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An investigation of volatile organic compounds from the saliva of healthy individuals using Headspace -Trap/GC-MS

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Abstract:

Analysis of VOCs from saliva, to aid disease diagnoses, has received little attention. This work aims to address the paucity of information on saliva volatiles and includes a database of newly identified compounds whilst incorporating a review of the literature. The volatile constituents in the headspace of whole saliva obtained from 10 healthy individuals were examined in a longitudinal study over a period of 10 days using Headspace-Trap Gas Chromatography-Mass Spectrometry (HS-Trap-GC-MS). A total of 317 compounds (268 identified) were found and compared across subjects and between samples with 34 VOCs being present in all 100 samples. The average number of compounds per subject ranged from 121 to 150 over a 10 day period and was fairly consistent for a particular individual (s.d. ranged from 3-15). However, the number of compounds present in an individual on all 10 days was substantially lower ranging between 65 and 109, showing that there is significant daily variation in salivary composition. A core group of ubiquitous VOCs were found with the remaining VOCs showing significant intra and inter individual variability. Saliva VOCs have been found to be readily analysed using the HS-trap technique and studies on the VOCs from healthy individuals should be of utility for comparison with VOCs analysed from samples from diseased groups.

1. Introduction

The first recorded use of the smell of saliva was undertaken by the ancient Chinese for tuberculosis diagnoses, spitting onto a hot stone was purported to produce a diagnostic tarry smell. Saliva is a readily available, exocrine fluid originating in the oral cavity. A typical human can produce 500-1500 ml day^{-1} at a rate of about 0.5 ml $minute^{-1}$ (Chiappin *et al* 2007, Pfaffe *et al* 2011).

Whole saliva includes a number of VOCs which could be derived from serum, blood, gingival exudates, skin lipids, microorganisms, commercial products, medicines, environmental pollution, and food debris (Kaufman and Lamster 2002a, Kaufman and Lamster 2002b, Kostelc *et al* 1981, Martin *et al* 2012), the oropharynx, nasal cavity, upper airway and from gastrointestinal reflux (Lima *et al* 2010, Pfaffe *et al* 2011, Soini *et al* 2010, Kusano *et al* 2011, Kaufman and Lamster 2002a, Alagendran *et al* 2010).

Over 300 bacterial species were reported to be present in the oral cavity (Soini *et al* 2010). Microbial activity in the mouth produce putrefactive compounds such as aliphatic amines, branched chain fatty acids, indole, phenol and volatile sulphur containing compounds (VSC) (Imamura 1982, Yang 2002, Calil *et al* 2008, Calil and Marcondes 2006, Kostelc *et al* 1981, Tonzetich & Carpenter 1971, Kakoei *et al* 2012). Stress and anxiety may induce changes in the oral microbiota which increases oral volatiles, especially the VSCs (Calil and Marcondes 2006).

Passive diffusion is the most common exchange methodology between blood and saliva (Kaufman and Lamster 2002b, Pfaffe *et al* 2011, Kidwell *et al* 1998, Chiappin *et al* 2007), although other methods such as ultrafiltration and active diffusion are also present. Saliva therefore reflects the biochemical and metabolic information in blood which makes saliva

analysis a potential tool for the study of physiological and pathological conditions. Nagler et *al* (2002) found a correlation between compounds in blood and saliva. Saliva has a lower concentration of constituents than blood due to the diurnal/circadian variations of certain compounds (Kumar *et al* 2009, Lima *et al* 2010). Lipophilic and neutral molecules diffused more easily into saliva than lipophobic, hydrophilic and ionised molecules (Lochner *et al* 1986).

VOCs in breath dissolve in saliva influencing its composition (Soini *et al* 2010). Humans are exposed to organic chemicals via inhalation, consumption, ingestion of contaminated food and transdermal absorption (Soini *et al* 2010, Sanchez *et al* 2012). Environmental exposure to chemicals that originate from commercial products such as; toothpastes, mouth washes, soaps, shampoos, cosmetics, medicines, plastics, paints, and pesticide formulations have been reported in the literature (Kumar *et al* 2009, Soini *et al* 2010) and lead to some of these chemicals being identified in saliva (Kostelc *et al* 1981). Also the exposure to air pollutants especially compounds derived from engine exhaust gas such as gasoline and diesel (Sheng *et al* 2006and Scultz *et al* 1999).

