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Comparison between Human Scent Compounds Collected on Cotton and Cotton Blend Materials for SPME-GC/MS Analysis

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Abstract

Human scent evidence has been used for centuries in various European countries and is now becoming more prevalent in the United States. Human scent evidence is collected either directly or indirectly and then used for scent discrimination with specially trained canines. The direct method allows the canine to smell an article of evidence, whereas the indirect method involves pre-scenting the canine with a sorbent material onto which traces of human scent have been previously collected. Even though there is no standardized collection material for human scent samples across various law enforcement agencies cotton based materials are commonly used. The purpose of this study was to investigate the ability of different types of sorbent materials, mainly cotton and cotton blend materials, to trap and release a combination of volatile organic compounds (VOCs) previously reported as human scent compounds. Volatile Organic Compounds in the headspace of the samples were extracted and identified using solid-phase micro-extraction gas chromatography / mass spectrometry (SPME-GC/MS). The results showed that cotton blend materials released significantly greater amounts of polar compounds when compared to the pure cotton materials. It appears that the chemical composition of the materials rather than the surface morphology plays the greater role in governing the trapping and releasing capabilities of the materials for human scent collection.

Keywords: Human scent; GC/MS; VOCs; Sorbent

Introduction

There are three types of canines used in human scent procedures; tracking dogs, trailing dogs and scent identification dogs [1]. Tracking dogs are trained to methodically follow odor on the ground caused by human or ground disturbance and are typically not pre-scented on an object. Trailing dogs have a propensity to either follow a ground disturbance odor and/or target odor plumes and are typically pre-scented on an object [2]. Scent identification canines are given a scent which is collected from the scene of a crime and are instructed to match this to a scent sample collected from a possible suspect, thus establishing an association between a suspect and an object or location, establishing *corpus delicti*. [3,4,5]. However, scent identification canines are not trained to track or trail, simply to match odor of one object to the odor of another and the training given to each type of canine determines the method used to match odors. Scent identification canines are given such a high degree of training that they may also be trained to match odor not just from different individuals but also from different areas of the body from the same individual [6]. This type of scent discrimination is possible as it is believed that persons have distinctive odors and canines have the ability to discriminate between these odors [7]. Preliminary studies conducted to determine the volatile organic compounds (VOCs) emanating from human skin were performed by Bernier et al. [8] to determine which VOCs were potentially mosquito attractants [8]. For this study, samples were collected on glass beads which were held by the subjects followed by gas chromatography mass spectrometry (GC/MS) analysis. Chromatograms were obtained that contained as many as 346 discernable peaks, 303 of which were identified as acids, alcohols, esters, aldehydes, aliphatics, aromatics, ketones, amides, amines and heterocycles [9]. Further studies conducted by Curran [10] and Hudson [11] dealt with the analysis of hand odor samples as this is the portion of the body which generally comes in contact with objects at a crime scene and is of the most significance from a forensic standpoint. In these studies, solid phase micro extraction gas chromatography mass spectrometry SPME-GC/MS was used to analyze the headspace of hand odor samples which were collected on sorbent materials. Curran [10] determined that the headspace consisted of various classes of compounds which were classified into seven groups: acids, alcohols, aldehydes, hydrocarbons, esters, ketones and nitrogen containing compounds, confirming the findings of Bernier et al. [9,10]. It is believed that these compounds are used by scent identification canines to match individuals. In order to create a match using scent identification canines, the canine must first be presented with an article containing the scent of a suspect. Pre-scenting a canine before a search is accomplished by having the canine smell the actual item of evidence or by introducing the canine to a sorbent material previously exposed to the article of evidence. The latter being the indirect method of collecting scent evidence which is preferred for its non-intrusive nature. Indirect collection of scent evidence onto a sorbent material can be done in one of three ways: wiping the sorbent material across the article of evidence, placing the sorbent material in close contact with the evidentiary material for a specific period of time or by using the scent transfer unit (STU-100) that uses airflow through a sorbent material to capture volatiles while in close proximity to the article of evidence [12]. The STU-100 is reported to offer an advantage over direct collection methods as it does not disrupt or contaminate other forms of trace evidence that may be present at the crime scene. The STU-100 is a portable hand held device which uses an electric fan to draw air through a modified inlet. The inlet is able to hold a sorbent material in place which traps volatiles as the STU-100 pulls air through when placed above an article of evidence [12].

