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The frequency of occurrence and discriminatory power of compounds found in human scent across a population determined by SPME-GC/MS

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Abstract

The composition of human scent collected from the hands is of interest to the medical community as a mechanism to diagnose disease and the forensic community as a means to investigate canine scent discriminations. An extensive survey of the volatile organic compounds (VOCs) identified in the headspace of hand odor samples utilizing solid phase micro-extraction gas chromatography/mass spectrometry (SPME-GC/MS) has been conducted to determine the constituents of the human base odor profile. Sixty-three compounds were extracted from the collected odor samples. The composition included acids, alcohols, aldehydes, hydrocarbons, esters, ketones and nitrogen-containing compounds. The majority of the compounds detected (79.4%) were present in less than one third of the individuals sampled. Spearman correlation coefficient comparisons at a match/no-match threshold of 0.9 produced a distinguish ability of 99.67% across the population.

Keywords: Human scent; Hand odor; VOCs; Canine; Human scent line-up

1. Introduction

The medical usefulness of volatile compounds produced by humans for the diagnosis of lung, breast, bladder, and skin cancer has been demonstrated through both instrumental [1–4] and biological analysis [5–7]. The value of compounds emanated by the body for diagnostic purposes relies upon a baseline determination of the presence and quantity of human odor compounds from an individual. The body odors of human individuals are determined by several factors, some odors are stable over time (genetically based) or they may vary with environmental or internal conditions. The authors have developed distinguishing terminology for these factors: the "primary odor" of an individual contains constituents that are stable over time regardless of diet or environmental factors; the "secondary odor" contains constituents which are also endogenous but are influenced by diet and environmental factors; and the "tertiary odor" contains constituents that are present due to exogenous sources (i.e., lotions, soaps, perfumes, etc.) [8].

The value of compounds emanated by individuals, collected as human scent evidence, are of importance to the law enforcement community. The Locard exchange principle proposes that a person cannot enter or leave an area or come in contact with an object, without an exchange of materials. In the case of scent evidence, the suspect leaves his scent in the location of the crime scene itself or on objects found therein. This form of trace evidence collected from a crime scene can be evaluated through the use of specially trained canines to determine an association between the evidence and a suspect.

The hypothesis that human scent is stable over time and distinguishable between individuals is the foundation on which canine identifications are based. Scientific research into the ability of canines to distinguish between individuals based on their scent supports this theory [9–12]. Thus far, there has been limited research as to the VOCs which comprise the human scent profile and their usefulness in distinguishing individuals by analytical methods [8,13–16]. To conduct such analysis, the frequency of

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occurrence of compounds extracted in human scent will require a larger population study to determine the variability of human-produced compounds among individuals.

Forensically, VOCs from the hand are of vast interest as it is the region of the body where known samples of human scent is most often collected by law enforcement for use by scent discrimination canines in comparison to collected samples from the crime. Hand odor is comprised of the secretions from eccrine and sebaceous glands plus odors from the microbial degradation of these secretions. Eccrine secretions are typically composed of 98% water, but also contain various organic and inorganic components [17]. Eccrine sweat originates in the extracellular fluid and therefore, reflects the chemistry of blood plasma [18]. The VOCs dissolved in blood include numerous alcohols, aldehydes, and alkanes [3]. Sebum from sebaceous glands consists of glycerides, free fatty acids, wax esters, squalene, and cholesterol. A wide variety of organic compounds can be found in the sebum, and may be influenced by diet and genetics [19]. It has been suggested that slight differences in the overall composition of the sebaceous fatty acid mixture may play a significant role in the unique individual odors in humans [19].

A significant portion of the scientific research into human odor has been conducted on secretions from the axillary (armpit) area [20,21] and the feet [22]. Identification of the compounds emanated by human hands that may influence mosquito host-seeking behavior have resulted in a listing of more than 300 compounds [23,24]. Many compound classes are present in human emanations including acids [8,14,15,20,21,24,25], alcohols [8,14,15,24–26], aldehydes [8,14,15,18,24–26], hydrocarbons [8,14,15,24,25,27], esters [14,15,24,25,28], and ketones [8,14,15,24,25,29]. The components of human secretions may not adequately represent the compounds, nor the abundances present in the headspace above this matrix. The headspace above skin on the forearm which comprises the odor has been directly sampled through solid phase micro-extraction gas chromatography/mass spectrometry (SPME-GC/MS) [29], and hand odor from a small subject population was also directly evaluated using an original sampling device and SPME-GC/MS [16].

The purpose of this paper is to conduct a large scale study of the volatile organic compounds (VOCs) present in the headspace of collected hand odors. The sampling method utilizes cotton gauze pads treated by supercritical fluid extractions (SFE) to remove the presence and possible interference of compounds in the background of the pads. Headspace SPME-GC/MS analysis of scent samples from the hands of 60 individuals (30 males and 30 females) provides a range of compounds extracted among individuals. This information can be used to assess the variation of these compounds across a population utilizing Spearman rank correlation coefficient comparisons, which has both diagnostic and forensic implications.

2. Materials and methods

2.1. Materials

Supercritical fluid extraction using methanol (HPLC grade, Fisher Scientific, Pittsburgh, PA) modified supercritical grade carbon dioxide (Air Products, Allentown, PA) was used as a pre-treatment for the gauze to create an "analytically clean" collection medium [14]. Gauze pads were DUKAL brand, 100% cotton, sterile, 2 × 2, 8ply, gauze sponges (DUKAL Corporation, Syosset, NY, USA). The vials used to hold the gauze were 10 ml glass, clear, screw top vials with PTFE/Silicone septa (SUPELCO, Bellefonte, PA, USA). All subjects used the same soap to wash the hands and forearms (Natural, Clear Olive Oil Soap, Life of the Party, North Brunswick, NJ, USA).

2.2. Pre-treatment of gauze pads by supercritical fluid extraction

An ISCO Model 260D Syringe Pump with an SFX 2-10 supercritical fluid extractor was used to perform the pre-treatment. Each supercritical fluid extraction began by filling the plastic extraction vessel with two pieces of sterile gauze pads. The optimum SFE conditions developed to extract organic volatile compounds from sterile absorbers were determined to be a 30 min static extraction time followed by a 10 min dynamic extraction time at an extraction temperature of 130 °C, pressure of 4500 psi, and a spike of 500 μl HPLC grade methanol directly into the extraction vessel. These samples were analyzed by identical SPME-GC–MS parameters for qualitative and quantitative analysis of the scent samples as described later in the text.

