JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification of Components Responsible for the Odor of Cigar Smoker's Breath

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Following smoking 1/2 of a cigar, the most odorous cigar tobacco smoke components extracted from the surface of the tongue by nylon-meshed swabs and then extracted from the swab headspace by solid phase microextraction were ethyl pyrrole, 2,3-dimethyl pyrazine, and 2-ethyl pyridine. Similar classes of compounds were identified from the headspace of an aqueous simulated saliva solution treated with cigar smoke. The most odorous compounds were 2,3,5-trimethyl pyridine, 2,5-dimethyl pyrazine, and 2-ethyl-3,5-dimethyl pyridine. Pyridines and pyrazines, the most prominent classes of odorous compounds identified in this experiment, may be generated during cigar pyrrolysis by cleavage of nicotine or by Maillard reaction.

KEYWORDS: Tobacco smoke; malodor

INTRODUCTION

More than 1.2 billion people worldwide smoke tobacco products (*1*). The lingering odor of residual pyrrolyzed tobacco that is responsible for smoker's breath creates a consistent demand for breath-freshening products. Successful strategies for the amelioration of breath malodor associated with tobacco smoke are particularly difficult to develop due to an estimated 4800 compounds generated upon pyrrolysis of tobacco (*2*) and pyrrolysis of additional substances found in cigarettes (599 different additives may be utilized in cigarettes in the United States; *3*). Additionally, odorous substances in tobacco products may be present at extremely low parts per billion levels and still exhibit noticeable odor; for example, pyridine has a reported odor threshold value of 3.7 parts per billion (*4*, *5*). It is readily apparent to smokers and their associates that pyrrolyzed tobacco volatiles are released from the oral cavity, air passageways, and lungs and are present in the breath in sufficient quantity to be perceived by others. These odors are also perceived as an aftertaste (denoted as self-perceived malodor) by the smoker but in a manner consistent with the concept of odor adaptation (constant exposure to odor decreases perception over time; *6*).

Gas Chromatography−Olfactometry (GCO). Previously, researchers investigated potential pathways for volatile generation via pyrrolysis of tobacco. One study listed major components generated in this process as benzene, phenol, toluene, and catechol (*7*). Less research has sought to determine the odor associated with identified components. GCO instrumentation has been successfully employed to identify key aroma impact components. Major GCO techniques include Osme (*8*), CHARM analysis (*9*), and aroma extraction dilution analysis (*10*). Once odorous compounds are identified using gas chromatography/mass spectrometry (GC/MS), model solutions that exhibit similar aroma profiles as the original substance can be developed.

Extraction of Volatiles from the Oral Cavity. Pioneering work in sampling human mouth air was conducted over 30 years ago when the endogenously formed malodorants hydrogen sulfide, methyl mercaptan, and dimethyl sulfide were detected by extraction of mouth air with a gastight syringe followed by separation with a packed column and detection with a flame photometric detector (*11*). Research into the origins of halitosis has demonstrated that many people develop a bacterial-mediated plaque on the posterial dorsal surface of the tongue, which is the principle oral malodor source (*12*). We also hypothesized that this area of the tongue would make an ideal surface for trapping cigar-related volatile compounds.

Solid phase microextraction (SPME) was successfully utilized to extract odorous components from the oral cavity for subsequent analytical measurement (*13*). SPME has proved to be an ideal tool for extracting volatile components from the headspace of unconventional odorous substances (*14*) for subsequent analysis for odor by GCO and GC/MS. Little sample preparation, no need for solvent, and relatively fast extract times are among the well-documented benefits.

Differences between Cigars and Cigarettes. In addition to the size differences between most cigars and cigarettes (a large cigar may contain as much tobacco as a pack of cigarettes), cigars are rolled in tobacco leaves whereas cigarettes are typically rolled in paper. Most cigars contain a dried burley or air-cured tobacco. The tobacco leaves are typically aged for a year and then fermented in a process that may take up to 5 months. This fermentation process imbibes cigars with unique flavors not typically found in cigarettes. A common means for discerning the flavor strength of a cigar involves assessing the tobacco leaf wrapper color. Generally, the lighter the wrapper color, the milder the taste and smell (the darker the wrapper, the stronger the taste and smell; *15*). The Connecticut River Valley, U.S.A., produces some of the world's finest wrapping tobacco. Cigarettes, on the other hand, may contain as many as

10.1021/jf0519109 CCC: $33.50
Published on Web 12/17/2005

599 additives (in the United States) including coffee, chocolate, ammonia, and vinegar (*3*). The primary goals of this research were to identify key odor impact components present in a tobacco smoker's oral cavity following smoking 1/2 of a cigar.