Saliva analysis offers an alternative solution to invasive, time-consuming, complicated, and expensive diagnostic methods using specimens such as blood, serum, urine and faeces. Saliva sampling requires minimal training and cost and no specialist equipment is required. The collection process is free of stress, discomfort and is more acceptable to patient than giving blood, faeces or urine samples. Saliva specimens can be collected without external stimulation by spitting in a vial or by passive drooling with no oral movements. (Martin *et al* 2012, Chiappin *et al* 2007).

Hydration, olfactory stimulation, exposure to light, body positioning, seasonal and diurnal factors can affect the quantity and composition of un-stimulated saliva (Martin *et al* 2012, Chippin *et al* 2007). Sample collection, storage, preparation and the degree of saliva stimulation can influence the observed composition (Gröschl and Rauh 2006; Schipper *et al* 2007, *Soini et al* 2010, Chiappin *et al* 2007). Volatiles can evaporate or become adsorbed/absorbed onto the surfaces of the materials during collection and storage, many VOCs degrade because of microbial activity after collection (Martin *et al* 2012). Higher bacterial loadings occur from spitting hence the passive drool approach is commonly adopted to minimize such effects (Martin *et al* 2010, Chippin *et al* 2007). Inhibitors and/or denaturing agents such as sodium azide can be used to stop bacterial growth, degradation to the molecules and any enzyme activity (Schipper *et al* 2007, Chiappin *et al* 2007, Martin *et al* 2012).

Several methodologies and techniques have been employed to study the volatile organic compounds in saliva. Larsson (1965) directly injected saliva headspace and breath samples into a GC/FID. They reported the presence of four compounds, tentatively assigned as acetone, acetaldehyde, ethanol and methanol and assessed the influence of food, alcohol, oral hygiene and smoking on their concentrations. Locher *et al* (1986) investigated saliva as a vehicle for the non-invasive detection of diabetes and liver disease, producing individual metabolic volatile profiles using solvent extraction/derivitisation combined with GC-MS. They compared saliva volatiles collected 12 hours apart. They identified 26 compounds and found significant differences in the metabolic profiles from diabetic and non diabetic individuals. Their study of a liver disorder group failed to produce any definitive differences. Alagendran *et al* (2010) used solvent extraction/derivatisation with GC-MS to identify salivary volatiles during the menstrual cycle to

assess ovulation and reproductive phases in women. They identified that 2-nonenal, acetic acid, acetaldehyde and dodecanol are ovulatory specific whereas butanoic acid, 2-propenyl ester, skatole and 3-hexanol were present in follicular and luteal phases.

Stir-bar extraction followed by GC-MS was used by Penn and Soini (Penn *et al*, 2007 and Soini, *et al* 2010) to investigate the volatile composition of sweat, skin and saliva from a large group of 197 individuals. They identified 88 volatile compounds in saliva. Half of the compounds were common to skin, sweat and saliva. Individual variability among the salivary VOC profiles was attributed to variation in diet, environment and genetics.

Dynamic headspace extraction using a Tenax trap combined with GC-MS (Kostelc *et al* 1981) was used to study headspace exogenous volatiles in stimulated saliva collected from 17 subjects (3 females and 14 males). They found 29 compounds in these subjects, attributing the majority to sources such as urban air, drinking water, diet and cosmetic preparations.

Several papers investigated sampling, extraction and enrichment techniques for use with saliva samples. Solid phase microextraction (Kumar *et al* 2009, Kusano *et al* 2011, Wang and Ming-Yen 2009, Buszewski *et al* 2007), headspace single-drop microextraction (SDME) (Jermak *et al* 2006) and a polydimethylsiloxane coupon (placed in the oral cavity), (Martin *et al* 2012) have been used in the study of saliva.