2.3. Method for hand odor sampling

A total of 60 subjects were evaluated, comprised of 30 males and 30 females ranging in age from 17–28 years old. The sampling protocol consisted of 30 s washing of the hands and forearms with olive oil based soap, a 2 min rinse of the areas with cool water, 2 min air drying, and 5 min of rubbing the palms of the hands over the forearms. A pre-treated 2 × 2 sterile gauze pad was removed from the 10 ml glass vial using tweezers rinsed previously with a 10% bleach solution. The gauze was placed in the palms of the subject's hands. The subjects sampled themselves by holding the pre-treated gauze between the palms of their hands as they walked outdoors for 10 min. At the end of that period, the gauze was re-sealed in the 10 ml glass vial. All samples were stored sealed in the 10 ml vials at ambient room temperature, and aged approximately 24 h prior to extraction. These storage conditions were chosen to simulate the conditions under which odor is collected for canine evaluation purposes, and no attempt was made to control microbial interactions with the substrate as it may make contributions to the overall odor profile. Samples were collected at an average temperature of 26.6 °C and an average humidity of 76%. Prior to the population sampling, the protocol was preliminarily tested five times each with a single male and a single female volunteer. These 10 samples were used to optimize the extraction time necessary when using the SPME fibers.

2.4. Determination of optimal SPME extraction time

Five samples each were collected from Male 1 and Female 1 on the same day following the previously described sampling

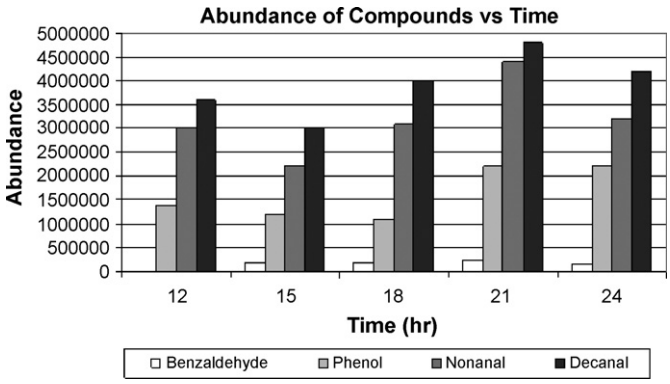

Fig. 1. Abundance of selected scent compounds as a function of extraction time for a single male individual.

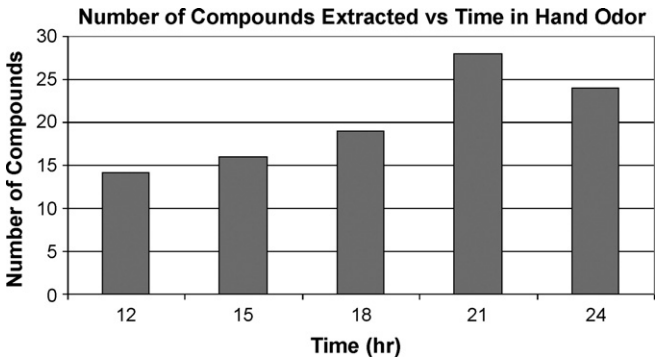

Fig. 2. Number of scent compounds as a function of extraction time for a single male individual.

protocol. A divinylbenzene/carboxen on polydimethylsiloxane (CAR/DVB on PDMS) 50/30 um fiber (SUPELCO, Bellefonte, PA, USA) fiber was exposed to the headspace above the 10 samples at room temperature for 12, 15, 18, 21, and 24 h per subject. The samples were analyzed on an Agilent 6970 gas chromatograph (GC) with a 5973 mass selective detector (MS). The column used was a 30 m × 0.25 mm, 0.25 μm film thickness HP5-MS. Helium was the carrier gas, flow controlled at 1.0 ml/min. The analytes were desorbed in the injection port of the GC using an inlet temperature set at 250 °C. The 33 min GC method began with an initial oven temperature of 40 °C for 5 min, followed by a ramp of 10 °C/min to 300 °C, and ending with a 2 min hold [14]. The quadrupole mass analyzer was operated in electron ionization (EI) mode, and scanned over a mass ranged of *m/z* 50–550 in full scan mode.

2.5. Extraction and analysis of hand odor samples (SPME-GC/MS)

DVB/CAR on PDMS fibers were used to extract the VOCs from the headspace of the vials containing the scented gauze which was aged approximately 24 h. SPME extractions were conducted at ambient room temperature for 21 h, which was determined to be the optimal extraction time based on a combination of the number and abundance of compounds recorded.