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The capability of the sorbent to collect and trap the required VOCs to be used as human scent evidence will be the focal point of this article. The sorbent material employed is dependent on the protocol of the specific country, although cotton based sorbents are generally used [13]. The Netherlands utilize a non-sterile cotton sorbent known as Kings Cotton; in Poland, cotton "scent tampons" manufactured solely for the police are used, while in Hungary, an "odor collecting cloth" the composition of which is not known is used. In the United States, the Federal Bureau of Investigation (FBI) uses a sterile Johnson and Johnson cotton gauze pad while research groups have used cotton Dukal brand gauze. As the VOCs being studied are on the order of 100 to 400 atomic mass units (amu), it is unlikely that the sorbents will physically trap these tiny molecules within their fiber matrices. Rather, it is being hypothesized that a chemical interaction between the sorbent fibers and the VOCs comes into effect much like the interaction of these molecules on the stationary phase of a capillary column or the fiber coating when being extracted with SPME.

Cotton is cellulosic in nature and is chemically known as poly 1,4-β-D-anhydroglucopyranose. It is formed by the condensation of glucose molecules (Figure 1). The cellulose structure has three hydroxyl groups attached to each glucose residue. Due to the presence of these hydroxyl groups, cellulose is capable of forming hydrogen bonds (Warner 1995). It is believed that these hydrogen bonds are the main chemical interacting force that gives these cotton based sorbents their ability to trap the VOCs being collected.

Rayon is a man-made fiber made by the dissolution of natural fibrous materials such as cotton or cotton derivatives (Figure 2). The most common way in which rayon is produced commercially is by dissolving cellulose in dilute alkali after it has been treated with caustic soda and carbon disulfide [14]. The end product is a synthetic fiber comprised of regenerated cellulose in which substituents have replaced not more than 15 percent of the hydrogens of the hydroxyl groups. The chemical properties of rayon are similar to that of cotton fibers as they are both cellulosic in nature [15]. However, with less available hydroxyl groups, the number of hydrogen bonds is reduced compared with that of cotton.

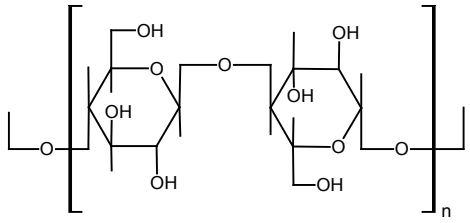

Figure 1: Repeat unit of cotton.

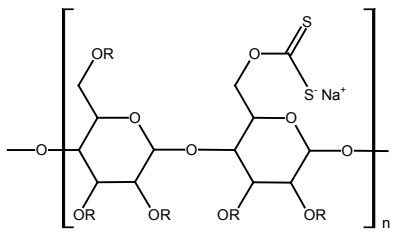

Figure 2: Repeat unit of Rayon.

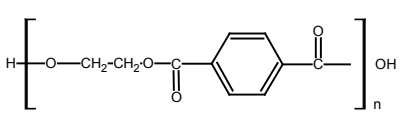

Figure 3: Repeat unit of Polyester

Polyester is an artificial fiber made up of long chain synthetic polymers composed of at least 85 percent by weight of an ester of dihydric alcohol and terephthalic acid. The most common polyester is polyethylene terephthalate (PET) (Figure 3) [16]. Polyester is hydrophobic and oleophilic in nature. The hydrophobic nature provides water repelling properties and rapid drying while the oleophilic nature allows it to absorb non-polar compounds easily but makes removal difficult possibly due to induced dipole dipole interactions of the VOCs along the polymer backbone.