MATERIALS AND METHODS

Tobacco Products Utilized for Aroma Active Component Identification. Cigars were chosen for this study because they contain more tobacco and take longer to smoke. Because a greater quantity of tobacco smoke volatiles flow into the oral cavity when smoking a cigar vs cigarette, it was believed that a stronger tobacco smoke breath malodor would also result. In addition, the many additives in cigarettes may confound the problem of volatile malodor identification associated with smoking tobacco products. Macanundo cigars were chosen due to their popularity and their rather mild flavor. Onyx cigars were chosen for testing due to a stronger, more robust flavor.

Multidimensional (MD) GC/MS. In addition to normal GCO instrumentation, a means for separating the thousands of compounds present in tobacco smoke and deposited in the oral cavity was necessary. Utilization of single or even dual columns with differing polarities was not sufficient for baseline separations. MDGC equipped with a heart-cutting valve and cryogenic focusing capacity and coupled with a mass spectrometric detector was made for a capable unit and permitted separation and identification of most compounds.

Experiment 1: Identification of the Odorous Components Responsible for Tobacco Breath—Oral Cavity Swabbing Followed by SPME/GCO/GC/MS. Testing for odorous compounds was conducted at the Microanalytics lab (Microanalytics, A MOCON Co., Round Rock, TX) and the Wrigley Research and Development facility (Wm. Wrigley Jr. Co., Chicago, IL). Macanundo (Macanundo robusto brand, Dominican Republic) and Onyx brand cigars (Onyx brand; Dominican Republic, mini Belicoso) were utilized to generate odorous compounds.

Panelists (smokers) did not eat, drink, or use oral hygiene products for 2 h prior to smoking. Panelists refrained from smoking or consuming any foodstuff except water 1 h prior to smoking. Neither panelist regularly smoked, and both possessed normal oral health. Two panelists smoked one-half cigar (approximately a 20 min smoke). Odorous tobacco smoke compounds were extracted from panelist oral cavities by swabbing the tongue's surface fore and aft, five strokes, with a nylon-stemmed, nylon mesh-coated swab (TX 714A, The Texwipe Co., Upper Saddle River, NJ). The polypropylene swab stem was cut off, and the swab head was sealed in a 40 mL glass vial with a plastic screw cap and Teflon septa for subsequent headspace sampling. Two swabs were placed in each vial (one from each smoker) for headspace sampling. Blank swabs were analyzed as controls (tongues were swabbed 10 min before tongues were exposed to cigar tobacco smoke).

Headspace was extracted utilizing SPME (Supelco, Bellfonte, PA) with a Carboxen−polydimethyl siloxane fiber (75 μMm, 23 gauge). The extraction time was 60 min. Fibers remained in the GC injection port for 5 min following injection. Only manual SPME extractions were conducted in the initial experimentation.

An Agilent 6890 GC/MS modified for multidimensional analyses and equipped with a sniff port and Aroma Trax software (Microanalytics) was utilized for analyses. The GC/MS operating parameters were as follows: He carrier gas flow rate, 6.5 mL/min; split mode (2:1); injector set at 250 °C; column 1 was 15 m, 0.53 mm i.d.; film thickness, 1 μm with 5% phenyl methylpolysiloxane stationary phase (SGE BP5) and operated with constant pressure mode at 16 psi; average velocity, 66 cm/s. Column 2 was a 30 m × 0.53 mm fused silica capillary column coated with poly(ethylene glycol) (WAX; SGE BP20) at a film thickness of 1 μm. The column pressure was 5.7 psi, and an average velocity of 56 cm/s was employed. The oven was programmed to hold at 40 °C for 3 min and then 7 °C/min to 220 °C and held for 20 min. The MS operated in the electron impact ionization mode at 70 eV ionization energy. Identification of components was conducted by matching unknown spectra with the Wiley database and by matching retention indices with authentic standards.