A small study (Sanchez *et al* 2011) coupled a headspace sampler (HS) directly with GC-MS for determination of biomarkers in 28 unstimulated saliva samples: 24 from healthy volunteers, and 4 from patients with different types of disease (including lung, colon and stomach cancer). Methyl tert-butyl ether and styrene were considered as biomarkers of exposure to environmental

pollutants and dimethyl disulfide, limonene and 2-ethyl-1-hexanol were considered to be biomarkers for diseases.

In the present study, the headspace-trap (HS-trap) technique in conjunction with GC–MS has been applied for the first time for determination of VOCs in human saliva. The HS-Trap is an enhanced static HS system that traps, focuses and pre-concentrates volatiles prior to gas chromatography without sample preparation. It has sensitivity and detection limits similar to the conventional purge and trap technique (Barani *et al* 2006). This method has been applied for trace analysis of volatile organic compounds in environmental samples, pharmaceuticals, soil, food products, and water (Barani *et al* 2006, Schulz *et al* 2007, Røen *et al* 2010, Meng *et al* 2009).

This pilot study aimed firstly to investigate the salivary headspace volatiles of normal healthy individuals to establish a "normal volatile profile", and provide a basis for the future use of salivary VOCs as a diagnostic medium for diseases. Secondly, this work aimed to assess the daily variation of the VOCs in healthy individuals over a 10 day period.

2. Materials and Methods

2.1. Participants:

Ethical approval for the study was granted by the university research ethics committee. Informed and written consent was obtained from all participants. Ten participants in good systemic health aged from 22-41 and of mixed gender (7 males and 3 females) took part in the study. They kept a record of their dietary intake over the 10 day sampling period. Participants did not have any dietary restrictions imposed. Subjects were requested to fast overnight (The cut off time for

overnight fasting was 8pm) and they were requested to only drink water prior to sampling. Alcohol consumption was not permitted during the period of the study. In addition, participants had to be free of fever and/or cold. Participants taking medication, smokers and ex-smokers were excluded from the study. The participants were asked not to undertake strenuous physical activity prior to sampling. This was only limited prior to the sample collection.

2.2. Sample Collection

The sample collection technique, handling, and storage were carefully designed to maximize measurement and validity of salivary volatiles and minimize the influence of extraneous factors on the salivary profile. A non-stimulated collection approach similar to (Martin *et al* 2012 & Mohamed *et al* 2012) was chosen in order to minimise the introduction of any contaminants to the samples. The sampling protocol was standardised to ensure consistency of results between samples and subjects. All saliva samples were collected between 9 – 11 am. They were allowed to brush their teeth as usual in the morning but were requested not to use mouthwash. Prior to sampling, participants were asked to rinse their mouths at least 20 times using 750 ml of still bottled water (Love One, Global Ethics Ltd. bottled at source at Powys/Wales by Radnor Hills Mineral Water Company Ltd. Quoted mineral composition: calcium 44mg/l, potassium 2mg/l, sodium 37 mg/l, sulphate 10.9 mg/l, magnesium 6.8 mg/l, nitrate 15 mg/l, chloride 13 mg/l, fluoride 0.09 mg/l and the total dissolved solids were 214). The volatile composition of the water was also tested to eliminate any influence on the volatiles in the mouth prior to sampling.

After the water rinse they waited 10 minutes before commencing collection to avoid sample dilution. The participants were instructed to sit in an upright position, to tilt their head forward to allow saliva to flow from their mouth into glass headspace vials (Perkin Elmer 22 ml vials with

aluminum crimped caps and PTFE lined silicone septa). They were asked to weigh 2 gm of their own saliva using an analytical balance. The samples of saliva per person which had accumulated in the vial weighed 2gm ± 0.1. The vials were then immediately crimped and without any processing stored in a freezer at -20 °C until analysis. Saliva specimens were analysed within 24 h of collection. All samples were collected in the same room at the same time of day. Samples of indoor lab air were conducted during the study to determine the lab air volatiles to eliminate any interference from the surrounding environment. For method validation 4g aliquots of saliva were portioned into two vials one was run immediately fresh and the other was frozen for 24 hours, subsequently defrosted at 20^{o}C for 20 minutes and then run using the same method as the fresh samples. Analysis showed that there was minimal difference between the two chromatograms. The same compounds were identified in both samples and the majority of the peaks present were at a similar level. The differences between fresh and frozen samples were in line with differences in the chromatograms observed when 4g samples of saliva were portioned into two vials and both run fresh or frozen.