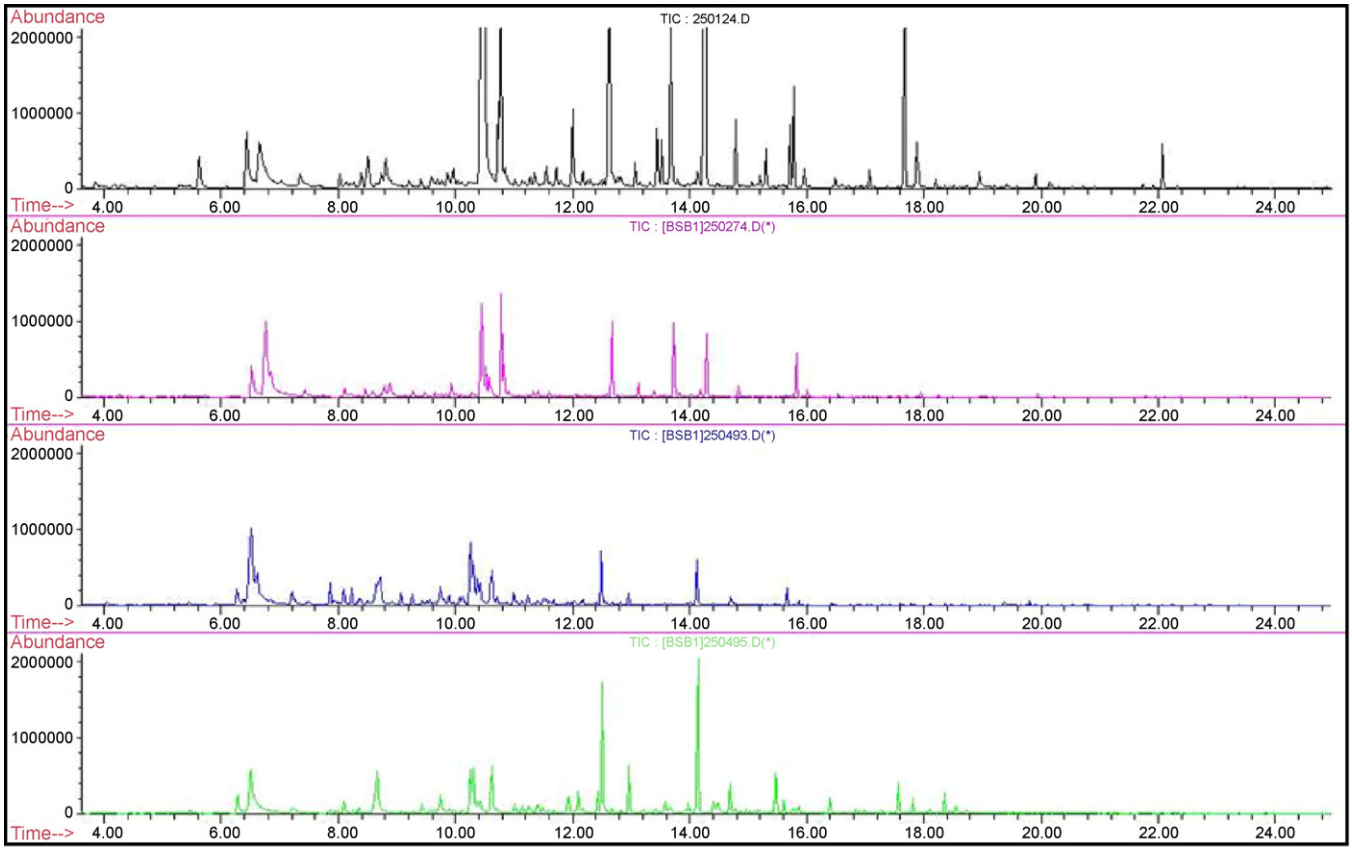

Fig. 3. Typical hand scent profiles for individual males.

All gauzes were pre-treated using SFE and extracted using the SPME-GC/MS method prior to use to verify that the background compounds were eliminated.

2.6. Spearman correlation coefficient comparisons

After extraction and analysis of the collected odor sample, compounds determined in the headspace of the samples were ranked according to their integrated peak areas in ascending fashion for each subject. These ranked data arrays were then compared using the Spearman Correlation, as seen in Eq. (1) below,

$$r_s = 1\frac{6\sum d^2}{n(n^2-1)} \quad (1)$$

where d is the difference between the ranked compounds and n is equal to the number of compounds being compared. Sixty subjects were considered producing 1770 possible pairings.

3. Results and discussion

3.1. Determination of optimal SPME extraction time

The five replicate hand odor samples from the individual male and individual female each were extracted after being aged for 24 h at five different times: 12, 15, 18, 21, 24 h. The resulting profiles were then evaluated on the basis of abundance and the number of odor compounds extracted to determine the optimal extraction time for the variety of compounds detected. Twenty-one hours was shown to be the optimal extraction time for these hand odor samples based on the evaluation criteria compared between the male and the female subject. Fig. 1 shows the resulting abundance of selected compounds as a function of extraction time and Fig. 2, the number of compounds extracted as a function of extraction time for a single male volunteer. As is evident from these figures, a 21 h extraction time produces the greatest quantity of compounds extracted and the collection of the highest abundances of the selected compounds.

3.2. Extraction and analysis of hand odor samples (SPME-GC/MS)

Figs. 3 and 4 display typical chromatograms obtained from the headspace above hand odor samples for males and females, respectively. These chromatograms are shown with the siloxane peaks that result from the SPME fiber coating and the column removed. The chromatograms of Fig. 3 are expanded to highlight the less abundant compounds produced by the male subjects; this results in peaks that are off-scale for phenol, 6-methyl-5-hepten-2-one, nonanal, decanal, and dodecanoic acid peaks. As can be seen in Figs. 3 and 4, there are similarities and differences in compounds observed in the chromatograms