By utilizing the sniff port to identify specific times of column eluant that exhibit odor characteristic of tobacco smoke, heart cuts (small segments) of chromatographic effluent that contained the odor peaks were selectively analyzed by the MS detector and sniff port for further evaluation and identification. The heart-cut valve was located between the first column and the second column. The second column eluant was split between the MS detector and sniff port (50:50) whereas eluant from the first column traveled exclusively to the FID unless selectively sent to the second column by the heart-cutting valve or unless purged.

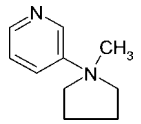

Figure 1. Nicotine or 3-(1-methyl-2-pyrrolidinyl) pyridine.

Additional testing and further refinement of aroma active compound identities were conducted at the Wrigley Chicago Research and Development facility with a Microanalytics GC/MS unit with identical features as that described above with the exception that the Chicago unit contained both a Leap Technologies CombiPal autosampler capable of utilizing automated SPME (Leap Technologies, Carrboro, NC) and also a direct connection between the output of the first column and the sniff port to make heart cutting more accurate and precise. Further explanation of this feature is necessary due to its importance in methodology. In the previous instrument, the sniff port was connected to the output of the second column only. The time utilized for heart cuts was less precise and was based on back-calculating when an odorous peak with a specific retention time at the MS and at the sniff port (at the output of the second column) had transited the first column (the heart-cut valve was located on the output of the first column). With the second instrument, the sniff port had plumbing that allowed for direct connection to the output of the first column; thus, precise heart cuts could be made based on odor detection time. The sniff port also had the option of connection to the output of the second column as before.

For components that exhibited odor activity but the identity of the component could not be firmly established, the heart-cut effluent was cryogenically focused onto the head of the second column (utilizing a feature of this instrument that contained a spray nozzle that utilized liquid CO_2) to provide additional peak separation. Headspace extraction times utilizing SPME were also extended up to 24 h to fully load the fiber with headspace volatiles. Other than specified, columns, oven, and other analytical parameters remained the same as previously discussed.

The odor intensity and character of compounds were rated based on a tier one, two, or three system where tier one was most odorous. Sniffing was done in triplicate by one expert odor judge.

Experiment 2: Identification of the Odorous Components Responsible for Tobacco Breath by Entrapment of Smoke Volatiles in Simulated Saliva Followed by SPME/GCO/GC/MS. A second experiment was conducted at the Wrigley facility for the purpose of identifying cigar smoke volatiles that were retained in saliva. Onyx brand cigars were selected for this experiment, as previously mentioned, because they were considered stronger, more robust in flavor, and resulted in greater perceived aftertaste and breath malodor as compared to Macanundo brand cigars (self-assessment).

Simulated saliva was prepared in accordance with an in-house method by dissolving sodium chloride, sodium bicarbonate, and potassium bicarbonate in deionized water (solution with electrolytes present in concentrations similar to saliva). This solution was utilized to measure residual tobacco smoke components following exposure in a manner similar to saliva exposure in the mouth. To prepare saliva-like solutions exposed to cigar smoke, a panelist drew smoke from a cigar and then gently blew four separate 2 s puffs with a standard drinking straw into the bottom of a 22.5 mL vial so that smoke bubbled through the solution. Liquid contents were then transferred to a separate vial (this step ensured any adsorbed smoke volatiles on glass surfaces were excluded). Analytical analyses were conducted in the identical manner previously described.

Odor activity and character of eluant were rated by two trained odor judges in triplicate utilizing a time intensity Osme-like device (*8*) that employed a 0−100 point scale (100 indicated strongest odor and 0