It has been reported previously that cotton based sorbents for human scent sample collection is very common among law enforcement agencies [13], but since instrumental analyses has shown that human scent possesses a wide variety of compounds with varying functional groups [10], it raises the question of the effect that different compositions of cotton based sorbents will have to collect human scent samples. It may be argued that the trapping and releasing capabilities of human scent collection sorbents may be enhanced or diminished depending on the functional groups present within the collection material. Although it has not yet been demonstrated whether the trapping and releasing capabilities of different materials improves or diminishes a canine's discrimination capability, the findings of this study will show that human scent profiles collected from the same individual and analyzed instrumentally varied based on the type of sorbent material used as the collection media. As there is presently limited scientific data that characterizes the ability of various sorbent materials to trap and release compounds previously reported as components of human scent, this article will investigate the trapping and releasing capabilities of pure cotton and cotton blend materials.

Materials and Methods

Materials

Sorbent materials used were DUKAL brand, sterile, 2x2 inch, gauze pads (DUKAL Corporation, Syosset, NY, USA), Kings Cotton, non-sterile, 2x2 inch sorbent material (Seafarma, NL) and Johnson and Johnson brand, sterile, 2x2 inch gauze pads (Johnson and Johnson, Consumer Products Company, China). The 2x2 inch Johnson and Johnson brand gauze pads were sectioned into quarters. This was only done for the Johnson and Johnson Gauzes to allow them to fit into the 10ml headspace vials used. The vials used to hold the gauzes were 10 ml glass, clear, screw top vials with PTFE/Silicone septa (SUPELCO, Bellefonte, PA, USA). The vials were cleaned using acetone followed by heating in an oven at 105°C (Isotemp Oven, Model 655G Fisher Scientific, Pittsburgh, PA, USA). The SPME fibers used for the extractions were 50/30μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (SUPELCO, Bellefonte, PA, USA). The temperature and the humidity of the environment were monitored using Thermochron I-Buttons (MAXIM, Dallas, Texas, USA).

Scanning Electron Microscope (SEM) morphological study

The sorbent materials were cut into small pieces and placed on an aluminum stub using a carbon adhesive. For the SEM imaging, the mounted samples were placed in a SPI sputter coater where the

materials were coated with gold-palladium. The surface morphology of the sorbent materials was observed using a scanning electron microscope (JEOL JSM – 59LV, Japan) at 25X magnification.

Pre-treatment of sorbent materials

All sorbent materials were pre-treated to ensure that they were free of compounds previously reported as human scent by spiking with 1000μl of methanol followed by heating at 105°C for 45 minutes in an Isotemp Oven, Model 655G (Fisher Scientific, Pittsburgh , PA, USA). The heating process removed compounds previously reported as human scents which were present on the sorbent materials prior to cleaning; these were mainly alkanes and alcohols.

Hand sampling procedure

Human subjects used for this study were required to wash hands and forearms with clear Olive Oil Soap for 30 seconds, rinse with water for 2 minutes, air dry for 4 minutes, then rub the palms of hands over forearms for 5 minutes [10]. Subjects then sampled themselves by holding the pre-treated 2x2 sorbent material between the palms of the hands for 10 minutes. The sample was then placed back inside the 10ml glass vial and sealed by the subjects. Triplicate samples were collected from each subject.

Volatile organic compound mix

Ten microliters of a 60ng/μl volatile organic compound (VOC) standard was spiked onto each of the sorbent materials. The VOC mixture was comprised of 39 compounds each at a 60ng/μl concentration and previously reported as human scent compounds (Table 1). The amounts of each compound used was determined from previous studies that simulated the levels of human scent VOCs commonly obtained from actual human scent samples [11]. The materials were immediately sealed in 10 ml glass screw top vials. Positive controls were made by placing 10μl of the VOC standard directly in 10ml glass vial (no sorbent material was present). The vials were allowed to equilibrate for 24 hours prior to SPME headspace sampling. Semi-quantitation of the recovered analytes was based on a five-point calibration curve (5-60ng/μl). Six replicate samples were analyzed for each material.

SPME-GC/MS procedure

Volatile organic compounds were extracted from the headspace of the vials containing the sorbent materials using 50/30μm DVB/CAR/PDMS fibers. Headspace extractions of the samples were performed at room temperature (20°C) for 21 hours [10]. The instrumentation used for the separation and analysis of the analytes was an Agilent 6890 GC / 5973 MSD with a 0.25 mm x 30 m HP5-MS column which had a 0.25μm phase film thickness. Helium carrier gas was maintained at a flow rate of 1.0 ml/min. The initial GC oven temperature of 40°C was held for 5 minutes, followed by a temperature ramp of 10°C per minute to a final temperature of 250°C which was held for 2 minutes. The mass spectrometer transfer line was maintained at 280°C and the source temperature was 230°C. Mass spectra were repeatedly scanned from 39-300 m/z [11].