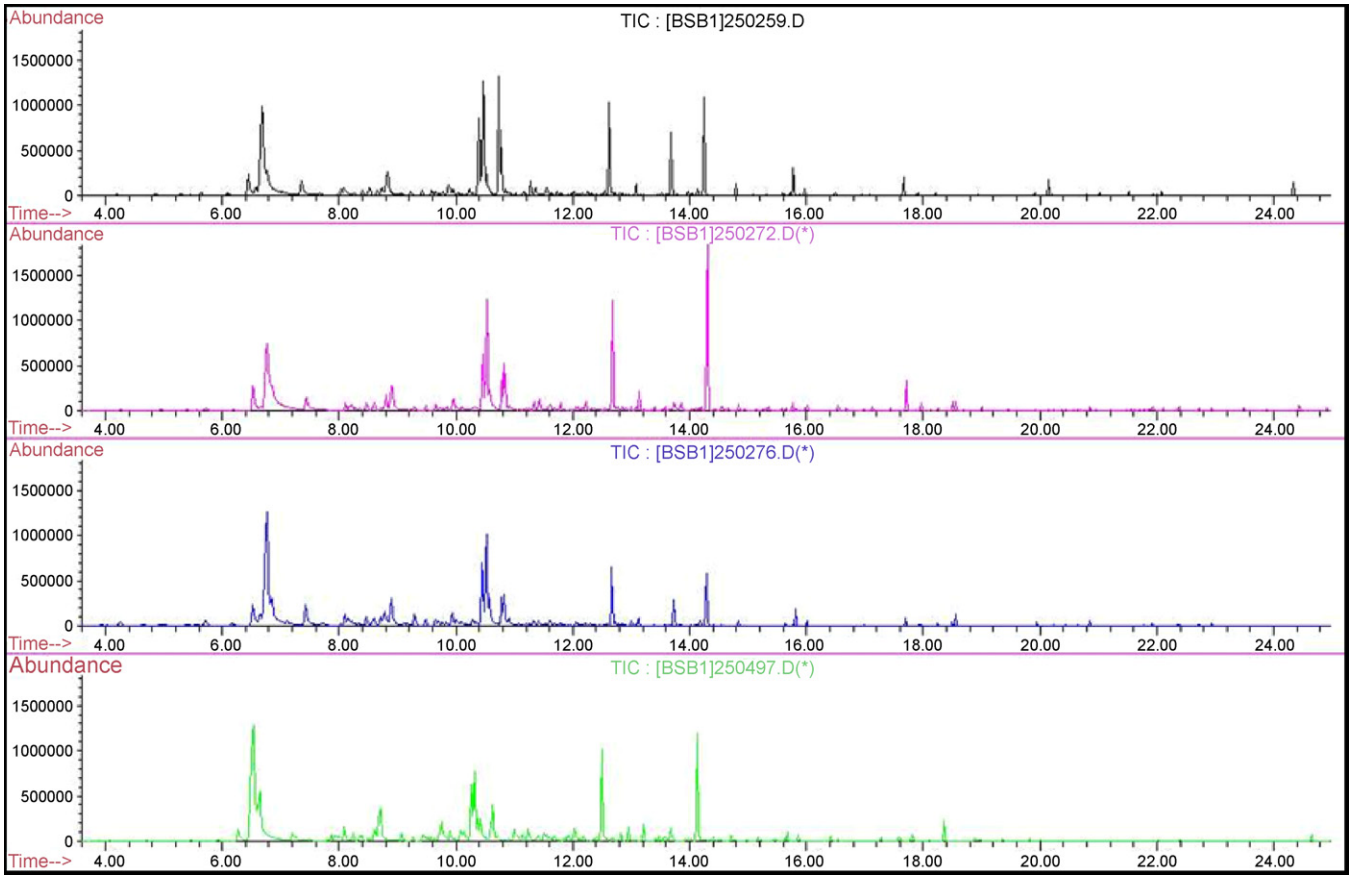

Fig. 4. Typical hand scent profiles individual females.

Table 1
Compounds extracted from the hand odor of 60 subjects

R.T.	Compound name	Frequency of occurrence			Percentage of occurrence (%)		
		Males	Females	Total	Males	Females	Total
3.86	Pyridine[a]	1	0	1	3.33	0.00	1.67
4.67	Toluene[a]	0	1	1	0.00	3.33	1.67
4.74	2-Butenal, 2-methyl-	2	2	4	6.67	6.67	6.67
5.45	Octane[a]	1	0	1	3.33	0.00	1.67
5.73	Hexanal[a]	0	1	1	0.00	3.33	1.67
6.66	2-Furancarboxaldehyde[a]	29	30	59	96.67	100	98.33
7.43	2-Furanmethanol[a]	24	25	49	80.00	83.33	81.67
7.78	Benzene, 1,3-dimethyl-[a]	0	2	2	0.00	6.67	3.33
7.82	*p*-Xylene[a]	2	2	4	6.67	6.67	6.67
8.32	Nonane[a]	6	6	12	20.00	20.00	20.00
8.51	Heptanal[a]	6	2	8	20.00	6.67	13.33
9.21	Propanedioic acid-dimethyl ester	17	17	34	56.67	56.67	56.67
9.75	Benzene, 1-ethyl-2-methyl-	3	0	3	10.00	0.00	5.00
9.87	Benzaldehyde[a]	6	3	9	20.00	10.00	15.00
9.89	Benzene, 1,3,5-trimethyl-	4	5	9	13.33	16.67	15.00
10.28	Furancarboxylic acid-methyl ester	2	3	5	6.67	10.00	8.33
10.15	Benzene, 1-ethyl-3-methyl-	0	2	2	0.00	6.67	3.33
10.42	Phenol[a]	30	30	60	100	100	100
10.49	5-Hepten-2-one, 6-methyl-[a]	8	16	24	26.67	53.33	40.00
10.67	1,2,4-Trimethylbenzene	0	1	1	0.00	3.33	1.67
10.78	Octanal[a]	4	6	10	13.33	20.00	16.67
11.10	Thiazolidine	1	1	2	3.33	3.33	3.33
11.26	Benzyl alcohol[a]	3	6	9	10.00	20.00	15.00
11.60	Benzene, 1,2,3-trimethyl-	1	2	3	3.33	6.67	5.00
12.13	1-Octanol	1	2	3	3.33	6.67	5.00
12.43	1,6-Octadien-3-ol, 3,7-dimethyl-[a]	5	7	12	16.67	23.33	20.00
12.54	Undecane[a]	4	2	6	13.33	6.67	10.00
12.64	Nonanal[a]	30	30	60	100	100	100
12.83	Octanoic acid-methyl ester	5	15	20	16.67	50.00	33.33
12.87	Phenylethyl alcohol[a]	0	3	3	0.00	10.00	5.00
13.47	Nonane, 1-chloro-	0	1	1	0.00	3.33	1.67
13.53	2-Nonenal, (*E*)-[a]	7	8	15	23.33	26.67	25.00
13.59	Nonanol	1	1	2	3.33	3.33	3.33
13.93	2-Decanone[a]	2	0	2	6.67	0.00	3.33
13.97	Naphthalene[a]	2	1	3	6.67	3.33	5.00
14.19	Dodecane[a]	8	12	20	26.67	40.00	33.33
14.28	Decanal[a]	30	30	60	100	100	100
14.51	Nonanoic acid-methyl ester	7	12	19	23.33	40.00	31.67
14.66	6-Octen-1-ol, 3,7-dimethyl-, (R)-	0	1	1	0.00	3.33	1.67
14.79	Hexanedioic acid, dimethyl ester	18	26	44	60.00	86.67	73.33
15.07	2-Decenal, (E)-	1	4	5	3.33	13.33	8.33
15.59	Tridecane[a]	9	8	17	30.00	26.67	28.33
15.68	Eicosane	0	2	2	0.00	6.67	3.33
15.71	Undecanal[a]	9	14	23	30.00	46.67	38.33
15.76	Tetradecanal	2	3	5	6.67	10.00	8.33
15.99	Decanoic acid, methyl ester	1	4	5	3.33	13.33	8.33
16.44	.Beta.-Pinene[a]	1	1	2	3.33	3.33	3.33
16.50	2-Octenal, (E)-	4	5	9	13.33	16.67	15.00
16.58	Decanoic acid	0	1	1	0.00	3.33	1.67
16.85	Tetradecane[a]	10	15	25	33.33	50.00	41.67
17.08	Dodecanal	2	0	2	6.67	0.00	3.33
17.67	6,10-dimethyl-5,9-undecadien-2-one[a]	19	20	39	63.33	66.67	65.00
18.10	Pentadecane[a]	0	1	1	0.00	3.33	1.67
18.37	Tridecanal[a]	2	1	3	6.67	3.33	5.00
18.52	Dodecanoic acid, methyl ester	7	5	12	23.33	16.67	20.00
18.96	Dodecanoic acid	6	7	13	20.00	23.33	21.67
19.47	Hexadecane[a]	0	1	1	0.00	3.33	1.67
20.36	Cyclotetradecane	0	4	4	0.00	13.33	6.67
20.60	Heptadecane[a]	0	3	3	0.00	10.00	5.00
20.76	Tetradecanoic acid, methyl ester	3	1	4	10.00	3.33	6.67
22.72	7-Hexadecenoic acid, methyl ester	0	1	1	0.00	3.33	1.67
22.94	Hexadecanoic acid, methyl ester	0	3	3	0.00	10.00	5.00
23.76	Cyclohexadecane	0	1	1	0.00	3.33	1.67

[a] Identity verified by standard comparison.

of the individuals. Primarily, there are qualitative similarities in the detected compound peaks, while quantitative differences in the peaks are more readily noted.