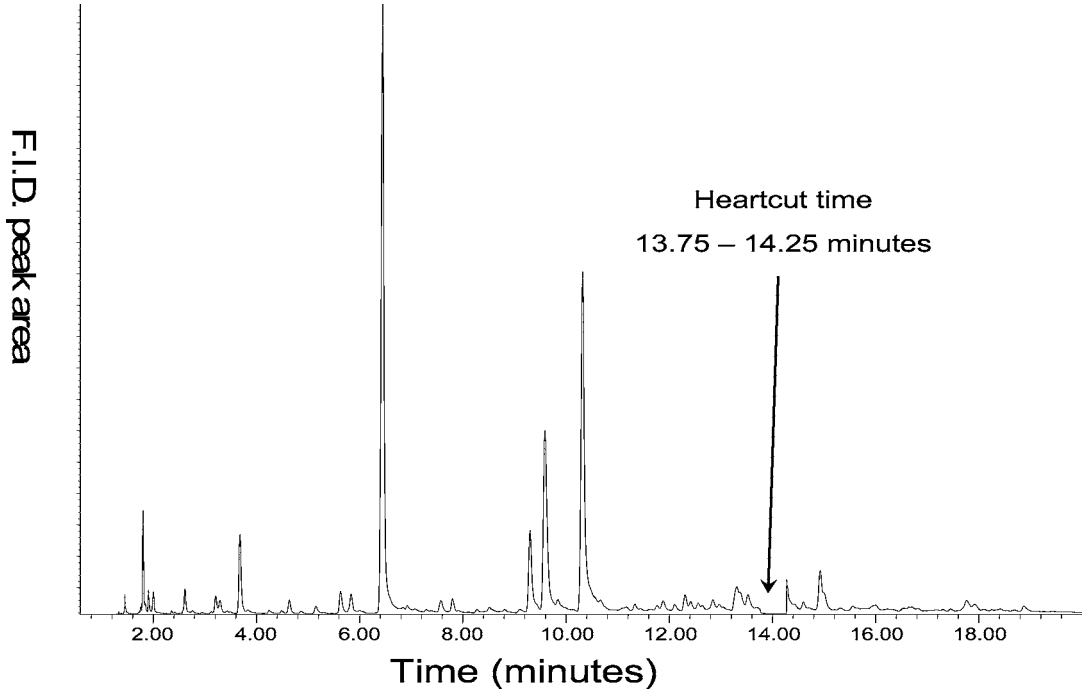

Figure 2. Extracted tobacco smoke components FID chromatogram showing heart cut from 13.75 to 14.25 min.

indicated no odor). Confirmation of peak identities was made as described above using the Wiley database and commercially available standards.

Judges (*2*) with previous GCO experience were trained on tobacco smoke extract by practicing on synthetic saliva impregnated with cigar smoke, including Onyx cigar smoke, in the manner previously described. Each judge sniffed six practice samples and developed a list of odor descriptors to choose from when evaluating the Onyx cigar smoke extracts (each extract was evaluated three times by each judge). Odorants that were perceived a minimum of two times out of three by both judges were included in the final list. Intensity scores were averaged to yield the final odor intensity score.

RESULTS AND DISCUSSION

Results from Experiment 1: Aroma Active Component Identification. Utilizing swabs and SPME for extracting residual cigar smoke components from the surface of the tongue revealed that the most odorous volatiles from both Macanundo and Onyx cigars were primarily aromatic and nitrogenous in nature (**Table 1**). Compounds were classified as tier one, two, or three based on aroma intensity with tier one components the most odorous.

The most odorous components extracted from oral cavities following the consumption of a Macanundo cigar were ethyl pyrrole, 2,3-dimethyl pyrazine, 2-methyl pyridine, 4-cyclobutyl pyridine, and diacetyl. These compounds were described as musty, savory, nutty, tobacco, and buttery. Ethyl pyrrole was considered the strongest odorant followed by 2,3-dimethyl pyrazine. The strongest odorants extracted from the tongues of Onyx cigar smokers were 4-methyl pyridine, pyridine, and ethyl pyrazine. These compounds were described as nutty, stench, and meaty.

With the exception of diacetyl that was found only in Macanundo cigars, odorants from both cigars were similar in character. Structural isomers 2-methyl pyridine (Macanundo, Kovats 830) and 4-methyl pyridine (Onyx, Kovats 930) were both among the most odorous compounds identified.