Statistical evaluation

One way ANOVA was performed using Microsoft Excel to compare the mean masses recovered from each of the sorbent materials. This was used to determine if there were significant differences in the mean masses of VOCs recovered from the sorbent materials at a 95% confidence level. A Fisher's Least Significant Difference post hoc test was used to compare the means if the null hypothesis was rejected using the ANOVA F-test [17].

Results

Sorbent materials

Prior to this study, six sorbent materials were investigated, however three of these contained high endogenous levels of human scent compounds that could not be reduced with our current analytical cleaning techniques. The sorbents that could be cleaned to remove endogenous human scent compounds and therefore used in this study were: Dukal brand gauze, Johnson and Johnson cotton blend gauze and Kings Cotton. Once the materials were cleaned, they were subjected to headspace SPME-GC/MS analysis to verify analytical cleanliness, indicated by the non-detection of compounds previously reported as being those from human scent. The sorbents that could not be cleaned and therefore not used for this study included Hungarian cotton, Polish cotton and Johnson and Johnson cotton gauze.

There are two major differences between these three materials; their chemical compositions and surface morphologies. The Dukal

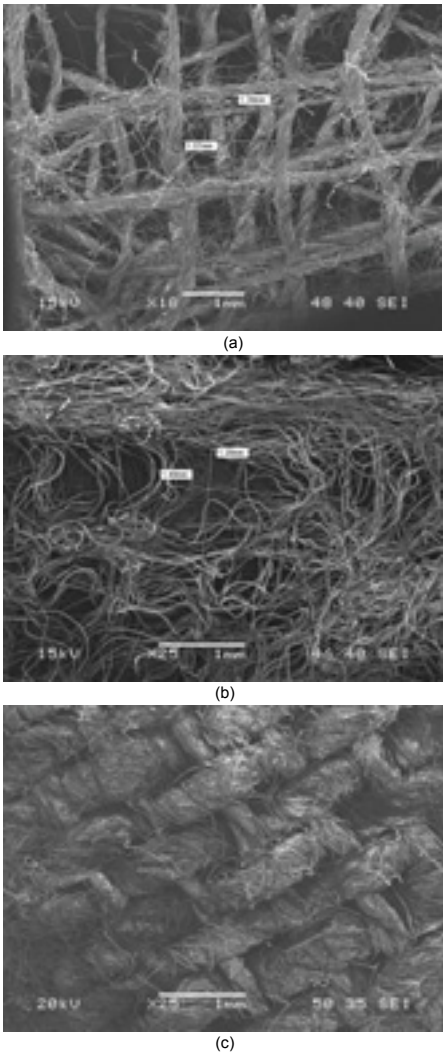

Figure 4: SEM Images of (a) Dukal brand gauze (b) Johnson and Johnson brand gauze (cotton blend) (c) Kings Cotton (Resolution x25).

brand gauze and the Kings Cotton are both 100 percent cotton materials, while the Johnson and Johnson brand gauze is a blend of cellulose, rayon and polyester. The Kings Cotton possesses the most tightly woven surface morphology with 96μm between wefts and wraps corresponding to Sieve size of 170 while the Dukal and Johnson and Johnson brands have less tightly woven surface morphologies with 1.5 mm and 1.4 mm respectively between wefts and wraps corresponding to a Sieve size of 14 as outlined by the American Standards of Testing and Materials [18] (Figure 4).

Hand odor sample collection

Collection of hand odor samples from the same individual on the selected materials showed differences in the functional groups of the acquired odor profiles. The odor profiles obtained from the 100 percent cotton materials showed primarily aldehydes and alkanes while odor profiles from the cotton blend material showed not just aldehydes and alkanes but also alcohols (Figure 5 and Figure 6). As the samples were collected from the same individual it was assumed that the same VOCs were deposited on all sorbent materials. The results obtained indicate that the blend material is releasing a wider variety of VOCs than the one 100 percent or pure cotton materials. These results were subjected to a one way ANOVA to test for variance at the 95% confidence level.