Table 1 lists the frequency that previously reported emanated compounds are detected in the headspace of the hand odor collected from the 60 subjects, as well as the gender-specific tabulation of detected compounds. The compounds are listed in order of elution. They are either identified by matching of mass spectra or by comparison to an injected standard. Table 1 contains the percentages of subjects within each gender that had detectable levels of these compounds. Some compounds that have been reported previously to be present in human-produced emanations, such as 2-ethyl-1-hexanol [16,26,30], lilial [16], and limonene [16,27], were not listed in Table 1, because they are likely to be "tertiary odors," e.g. skin lotion, perfumes, clothing, etc. Methyl salicylate (present in less than 5% of the population) has also been disregarded, although it has been previously reported [16], as it is most likely a "secondary odor" component possibly present due to the consumption of aspirin. It may also be possible that compounds extracted in only one individual within the population are of tertiary origin. Across the 60 subjects there are six compounds present at high frequency (100–67% presence), seven at medium frequency (66–33% presence), and 50 at low frequency (32–1% presence) in the study population. Fig. 5 is a histogram of the frequency of occurrence of the VOCs present in hand odor among the population studied.

The compounds extracted can be divided into seven groups: acids, alcohols, aldehydes, hydrocarbons, esters, ketones, and nitrogen containing compounds. The six high frequency compounds for both males and females include 2-furancarboxaldehyde, 2-furanmethanol, phenol, nonanal, decanal, and hexanedioic acid-dimethyl ester. Of these compounds, nonanal and decanal were previously reported as high frequency compounds in the headspace above the forearm skin of females [29] and have also been reported previously as the most abundant straight chain aldehydes in humans. The seven medium frequency compounds across the males and the females include: propanedioic acid-dimethyl ester, 6-methyl-5-hepten-2-one, octanoic acid-methyl ester, dodecane, undecanal, 6,10-dimethyl-5,9-undecadiene-2-one, and tetradecane. Tetradecane was also previously reported as a high frequency compound present in the headspace above the forearm skin of females, however, 6-methyl-5-hepten-2-one was mentioned as a low frequency compound [29].

Fifteen of the 63 compounds extracted were aldehydes, some of which have been shown to be produced by oxidative degradation of sebaceous secretion components [26]. The unsaturated aldehyde, E-2-nonenal, was detected in 25% of the subjects studied, which included volunteers of 17–28 years. This agrees with earlier studies of the VOCs of armpit odor [8,14,15], and indicates that E-2-nonenal may not be a suitable odor marker for individuals over the age of 40, as has been previously reported [26]. The volatile organic compounds present in the headspace of collected hand odor from children have also revealed the presence of E-2-nonenal [31]. Hexanal, heptanal, and phenol were extracted among the population and have been shown to be volatile components of the blood [3,32] as well as human emanations [8,14,15,24].

It is a reasonable assumption that the fresher the scent sample is, the higher the probability will be that compounds with greater volatility are present. In aged samples, these types of compounds may have dissipated, or have been altered by microbial

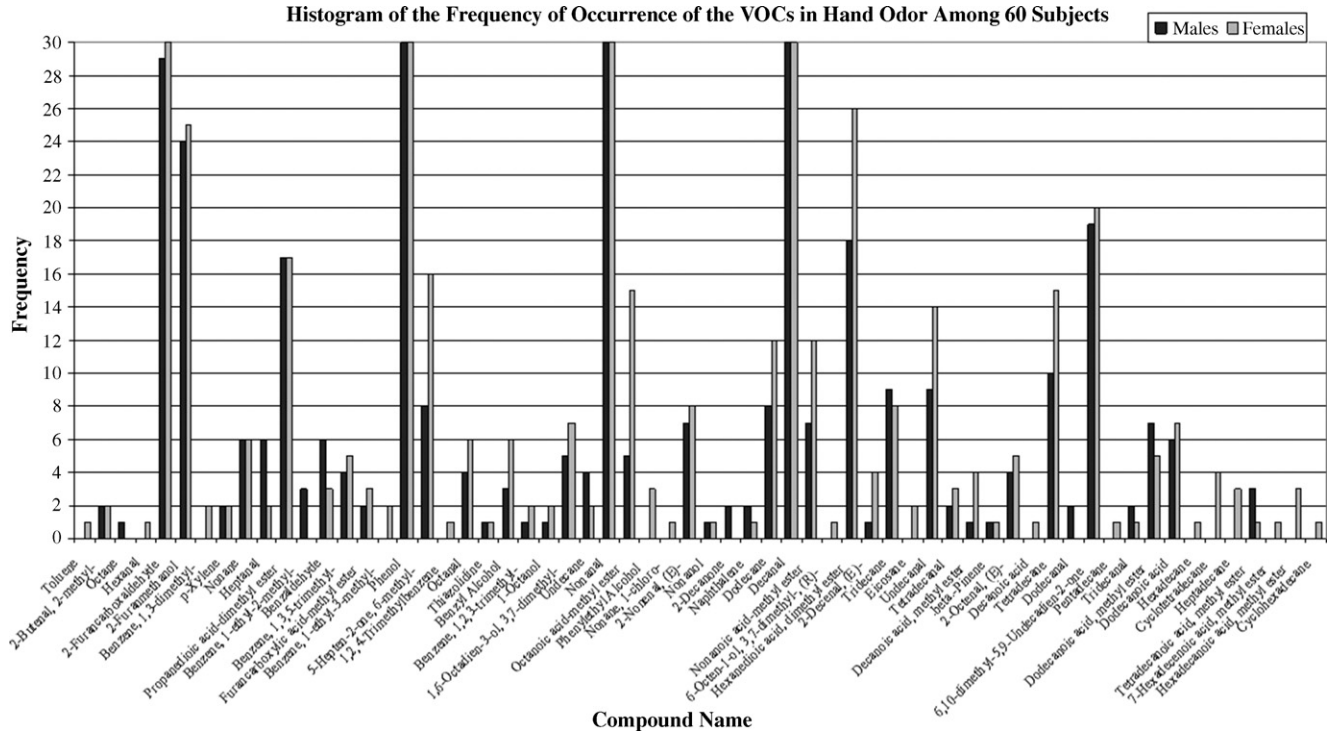

Fig. 5. Histogram of the frequency of occurrence of the human compounds among the 60 subjects.