2.3. Volatile sampling using HS-Trap GC-MS

A relatively new type of headspace sampler, the Turbomatrix HS40 Trap (Perkin Elmer, Wellesley, MA), which combines headspace sampling with a trap was utilised in this study. This was interfaced to a Clarus 600 GCMS system (Perkin Elmer). The HS40 Trap instrument has a good LOD similar to purge and trap methods, good repeatability similar to static headspace techniques and enables water to be removed prior to the chromatographic stage. A good overview of the method is described in Barani *et al.* (Barani et al 2006). The HS 40 Trap works

with the pulsed-pressure approach, combining a slight modification of the balanced pressure principle with the use of an on-board cold and packed trap to extend its detection limits. The following steps are undertaken during the analysis:

Thermal equilibriation of the sample

The sample vials were heated in the HS trap to 80 °C for 20 minutes to ensure that the vapour reached chemical equilibrium.

Pressurization step

The needle pierces the septum, and carrier gas (helium at 31psi) is used to pressurize the vial to a user defined value. Simultaneously, a valve isolates the GC column, minimising column pressure change. The column isolation flow is manually set by the operator 10mL/min higher than the column flow.

Trap load stage

The headspace of the vial is sent to the cold packed trap, allowing a flow of headspace through the trap. There is the option to repeat this trap load step upto four times for each vial.

Trap dry purge

The cold packed trap is purged with helium to eliminate water.

Trap desorb and trap hold

The trap temperature is increased to the set level at a rate of 400oC/min to release the trapped analytes. It is the kept at that value for a specified time to clean the trap. Once the trap is heated the isolation of the column from the carrier gas flow is ceased and the GC run starts. THe volatile are introduced into the GC via a fused silica transfer line maintained at 120°C.

Extensive method development was undertaken in order to determine the correct instrument parameters to optimize the analysis of saliva and other biological fluids. Table 1. gives a summary of the instrumentation and the various instrument parameters used during the salivary analysis.

Table 1. Experimental conditions and instrument parameters.

Parameter	Setting
	Headspace Trap Conditions
Sample Introduction	PerkinElmer TurboMatrix HS 40 Trap
Thermostatting	80 °C for 20 min
Needle	100 °C
Transfer Line	120 °C, 2 m × 320 µm
Carrier gas	Helium at 31 psi
Dry Purge	Helium, duration 15 min
Trap	Air toxic trap (supplied with HS40-Trap instrument), Trap low temp: 40 °C, Trap high temp:320 °C, Trap hold: 10 min
Extraction cycle	1 cycle at 45 psi
	Chromatography Conditions
GC	PerkinElmer Clarus 600 GC
Column	Zebron Capillary ZB-624, Dimensions: 60 m length, 0.25 mm I.D., 1.4 µm film thickness
Oven	35 °C for 10mins, then 8 °Cmin^{-1} to 200 °C no hold, 15 °Cmin-1 to 220°C for 12 min
Injector	Split Splitless (PSS), Split control/OFF, Flow mode
	Mass Spectrometry Conditions
MS	PerkinElmer Clarus 600 MS
Mass range	33-300 Dalton
Scan Time	0.25 s
Interscan Delay	0.08 s
Inlet Line	220 °C
Source Temperature	180 °C
Multiplier	400
	Software
Software	TurboMass 5.4.2
Search Library and Database	Compounds were identified by comparing their mass spectra with those contained in the NIST 2008 version, peak was searched manually (baseline subtraction and averaging over the peak).Forward and reverse match quality of at least 800/1000 was used as a lower match threshold, otherwise the compound was labeled unknown. Identity of x compounds were confirmed using standards.

3. **Results**

The volatile constituents in the headspace of whole saliva obtained from 10 individuals were examined in a longitudinal study over a period of 10 days. A total of 268 identified compounds (plus 49 unassigned peaks) were found and compared across subjects and between samples. (See Table 2 for more details). 34 Compounds were found to be ubiquitous to all 100 samples.

