08
	12	13.50	1.01 ± 0.05	1.53 ± 0.09	2.16 ± 0.09
	13	13.54	0.52 ± 0.04	0.94 ± 0.09	1.66 ± 0.18
	14	11.10	0.30 ± 0.01	0.65 ± 0.03	0.63 ± 0.04
	15	12.32	0.46 ± 0.02	0.87 ± 0.03	0.99 ± 0.04
	16	13.51	0.26 ± 0.07	0.42 ± 0.04	3.45 ± 0.07
	17	11.94	0.48 ± 0.03	0.86 ± 0.02	1.13 ± 0.06
cigar	1	8.03	0.19 ± 0.01	0.25 ± 0.01	ND
	2	9.25	0.05 ± 0.00	0.07 ± 0.01	ND
	3	9.58	ND	ND	ND
	4	8.36	0.06 ± 0.00	0.05 ± 0.00	ND
	5	9.19	0.12 ± 0.01	0.11 ± 0.00	ND
	6	12.98	0.24 ± 0.00	0.26 ± 0.01	ND
	7	12.78	0.54 ± 0.01	0.50 ± 0.01	ND
	8	11.28	0.06 ± 0.01	0.08 ± 0.00	ND
	9	10.68	0.04 ± 0.01	0.06 ± 0.01	ND
	10	11.73	0.04 ± 0.01	0.07 ± 0.00	ND
	11	11.75	0.05 ± 0.00	0.06 ± 0.00	ND
	12	12.64	0.03 ± 0.00	ND	ND
	13	11.71	ND	ND	ND

[a] ND, none detected.

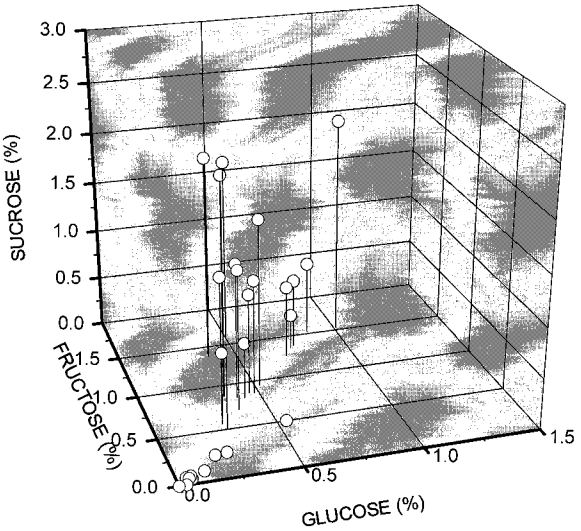

Figure 8. Three-dimensional plot of % glucose vs % fructose vs % sucrose for all tested tobaccos.

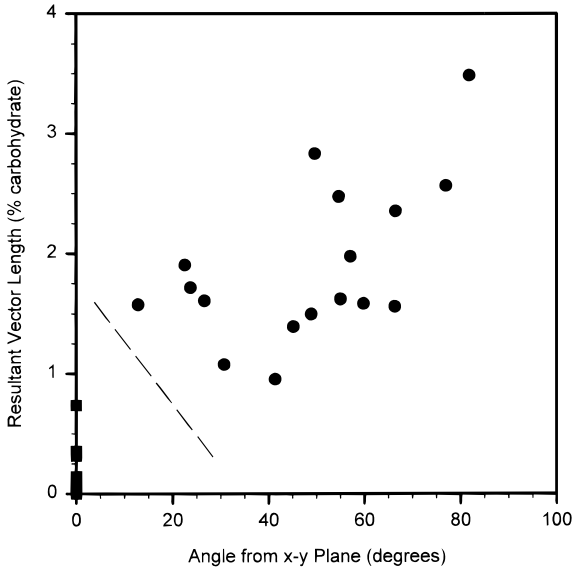

Figure 9. Vector plot of all (●) cigarette and (■) cigar tobaccos. An arbitrary line is shown to delineate the two regions of tobacco classification.

be seen in Figure 9, where vector length is plotted on the ordinate, while the angle it makes with the $x-y$ plane (or the % glucose−% fructose plane) is plotted on the abscissa.

The samples plotted fall into two distinct regions. These correspond to a region for cigarettes (●) and a region for cigars (■). These results are in perfect agreement with those from ATF Procedure 76-2, which is the present method for chemically differentiating between these tobaccos. When analyzing an unknown tobacco sample, inclusion in one of these two regions would indicate the type of product from which the tobacco came. Known samples, analyzed in the same fashion, would be used to augment the database. In this way, the regions should become more well-defined.

CONCLUSIONS

Determination of the carbohydrate profiles of cigars and of cigarettes provides a means for differentiation between these two closely related products. Following a simple water extraction from the tobacco, the carbohydrates are separated using high-performance anion exchange chromatography and directly detected using pulsed amperometric detection. Classification of tobacco is based on the total sugar content and relative amount of the targeted carbohydrates. Total sugar content of cigarettes is greater than that of cigars, and the presence of sucrose is virtually exclusive to cigarettes. At

present, this method is applicable to cigar and cigarette tobaccos made predominantly from flue-cured tobacco or tobacco blends, which account for the vast majority of cigarette sales in the United States. Tobacco products made exclusively from burley and black tobaccos have not been tested. Further, it is conjectured that the amino acid profile of the extracts may also reveal substantial information as to tobacco processing.

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