Table 2
Approximate vapor pressures calculated for the 63 compounds extracted among the 60 individuals

Frequency (H/M/L)	Compound name	Vapor pressure (Torr)	Molecular weight (g/mol)
Acid			
L	Dodecanoic acid	8.991E−07[a]	200.32
L	Decanoic acid	1.338E−16[a]	172.27
Alcohol			
H	2-Furanmethanol	6.098E−01[a]	98.10
H	Phenol	2.199E−02[a]	94.11
L	3,7-Dimethyl-1,6-octadien-3-ol	7.303E−03[a]	154.25
L	Benzyl alcohol	6.379E−03[a]	108.14
L	Phenylethyl alcohol	6.246E−03[a]	122.17
L	1-Octanol	4.911E−03[a]	130.23
L	3,7-Dimethyl-6-octen-1-ol	3.086E−03[a]	156.00
L	Nonanol	8.088E−04[a]	144.25
Aldehyde			
L	2-Methyl-2-butenal	1.705[a]	84.12
H	2-Furancarboxaldehyde	1.870E−01[a]	96.09
L	Heptanal	1.829E−01[a]	114.19
L	Benzaldehyde	6.011E−02[a]	106.12
L	Hexanal	5.310E−02[b]	100.16
L	Octanal	4.339E−02[b]	128.21
H	Nonanal	3.330E−02[b]	142.24
H	Decanal	9.570E−03[a]	156.27
M	Dodecanal	1.792E−03[a]	184.32
L	Tetradecanal	3.445E−04[a]	212.37
L	*E*-2-decenal	Not available	126.20
L	(*E*)-2-nonenal	Not available	140.22
M	Undecanal	Not available	154.25
L	(*E*)-2-octenal	Not available	170.29
L	Tridecanal	Not available	198.35
Hydrocarbons			
L	Toluene	2.822[a]	92.14
L	Octane	1.037[a]	114.23
L	1-Chlorononane	1.037[a]	162.70
L	*p*-Xylene	6.595E−01[a]	106.17
L	Nonane	2.532E−01[a]	128.26
L	Benzene, 1-ethyl-3-methyl-	2.086E−01[a]	120.19
L	Benzene, 1-ethyl-2-methyl-	1.759E−01[a]	120.19
L	Benzene, 1,3,5-trimethyl-	1.710E−01[a]	120.19
L	Benzene, 1,2,3-trimethyl-	1.039E−01[a]	120.19
L	β-Pinene	4.328E−02[b]	136.24
L	1,2,4-Trimethylbenzene	4.011E−02[b]	120.19
L	Undecane	2.583E−02[a]	156.31
L	Naphthalene	1.739E−02[a]	128.17
M	Dodecane	7.203E−03[a]	170.34
L	Pentadecane	6.379E−03[a]	212.42
L	Tridecane	2.008E−03[a]	184.36
L	Tetradecane	5.354E−04[a]	198.39
L	Hexadecane	3.616E−05[a]	226.44
L	Heptadecane	5.123E−06[a]	240.47
L	Cyclotetradecane	Not available	196.00
L	1,3-dimethyl-benzene	Not available	106.17
L	Eicosane	Not available	224.43
L	Cyclohexadecane	Not available	282.55
Ketones			
M	6-methyl-5-hepten-2-one	Not available	126.20
M	6,10-dimethyl-5,9-undecadien-2-one	Not available	156.27
L	2-Decanone	Not available	194.32
Esters			
M	Propanedioic acid, dimethyl ester	3.254E−02[a]	132.00
M	Octanoic acid, methyl ester	2.427E−02[a]	158.24
L	Decanoic acid, methyl ester	3.055E−03[a]	186.29
H	Hexanedioic acid, dimethyl ester	1.506E−03[a]	174.00

Table 2 (*Continued*)

Frequency (H/M/L)	Compound name	Vapor pressure (Torr)	Molecular weight (g/mol)
L	Dodecanoic acid, methyl ester	5.466E−04[a]	214.35
L	Tetradecanoic acid, methyl ester	1.304E−04[a]	242.40
L	Hexadecanoic acid, methyl ester	2.598E−05[a]	270.45
L	Furancarboxylic acid, methyl ester	Not available	126.00
L	Nonanoic acid, methyl ester	Not available	172.27
L	7-hexadecenoic acid, methyl ester	Not available	268.00
Amines/amides			
L	Pyridine	2.051[a]	79.10
L	Thiazolidine	Not available	89.16

[*] *Note*: (a) vapor pressures calculated using the Antoine Equation from Knovel Critical Tables, (b) extrapolated from data found in the Handbook of Chemistry & Physics (www.hbcpnetbase.com).

action, e.g. the formation of methyl esters from carboxylic acids. Loss by dissipation (evaporation) has been supported anecdotally from the behavior of bloodhounds when following a scent trail. A fresh trail is followed with the head in an upright position suggesting that more volatile compounds are being utilized whereas an old trail is followed with the nose to the ground suggesting that less volatile compounds are being utilized. The ability of human scent line-up canines to match humans based on scents after having been collected and stored in a glass jar for more than seven years to freshly collected samples suggests that a steady state is created within the container that limits evaporation of the volatile odor components [34]. The samples collected for this study were also stored in a sealed glass container and the high and medium frequency compounds extracted among the studied population have vapor pressures that fall in the semi-volatile range, unlike the low frequency compounds, as can be seen in Table 2. Canines have the demonstrated the ability to smell trinitrotoluene (TNT) (v.p. = 3.0×10^{-6} Torr) and TNT can also be readily extracted by headspace SPME [33]. Therefore, it is reasonable to assume that all 45 compounds listed with vapor pressures in the 10^{-6} Torr or greater range can also be detected by canines. It is possible that that other substances which have a relatively low volatility or are present in low concentrations may also contribute to human odor yet are not readily extracted by this method, and inaccuracy in abundances may be present as a consequence of the long SPME fiber exposure times in the vial headspace leading to compounds with low vapor pressures appearing in higher quantities.

The compounds detected in the headspace of hand odor samples were produced from sebaceous and eccrine secretions, without the influence of the apocrine glands, as seen in armpit odor. The ability of canines to distinguish the odors of humans over long periods of time [34] suggests that human scent is stable over time, or that portions of an individual's odor profile are stable even though elements of the odor may change. Alterations to portions of the odor of an individual may occur due to the influence of illness, the onset of puberty, the menstrual cycle in females, etc. Many of these factors directly affect the apocrine gland. The secretions obtained from the eccrine and sebaceous glands are less likely to be influenced by these changes, thereby more likely to produce the stable odor of an individual.