Total mass recovered from sorbent materials

To investigate the reasons for the observed differences in the odor profiles and to remove all uncontrolled variances obtained when using human subjects, a standard VOC mixture comprised of 39 compounds previously reported as human scent components

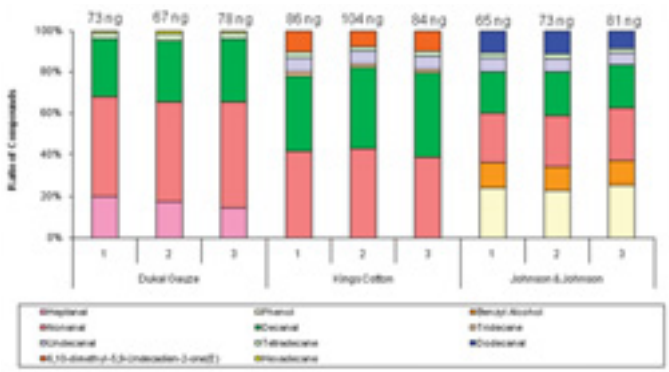

Figure 5: VOCs present in hand odor samples collected from subject 1 on different sorbent materials.

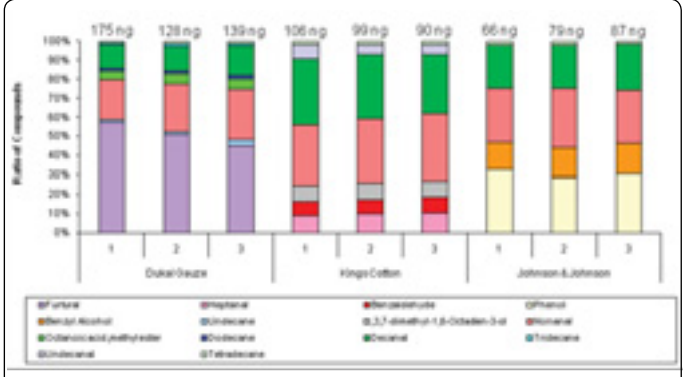

Figure 6: VOCs present in hand odor samples collected from subject 2 on different sorbent materials.

Compound Name	MW	BP (°C)
Acids		
Dodecanoic Acid	200	225
Pentadecanoic acid	242	257
Decanoic Acid	172	269
Alcohols		
2-Furanmethanol	98	171
Phenol	94	182
3,7-dimethyl-1,6-octadien-3-ol	154	198
Benzyl alcohol	108	205
Phenylethyl alcohol	122	219
1-octanol	130	195
Nonanol	144	215
Aldehydes		
2-Furancarboxaldehyde	96	162
Heptanal	114	153
Benzaldehyde	106	178
Octanal	128	163
Nonanal	142	93
Decanal	156	207
Dodecanal	184	240
Undecanal	170	223
(E)-2-Octenal	126	84
Tridecanal	198	132
Aliphatics/Aromatics		
Octane	114	125
Nonane	128	151
Undecane	156	196
Naphthalene	128	218
Dodecane	170	216
Pentadecane	212	268
Tridecane	184	234
Tetradecane	198	253
Hexadecane	226	287
Heptadecane	240	302
Ketones		
6-methyl-5-hepten-2-one	126	73
6,10-dimethyl-5,9-undecadien-2-one	194	254
2-Decanone	156	209
Esters		
Propanedioic acid, dimethyl ester	160	183
Octanoic acid, methyl ester	158	79
Hexanedioic acid, dimethyl ester	174	109
Dodecanoic acid, methyl ester	214	261
Methyl Tetradecanoate	242	323
Hexadecanoic acid, methyl ester	271	185

Table 1: Compounds previously reported as components of human scent that were used in volatile organic mixture.