The classes of VOCs found in saliva and their percentage compositions were; 26 aldehydes (8%, 3 ubiquitous), 22 alcohols (7%, 6 ubiquitous), 117 hydrocarbons (38%, 13 ubiquitous), 12 esters (4 %), 3 ethers (1 %), 40 ketones (13%, 5 ubiquitous), 30 aromatic compounds (9%, 4 ubiquitous), 9 Volatile Sulfur Compounds (VSCs) (3%), 5 Nitrogen containing compounds (2%, 2 ubiquitous), 1 acid (0.3 %), 3 others (1 %), and 49 unknowns (15%, 1 ubiquitous). Hydrocarbons were the largest group with 117 compounds which included 46 terpenes, 12 cycloalkenes, 17 branched alkenes, 18 straight chain alkenes, 10 cyclic alkanes, 6 branched chain alkanes, 8 straight chain alkanes.

The mean total number of VOCs identified, per subject, was 192 (SD 13, range 171 to 211) see Table 3 for details. The mean number of VOCs identified per person per day as illustrated in Table 3, was 137 (SD 3.6) (range 121 to 150). The number of compounds identified for each individual per day was quite consistent illustrated by the relatively small standard deviation for most subjects (range 3-15). However, if you consider the compounds that are consistently present in each individual over the 10 day period then the mean is much lower 87 (SD16) (range 65-109). This shows that despite the relatively consistent numbers of compounds per individual per day, the types of volatiles identified are subject to daily variation.

The inter subject and the overall variability in the abundance (peak area) of ubiquitous compounds is shown in Table (4), with selected compounds also included in Figure 1. There is

not an overall trend in the abundances of the 34 ubiquitous compounds although analysis of figure 1 and table 4 show that changes in the abundance of certain compounds seem to be closely linked for certain individuals. Some of these are discussed specifically below. There are 3 predominant behaviours observed (see Table 4). Firstly a pattern of low to moderate variability where all subjects are broadly overlapped. Compounds in this group have relatively low individual and overall variation in measured peak areas. Secondly, a similar pattern but where 1 or more individuals exhibit a peak on a certain day (or raised response over a number of days returning gradually to the baseline). Compounds in this group have high variability for certain individuals contributing to a high overall variability. Thirdly where one or more subjects have a high baseline level of a certain compound over the 10 day period, but where the variability is comparable to the other subjects. This group is characterized by relatively low individual variability but a high overall variability.

2-methyl-1-butanol (figure 1a) shows limited variation between individuals and limited overall variation showing that the salivary profiles of the subjects over a 10 day period are broadly overlapped.

Inter subject variability of the peak area for styrene (Figure 1b), o-xylene and 2-heptanone were low, except for subject 2 on day 6 where there was a large peak. In the case of isopropyl alcohol (Figure 1c) and acetone (Figure 1d) most subjects exhibited a low variability; however subject 5 exhibited a peak on day 2, which returned to the "baseline" value by day 6. For the case of ethanol a number of the subjects exhibit peaks on different days (see Figure 1e), with subject 6 giving a large peak on day 6.

It can be seen that subject 4 has higher baseline levels of limonene (Figure 1f) and 1-propanol (Figure 1g). In addition the following compounds were observed to follow this same general trend for subject 4, p-cymene, dehydro-p-cymene, alpha-pinene, 3-(2-propenyl)-cyclohexene, 1-butanol, 1-propanol and p-xylene. However table 4 shows that this subject doesn't have significantly higher variability when compared to the other subjects. In fact in the case of p-cymene, dehydro-p-cymene and limonene, subject 9 exhibits a more significant individual variation compared to the other subjects.

Octane (Figure 1h) was unusual as 7 of the 10 individuals exhibit a relatively high individual variation.

4. **Discussion**

Table 2 lists all the VOCs found in this study with references to previous studies where the same volatiles have been identified previously.