Results from Experiment 2. Utilizing synthetic saliva for trapping and SPME for extracting residual Onyx cigar smoke components revealed a different list of odorous compounds

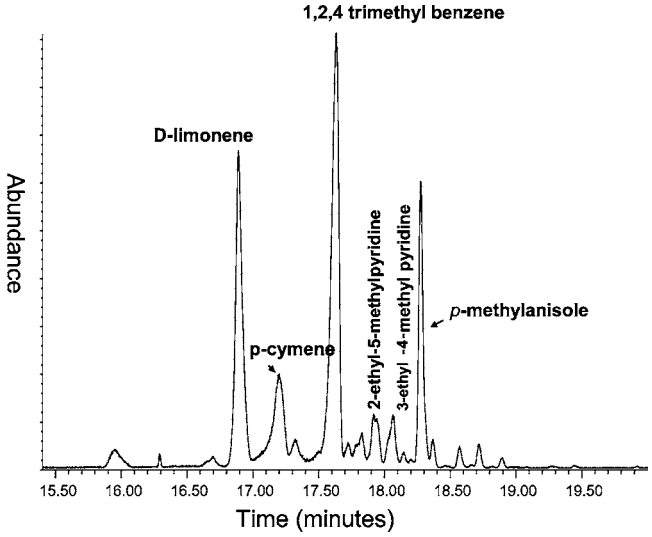

Figure 3. Abundance of heart cut effluent from the output of column 1 from 13.75 to 14.25 min.

(**Table 2**); however, all but one are pyridines or pyrazines. Di- and trisubstituted pyridines and pyrazines were the most odorous with 2,3,5-trimethyl pyridine judged the most intense. It has with a characteristic tobacco, musty aroma (judged 67 on scale of 0−100). Following this was 2,5-dimethyl pyrazine with a characteristic savory aroma and an odor score of 58. Acetophenone (1-phenylethanone) was also identified as an important odorant in this extract and possessed a floral aroma. It was the only nonpyridine or pyrazine component with odor activity and the only floral component identified.

Source of Odorants. Pyridines, compounds judged to be the most odorous and arguably responsible for the most offending odor from cigar smoke, may be byproducts of tobacco nicotine pyrrolysis due to cleavage of the covalent bond between carbon #3 on pyridine and carbon #2 on the pyrrolidine moiety (**Figure 1**). An additional well-known chemical mechanism responsible for the generation of pyridines and pyrazines is described by

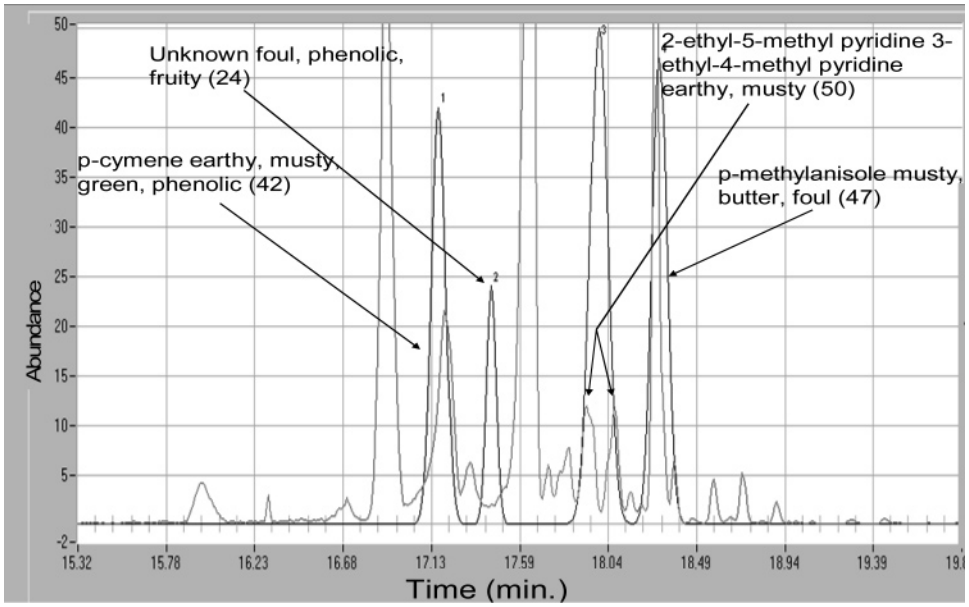

Figure 4. Overlay of aromagram and MS analysis of effluent from SPME headspace extraction of artificial saliva (23 h of extraction time at room temperature) followed by heart cutting (30 s heat cut) and cryogenic focusing (30 s before, during, and 30 s after the heart cut) with odor intensity score and odor character displayed.