Materials	Properties
Dukal Brand Gauze	Sterile, 100% Cotton
Johnson and Johnson Brand (Cotton Blend)	Sterile, Rayon/Polyester/Cellulose
Kings Cotton	Non-sterile, 100% Cotton

Table 2: Properties of the sorbent materials studied.

was created and used to spike the different sorbent materials (Table 1). Liquid samples were used as these could more accurately be manipulated to ensure confidence in the amount of compounds being deposited directly onto the sorbent materials. A positive control was used that consisted of 10μl of the 39 component mixture with each component at a concentration 60ng/μl in dichloromethane spiked in a 10 ml Headspace vial without any sorbent material present. Of the total mass of VOCs spiked (23.4 mg) into the positive control 10 ml glass vial, 2040 ng was recovered using SPME-GC/MS analysis. 591 ng were recovered after spiking the 39 component mixture onto Dukal brand gauze, 581 ng after spiking Kings Cotton and 857 ng from the Johnson and Johnson brand gauze (Figure 7).

A one way analysis of variance (ANOVA) is a statistical technique which is used to compare the means of three or more samples. This statistical analysis tool is used to detect whether or not the mean values from the groups being tested are significantly different. Using a one way ANOVA, it was determined that the recovered masses for the sorbent materials were significantly less than that of the positive

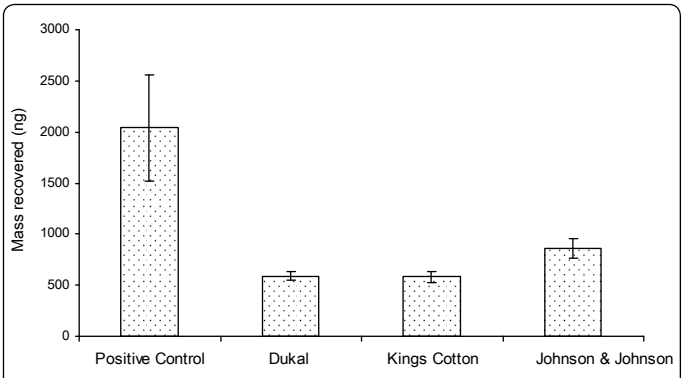

Figure 7: Average mass of VOCs recovered from positive control and sorbent materials.

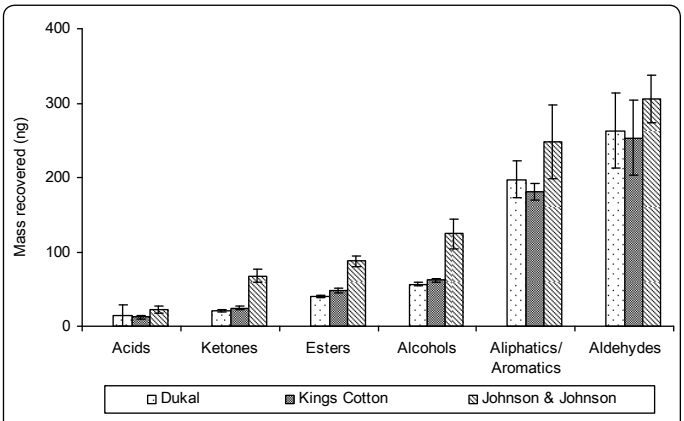

Figure 8: Average mass of functional groups recovered from each sorbent material.

control demonstrating that the sorbent materials are retaining significant amounts of VOCs.

A one way ANOVA performed to compare the mean masses recovered from the sorbent materials showed that the recovered masses were significantly different as the calculated F value was greater than the critical F value at a 95 percent confidence level. This resulted in the rejection of the null hypothesis which stated that there is no difference in the masses obtained from the different materials. A Fishers least significant difference (LSD) post hoc test was subsequently performed upon rejection of the null hypothesis. Pair wise comparisons of the difference between the means obtained from each sorbent material were compared to the LSD. All pairs of interest were tested: Dukal/KC, Dukal/J&J and KC/J&J.