A total of 147 salivary VOCs have been reported in the literature, of which 35 VOCs were common with this work. Classes of compounds in the literature and their percentage composition were; 12 aldehydes (8 %), 15 alcohols (10 %), 30 hydrocarbons (20 %), 20 esters (14 %), 4 ethers (3 %), 19 ketone and lactones (13 %), 10 aromatics compounds (7 %), 3 VSCs (2 %), 27 acids (18 %) and 7 Nitrogen containing compounds including an amine and 5 amides (5%) . Only 9 of the 34 ubiquitous compounds from this study were identified in the combined literature studies. These were ethyl alcohol, isopropyl alcohol, indole, phenol, styrene, dodecanal, acetone, 2-heptanone and limonene.

Methodologies examining VOCs in the literature showed distinctly different selectivity for specific compounds and different volatile compositions, when compared with that of the HS-Trap-GC-MS described here. There was the use of different sample preparation techniques such as solvents extraction, derivatisation (Locher *et al* 1986, Alagendran *et al* 2010). The methodologies reported in the literature also utilised volatile extraction steps using sorbent traps such as tenax (Kostelc *et al* 1981) and stir-bar sorptive extraction (Penn *et al* 2007 and Soini *et al* 2010). The methods also involved the use of different analytical equipment and different analytical methods, such as column type, column conditions, detector type etc. All this might explain the different proportions of VOCs identified. As mentioned some of the methods such as solvent extraction were able to identify semi-volatile and even relatively non-volatile compounds which this method would not have identified. The HS-Trap-GC-MS method involved no sample preparation steps and was found to detect a wide array of volatile compounds especially hydrocarbons. This method was not as selective to polar compounds such as carboxylic acids, amines and amides.

The earliest report on the analysis of saliva was the injection of headspace vapour into a GC/FID (Larsson 1965) using a glass capillary column. They only detected 4 compounds a ketone, 2 alcohols and an aldehyde. Using a dynamic headspace extraction method utilising a Tenax trap coupled with GC-MS (Kostelc *et al* 1981) identified 29 VOCs including 2 ketones, 5 hydrocarbons/terpenes, 6 alcohols, an aldehyde, 3 esters and 3 VSCs, 8 aromatic compounds and an amine. Using solvent extraction/derivitization combined with GC-MS (Locher *et al* 1986) identified 26 compounds including 15 straight and branched chain, saturated and unsaturated acids, 7 long chain alkane hydrocarbons, 3 fatty alcohols and 1 aromatic compound. Alagendran

et al (2010) used solvent extraction/derivatisation with GC-MS and identified 15 VOCs, 4 carboxylic acids, 2 branched alkanes, 2 long chain alcohols, 2 aldehydes, 3 aromatic compounds an ester and an amine. Stir-bar extraction followed by GC-MS identified. 88 VOCs (Penn *et al* 2007 and Soini *et al* 2010), including 15 ketones, 15 carboxylic acids, 19 hydrocarbons including terpenes, 3 alcohol, 10 aldehydes, 4 ethers, 17 esters, 2 aromatic and 3 amides. This method exhibited higher selectivity to acids and esters. Sanchez *et al,* (2011) report a HS GC-MS method for saliva analysis although they did not analyse whole saliva samples. It is expected that fewer VOCs would be detected using this technique due to the absence of the trapping, focusing and concentration steps.

117 hydrocarbons identified by the HS-Trap-GC-MS, represent 37 % of the total of VOCs detected. 13 of which were ubiquitous (Octane, Limonene, p-Cymene, Dehydro-p-cymene, 1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]dodec-8(9)-ene, Hexane, E or Z-1,3-Octadiene Stereoisomers1, E or Z-1,3-Octadiene Stereoisomers2, 3-(2-propenyl)-Cyclohexene, 1R-α-Pinene, trans-Carane, Calamenene isomer 1, Z -2-Octene). These compounds particularly terpenes are mostly attributed to food from plant origin such as fruit, vegetables and herbs (Belitz *et al* 2009). They are also found in fragrances, perfumes and personal care products (Breitmaier 2006). However, many of these compounds are also common air pollutants derived from diesel and petrol fumes, such as the long chain n-alkane derivatives hexane, octane, decane, undecane, and hexadecane. (Sheng *et al* 2006 and Schulz *et al* 1999).