It is uncertain whether scent identity is distinguishable merely by ratios of the common compounds between individuals, the presence or absence of compounds which vary significantly between individuals, or if it is a combination of the two factors. It was previously shown that VOCs from collected armpit samples could be distinguished for individuals based on relative peak area ratios of common compounds between multiple samplings of individuals, and that greater variability in scent profiles among individuals can be achieved when the human compounds that differ between individuals are also considered [14,35].

Human scent profiles contain a varying number of compounds depending, among other factors, on the subject being analyzed. Due to the fact that several variables are being measured within each person and among populations, these analyses yield multivariate data. Multivariate data can be used for differentiation between samples where each is characterized by a set of measurements. In this case, the samples are individual's scent profile that is characterized by a set of volatile compounds. Correlation tests are used to determine relationships between two or more variables. Many correlation determinations require an assumption that the variables have normal distributions, since that assumption cannot be made in the case of a component of a scent profile nonparametric methods of correlation are required. One of the most common multivariate, nonparametric methods of measuring correlation is the Spearman rank correlation coefficient. In a nonparametric correlation, an integer value is assigned to each variable measured, which is determined by its rank, or size, among the other measurements in the array.

Spearman correlation coefficient comparisons were conducted utilizing all of the human odor components detected in the headspace of the hand odor samples. The comparison of 60 subjects generates 1770 possible pairs. As can be seen from Table 3, when considering a correlation threshold of 0.9 the individuals were distinguished in 99.66% of the cases, when considering a correlation threshold of 0.8 the individuals were distinguished in 94.24% of the cases, and for a correlation threshold of 0.7, the individuals that were distinguished drops to 83.28%. These results are in agreement with studies conducted on armpit odor as a mechanism to distinguish profiles among individuals [14,35]. The variation revealed among the sample population demonstrates the importance of determining human odor baseline on an individual basis when using human odor as a diagnostic tool in addition to supporting the individual odor theory set forth by canine research.

Table 3
Summary of Spearman correlation coefficient comparisons across the population

Subject	Identified	Confused with at correlation threshold	
	(Y/N)	>0.9	>0.8
M1	Y	0	0
M2	Y	0	0
M3	Y	0	0
M4	Y	0	0
M5	Y	0	0
M6	Y	0	F15
M7	Y	0	F6
M8	Y	0	M25
M9	Y	0	0
M10	Y	0	M13
M11	Y	0	0
M12	Y	0	0
M13	Y	0	M23, M14, F26, F17, M15, M21, F20, M17, F27, M10
M14	Y	0	M13, M23, F17, F27, F26, M15
M15	Y	F17	F17, F20, M13, M23, M25, M14, F27
M16	Y	0	0
M17	Y	0	M23, M21, F26, M13, M25, F20, F17
M18	Y	0	F29, F25
M19	Y	0	F18, F21
M20	Y	0	0
M21	Y	0	M13, M17, M23, F26
M22	Y	0	0
M23	Y	F17	F17, M13, M14, M17, M15, F26, F27, M21, M25
M24	Y	0	0
M25	Y	0	F20, F17, M15, M17, M8, M23
M26	Y	0	0
M27	Y	0	0
M28	Y	0	0
M29	Y	0	0
M30	Y	0	0
Male errors		2	57

Subject	Identified	Confused with at correlation threshold	
	(Y/N)	>0.9	>0.8
F1	Y	0	0
F2	Y	0	0
F3	Y	0	0
F4	Y	0	0
F5	Y	0	0
F6	Y	0	M7
F7	Y	0	0
F8	Y	0	0
F9	Y	0	0
F10	Y	0	0
F11	Y	0	0
F12	Y	0	0
F13	Y	0	0
F14	Y	0	0
F15	Y	0	M6
F16	Y	0	0
F17	Y	M15, M23	M15, M23, F20, M13, F19, M14, M25, M17
F18	Y	0	F21, F23, M19, F25
F19	Y	0	F20, F17
F20	Y	0	F17, M15, F19, M25, M13, M17
F21	Y	F25	F25, F23, F18, M19
F22	Y	0	0
F23	Y	0	F21, F25, F18
F24	Y	0	0
F25	Y	F21	F21, F23, M18, F29, F18
F26	Y	0	M13, M23, M17, M14, M21
F27	Y	0	M23, M14, M13, M15
F28	Y	0	0
F29	Y	0	M18, F25
F30	Y	0	0
Female errors		4	45

Table 4
Comparison of compounds extracted from a single Male (13) to those present in ten subjects that are not distinguishable at the 0.8 correlation level

	Compound Name	M13	M23	M14	F26	F17	M15	M21	F20	M17	F27	M10
	2-Butenal, 2-methyl-, (E)-								X			
	Octane											X
	2-Furancarboxaldehyde	X	X	X	X	X	X	X	X	X	X	X
	2-Furanmethanol	X	X	X	X	X	X	X	X	X	X	X
	Nonane	X										X
	Propanedioic acid-dimethyl ester	X	X	X		X	X	X	X	X		X
	Benzaldehyde				X							
	Phenol	X	X	X	X	X	X	X	X	X	X	X
	5-Hepten-2-one, 6-methyl-										X	
	Thiazolidine									X		
	Benzyl Alcohol				X					X		
	Nonanal	X	X	X	X	X	X	X	X	X	X	X
	Octanoic acid-methyl ester			X			X					
	Phenylethyl Alcohol				X							
	Dodecane							X			X	
	Decanal	X	X	X	X	X	X	X	X	X	X	X
	Nonanoic acid-methyl ester		X					X	X			
	Hexanedioic acid, dimethyl ester		X			X	X	X	X	X		
	Tridecane	X										
	Tetradecane					X					X	
	6,10-dimethyl-5,9-Undecadien-2-one											X

Table 5
Compounds extracted from Female 17 and the eight subjects that are not distinguishable at the 0.8 correlation level

	Compound Name	F 17	M 15	M 23	F 20	M 13	F 19	M 14	M 25	M 17
	2-Butenal, 2-methyl-, (E)-				X					
	2-Furancarboxaldehyde	X	X	X	X	X	X	X	X	X
	2-Furanmethanol	X	X	X	X	X	X	X	X	X
	Nonane					X				
	Propanedioic acid-dimethyl ester	X	X	X	X	X	X	X		X
	Phenol	X	X	X	X	X	X	X	X	X
	Thiazolidine									X
	Benzyl Alcohol									X
	1,6-Octadien-3-ol, 3,7-dimethyl-								X	
	Nonanal	X	X	X	X	X	X	X	X	X
	Octanoic acid-methyl ester		X				X	X		
	Decanal	X	X	X	X	X	X	X	X	X
	Nonanoic acid-methyl ester			X	X					
	Hexanedioic acid, dimethyl ester	X	X	X	X		X			X
	Tridecane					X				
	Undecanal						X			
	Tetradecane	X								
	6,10-dimethyl-5,9-Undecadien-2-one								X	
	Dodecanoic acid, methyl ester								X	
	Dodecanoic acid								X	