Comparison of the differences in means to the LSD showed that the amount of VOCs recovered by the Dukal brand gauze and the Kings Cotton were not significantly different but the amount recovered from the Johnson and Johnson Brand was significantly greater from the other two sorbent materials. One difference between the Johnson and Johnson brand gauze and the two other sorbent materials is its chemical composition. The Johnson and Johnson brand is a synthetic material comprised of cellulose, rayon and polyester while the Dukal brand and Kings Cotton are both 100 percent cotton materials comprised primarily of cellulose. These results suggest that the chemical property of the various materials does play a role in the capabilities of the materials to release the VOCs loaded into them, with the cotton blend material releasing significantly greater quantities of VOCs compared to the 100 percent cotton materials.

Functional groups recovered from sorbent materials

Various functional groups were recovered from both the positive control and the sorbent materials in the following order, Aldehydes > Aliphatics/Aromatics > Alcohols> Esters > Ketones > Acids. The differences observed in the functional groups released by each of the sorbent materials are shown in Figure 8. For all functional groups studied, the Johnson and Johnson brand gauze released greater masses of VOCs than the Dukal brand and Kings Cotton. A one way ANOVA showed significant differences in the masses of alcohols, esters, aliphatics/aromatics and ketones released by the Johnson and Jonson brand compared to the 100 percent cotton materials.

Ketones and alcohols are polar compounds which are expected to have very strong interactions with the polar surfaces present on the cellulose backbone of the cotton materials. More specifically by the formation of hydrogen bond interactions between the cellulose backbone of the 100 percent cotton materials and the hydroxyl portions and carbonyl portions of the alcohols and ketones respectively. The Johnson and Johnson brand gauze is not entirely comprised of cellulose so it released alcohols and ketones more readily than the 100 percent cotton materials. The observed differences in the trapping and releasing capabilities of the 100 percent cotton materials and the cotton blend material could be a result of the former having predominantly polar surfaces while the latter possesses a combination of both polar and non-polar sites due to the presence of the rayon and polyester along with cotton weaved in to the sorbent material.

Conclusion

One marked difference in the odor profiles for the pure cotton and cotton blend sorbents was the lack of or reduced amount of polar alcohol compounds being detected when hand odor or spiked samples were extracted from pure cotton based sorbents. The cellulose backbone of the 100 percent cotton materials has a high affinity for polar compounds such as alcohols, which may result in these compounds being poorly released; hence these compounds were not available for extraction by SPME and subsequently not detected by our instrument. However, these polar compounds were able to be detected after being deposited on the less polar cotton blend material. As such, the non-detection of more polar compounds from the pure cotton materials is no indication of their absence on these sorbent materials during collection of scent from human subjects. Since these pure cotton sorbents have higher affinities for polar compounds they are possibly being released in quantities which are below the detection limit of the GC/MS used for the analyses. This would also explain the differences in the human scent profiles obtained for the same individual on different materials (Figure 5 and Figure 6). Even though this may be a drawback to matching the scent profiles of individuals instrumentally, it may not be as significant when using canines for matching odor from individuals. Canines' sense of smell is reported to be several orders of magnitude lower, parts per trillion to parts per billion [19,20], than the limit of detection of the analytical instrument used in this study, which has detection limits in the parts per million range. Additionally, canines may use compounds other than the ones observed instrumentally to differentiate between individuals.

From the results obtained, it does appear that the chemical composition of the materials rather than the surface morphology played the greater role in governing the trapping and releasing capabilities of the materials for human scent collection. Both

the Johnson and Johnson and Dukal gauze had similar surface morphologies each with a Sieve size of 14 and one would expect that they would have behaved similarly, however this was not the case indicating that the surface morphology had a limited role to play in the capability of the sorbents to release the VOCs deposited on them. Other factors such as the total surface area of the materials could also come into effect as not all the sorbents tested had the same apparent density and thickness, however this was not investigated. Field tests with scent discrimination canines would also be an excellent tool by which to prove or disprove these findings as one sorbent which could not be included in the study, Johnson and Johnson cotton gauze, is routinely used by the FBI to collect human scent which is then presented to canines for trailing of suspects. In conclusion, these findings do indicate that cotton blend sorbents containing polyester and rayon release compounds with polar functional groups more readily than do pure cotton sorbents. This should be taken into account in developing protocols for work with human scent discrimination canines when conducting indirect collection of scent evidence. As the type of sorbent used may affect the odor profile being presented to the canine and ultimately the probability of correctly identifying a suspect.

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