Ketones and lactones represent 13 % of the VOCs in saliva, 5 of these were ubiquitous (2,3-Butanedione, 2-Heptanone, Acetone, 2,4-dimethyl-3-Pentanone, 2,3-Pentanedione). Diet is a possible source for some of these ketones, such as 2-heptanone which was found in cheese (Jelen

et al 2002). Ketones such as acetone and 2-butanone can be formed endogenously from fatty acids and carbohydrates metabolisms (Garner *et al* 2007). Others could arise from bacterial metabolism such as 2,3-butanedione from lipids and 2,3-pentanedione from carbohydrates (Ott *et al* 2000).

Aromatics compounds represent 9 % of the VOCs in saliva. 4 of which were ubiquitous (Phenol, o-Xylene, Styrene, p-Xylene). The sources of some of these compounds were mainly dietary such as estragole, anethole (Mudge et al 2008). Phenol and Indole could arise from the metabolism of aromatic amino acids tyrosine, phenylalanine and tryptophan by the action of bacteria and fungi (Calil *et al* 2006, Kriek *et al* 2007, Garner *et al* 2007, Smith and Macfarlane 1997). Benzaldehyde was found in foods such as almond and cherry (Pićurić-Jovanović and Milovanović 1993, Yang Sun *et al* 2010) but is also a common air pollutant derived from vehicle emissions (Sheng *et al* 2006 and Schulz *et al* 1999). Many of the other compounds are also common outdoor air pollutants derived for example from diesel or petrol fumes. These emissions contain higher abundances of the lower-boiling aromatic hydrocarbons, such as benzene, toluene, ethylbenzene, xylenes, styrene, 1,2,4-trimethylmethylbenzene.(Sheng *et al* 2006 , Schulz *et al* 1999, Kusano *et al* 2011, Kostelc *et al* 1981).

Aldehydes represent 8 % of the VOCs detected of which 3 were ubiquitous. Some of these aldehydes can be attributed to dietary sources, such as dodecanal which could be naturally derived from plant or animal oils and fats (Mudge *et al* 2008) and were found in dairy, fruit and fish and in some bacterial cells (Varlet *et al* 2007). Volatiles such as hexanal and nonanal were considered a general marker for oxidative stress. (Fuchs *et al* 2010). Both could be endogenously

produced from membrane lipid oxidation (Frampton *et al* 1999) and both were reported to be in food (Delacy Costello *et al* 2001).

Alcohols represent 7 % of the total VOCs identified in this study of saliva, with 6 ubiquitous compounds in each group (Ethyl alcohol, Isopropyl Alcohol, 1-Propanol, 2-methyl-1-Propanol, 1-Butanol, 2-butyl- 1-Octanol). The sources of some these compounds were mainly dietary such as 2-butyl-1-octanol which is found in plant and animal oils (Mudge *et al* 2008). Ethanol can be found in alcoholic beverages, food, personal care products, perfumes and be derived from the metabolism of various microorganism's (McKarns *et al* 1997, Garner *et al* 2007). 1-propanol and 1-butanol were considered to arise from the biosynthesis of the amino acid isoleucine (Lamsen *et al* 2012).

Esters represent 4% of the total VOCs identified. These compounds are naturally occurring in food and used in flavor, fragrances and essential oils (Belitz *et al* 2009).

Volatile sulfur compounds represent 3% of the total compounds identified although none were found to be ubiquitous. Members of alliceae family, such as garlic and onion will introduce some sulphur containing compounds such as allyl mercaptan, allyl methyl sulfide, dimethyldisulfide, 1- 1-(E)-/ (Z),(methylthio)-propene and 1-(methylthio)-propane (Kostelc *et al* 1981, Belitz *et al* 2009, Lawson and Wang 2005) all were detected in this study. However, many of these compounds such as dimethyl disulfide have been identified in association with common oral anaerobic bacteria (Khalid et al 2013). The brassicaceae family which includes broccoli and cabbage will introduce some other compounds such as allylisothiocyanate and isothiocyanatocyclopropane (Olivier *et al* 1999, Egami *et al* 2011), which were also present in this investigation. Therefore the 9 VSCs found in this study possibly have a dietary origin.