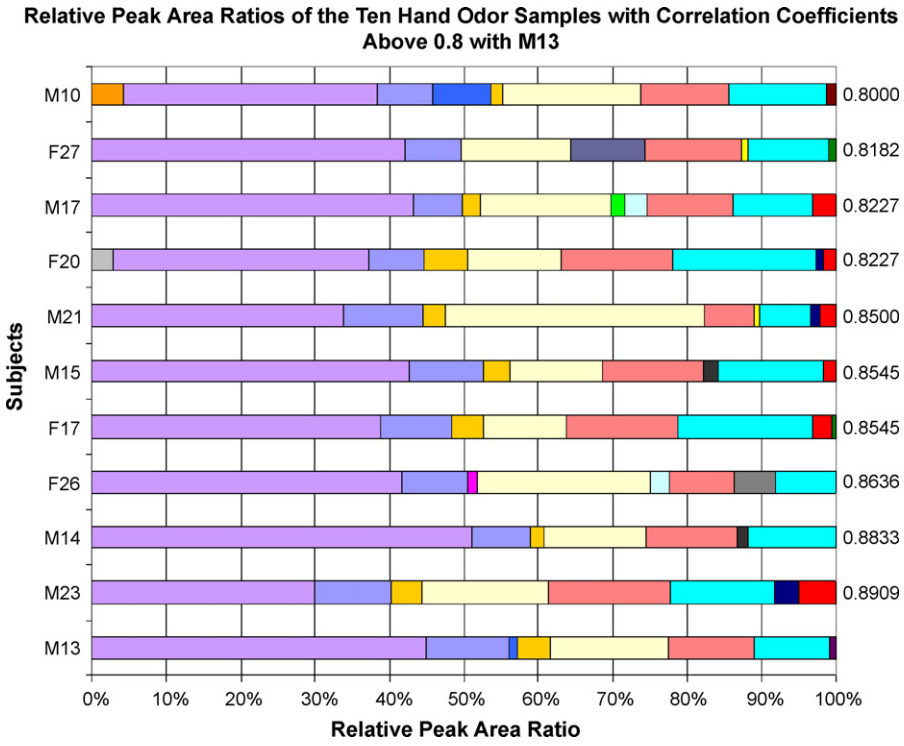

Fig. 6. Color odor chart of the relative peak area ratios of the human compounds extracted from M13 and the ten subjects that are not distinguishable at the 0.8 correlation level.

The male and female with the highest correlation to other individuals in the study are Male 13 and Female 17. As can be seen from Table 3, Male 13 correlates to ten subjects above 0.8 and Female 17 correlates to eight. Tables 4 and 5 display the compounds extracted between Male 13 and Female 17, respectively, as well as other subjects which closely correlated. Figs. 6 and 7 demonstrate in semi-quantitative fashion the relative ratios of the peak areas of the human compounds extracted in the headspace above the collected hand odor samples for Male 13 and Female 17, respectively, as displayed with the subjects to whom they were confused and the corresponding correlation coefficients. The colors which correspond to the compounds are also noted in Tables 4 and 5. The presence of different compounds for individuals with correlations above the 0.8 level is easily seen. Through the use of the color coded scent charts, differences in the ratios between subjects are evident even for the high frequency compounds that are the major components of the scent profiles of individuals.

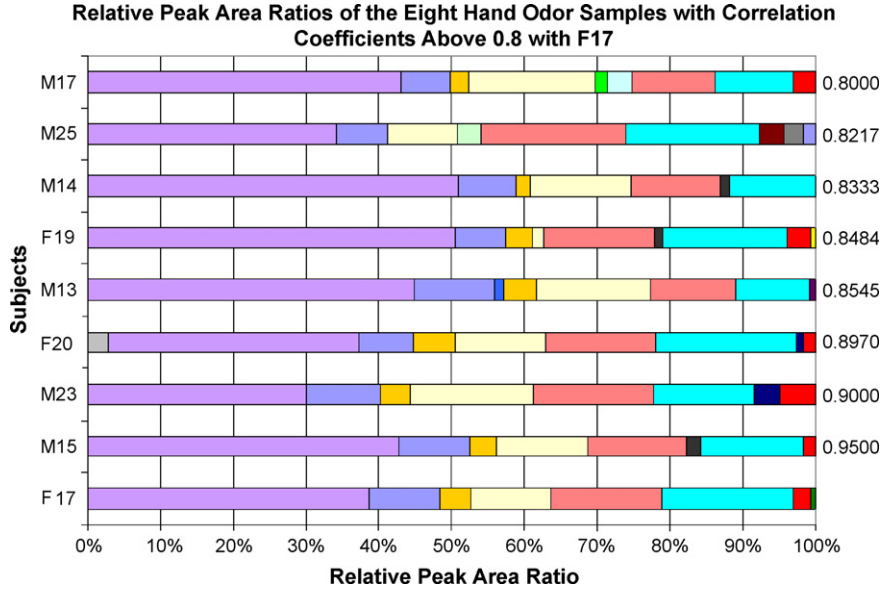

Fig. 7. Color odor chart of the relative peak area ratios of the human compounds extracted from F17 and the eight subjects that are not distinguishable at the 0.8 correlation level.

4. Conclusions

A headspace SPME method has been developed that uses DVB/CAR on PDMS fibers to extract hand odor samples which have been aged for 24 h, for subsequent extraction and identification of the compounds. The optimal exposure time of 21 h for this SPME extraction at room temperature and analysis by GC/MS provided qualitative and semi-quantitative information about the VOCs that comprise human hand odors. The compounds identified can be classified into seven groups: acids, alcohols, aldehydes, hydrocarbons, esters, ketones, and nitrogen containing compounds. For the 60 volunteers involved in this study, 63 human-produced compounds were identified with a high degree of variability, six of these were high frequency compounds, seven medium frequency compounds, and fifty low frequency compounds. Spearman rank correlation coefficient comparisons of human scent compounds among individuals was shown to be a viable method to distinguish individuals based on the VOCs present in collected human odor samples. Comparison of the closely correlated individuals revealed differences between the subjects. Further, refinement of this technique may allow for an improved means of individuals based on their hand-scent profile.

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