Table 1. Identification of Volatile Compounds Extracted from the Tongue (by Swab) Following Smoking 1/2 Cigar

odor intensity level	retention indices (Kovats)	descriptor	identity
tier 1[a]	979	musty	ethyl pyrrole[c]
tier 2[a]	856	savory	2,3-dimethyl pyrazine[c,d]
tier 3[a]	830	nutty	2-methyl pyridine[c,d]
tier 3[b]	923	nutty	4-methyl pyridine[c,d]
tier 3[a]	945	tobacco	4-cyclobutyl pyridine[c,d]
tier 3[a]	525	buttery	diacetyl[c]
tier 3[b]	798	stench	pyridine[c,d]
tier 3[b]	879	meaty	ethyl pyrazine[c,d]

[a] Aroma activity of compounds measured at Microanalytics following smoking 1/2 Macanundo cigar utilizing swabs and assigned positions in 1st, 2nd, or 3rd tier, according to aroma intensity only. [b] Aroma activity of compounds measured at Wrigley following smoking 1/2 Onyx cigar utilizing swabs and assigned positions in 1st, 2nd, or 3rd tier, according to aroma intensity only. [c] Compounds identified by comparison with the Wiley mass spectrometry database. [d] Compounds identified by matching retention indices of authentic standards.

Table 2. Identification of Compounds Responsible for the Oral Malodor of Tobacco Smoke

odor intensity (0–100)	retention indices (Kovats)	descriptor	identity
67[a]	1046	tobacco, musty	2,3,5-trimethylpyridine[b,c]
45[a]	945	tobacco, earthy	3-methyl-4-ethyl pyridine[b,c]
50[a]	967	nutty	2-ethyl-3,5-dimethyl pyridine[b,c]
58[a]	844	savory	2,5-dimethyl pyrazine[b,c]
47[a]	972	nutty	2,6-diethyl pyrazine[c]
45[a]	1034	tobacco, musty	2,4,6-trimethyl pyridine (collidine)[b,c] and 2-ethyl-5-methyl pyridine[b]
45[a]	401	floral	acetophenone[b,c]

[a] Aroma active compounds measured at Wrigley Chicago utilizing simulated saliva blend and Onyx cigars. [b] Compounds identified by comparison with the Wiley mass spectrometry database. [c] Compounds identified by matching retention indices of authentic standards.

the Maillard reaction, and these compounds have been identified after product pyrrolysis (*16*).

Method Comparison. Liquid saliva provided much greater concentrations of volatiles as opposed to tongue swabs. This made the task of volatile identification and characterization easier. As indicated in **Figure 4**, the most odorous compounds may be present at relatively low levels as compared to other eluting compounds. Swabs exhibited less odor, and the odor dissipated rapidly, perhaps due in part to absorption effects of the swab's nylon stem and mesh.

Differences between the two methods for extracting residual smoke components (swabs vs solution) were at least partly responsible for differences in components listed in **Tables 1** and **2**. Swabs may remove some saliva from the tongue, but they also remove residual tobacco smoke that had adsorbed to the surface of the tongue. Generally, panelists reported dry mouth conditions that indicated a decreased salivary level following smoking a cigar. Simulated saliva may be more representative of components trapped in saliva and not components that adsorb to the oral cavity tissue. Nevertheless, the similarities in structures (both Tables listed primarily pyridines and pyrazines) indicated that these components predominate and are probably responsible for aftertaste and oral malodor associated with smoking cigars. Future studies will address sensory analysis related to oral cavity extracts.

In summary, pyridines and pyrazines were the most odorous components identified from tobacco smoke deposits on the tongue and in simulated saliva following smoking a Macanundo and/or Onyx cigar. Precursors for the compounds are present in tobacco and include nicotine and the Maillard reactants amino acids and reducing sugars. Future research will seek to develop methods for measuring residual cigar smoke odor amelioration.

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Received for review August 4, 2005. Revised manuscript received November 21, 2005. Accepted November 22, 2005.

JF0519109