Nitrogen containing compounds NCC represent 2% of VOCs identified in saliva (2 are ubiquitous, pyrrole and indole). Pyrrole and indole may arise from microbial action in the saliva (Imamura T 1982). 2-methyl pyridine and 3-methyl pyridine can be found in food (Newkome 1984) and both are environmental contaminants (ref) with sources such as pharmaceuticals and agricultural industries. (ref).

Physical exercise such as cycling and walking might possibly alter the level of some volatiles such as acetone and indole levels (Yami *et al* 2009, Cooke *et al* 2003). Subject 8 especially on days 1 and 3 had done more physical exercise when compared to the other volunteers prior to sampling and was observed to have higher levels of acetone on those days. We found that for most subjects the levels of acetone and isopropyl alcohol followed similar trends. Acetone is formed via the decarboxylation of acetoacetate and the dehydrogenation of isopropylalcohol (Kalapos 2003).

We have reported the application of a new technique (HS-Trap GC-MS) for the analysis of biological samples, specifically saliva. Our study has identified higher total numbers of VOCs than previously reported. Many of these volatiles had not been previously identified in saliva. The method is automated and traps headspace volatiles directly onto a cold trap thus removing certain selectivity issues that arise with other methods such as SPME, ATD etc. In addition this study was able to assess the change in volatile profile over a 10 day period for a number of volunteers. An in depth analysis of 34 ubiquitous compounds and the variation in their relative abundance was also undertaken. This work helps in establishing a baseline profile for salivary volatiles in healthy adults, although there is scope for more extensive studies to increase the database. It is important to have information on the composition and inter and intra subject

variability for key volatiles if studies to investigate the use of saliva for disease diagnosis are to be undertaken.

4. Conclusion

To our knowledge this longitudinal investigation is the most comprehensive study in the analysis of VOCs in saliva with 317 (268 identified) compounds reported to be found in the headspace The average number of compounds per subject over a 10 day period was found to be relatively consistent although the actual composition of these volatiles was subject to a degree of daily variation, with approximately 60-70% of volatiles consistently present in all 10 samples of a given individual.

The HS-Trap-GCMS method used in this study has shown great utility for use in the detection of VOCs directly from the headspace of biological samples. This technique has great potential to be used for the detection of VOCs associated with disease. . This is a relatively small pilot study addressed only at a small number of healthy individuals. In the future this study could be extended to include a larger number of individuals and measure them for a longer period of time or an extended number of time points during the day.

The volatile profiles of salivary headspace from healthy volunteers were established and should provide a basis for the future use of salivary VOCs as a diagnostic medium for disease

Table (2): A longitudinal study of volatile organic compounds detected in saliva of healthy individuals over 10 days and a comparison with literature data (see references column)

(In the RT confirmed column, if the compound has been identified by running a pure standard then 1 is inserted otherwise a 0 is inserted.

	Compound Name	RT confirmed	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10	Presence %	References
1	Ethyl alcohol	1	10	10	10	10	10	10	10	10	10	10	100	F,G
2	Isopropyl alcohol	1	10	10	10	10	10	10	10	10	10	10	100	A
3	1-Propanol	1	10	10	10	10	10	10	10	10	10	10	100	
4	2-methyl-1-propanol,	1	10	10	10	10	10	10	10	10	10	10	100	
5	1-Butanol	1	10	10	10	10	10	10	10	10	10	10	100	
6	Hexanal	1	10	10	10	10	10	10	10	10	10	10	100	
7	Nonanal	1	10	10	10	10	10	10	10	10	10	10	100	
8	2,3-Butanedione	1	10	10	10	10	10	10	10	10	10	10	100	
9	2-Heptanone	1	10	10	10	10	10	10	10	10	10	10	100	A
10	Octane	1	10	10	10	10	10	10	10	10	10	10	100	
11	Limonene	1	10	10	10	10	10	10	10	10	10	10	100	F
12	P-cymene	1	10	10	10	10	10	10	10	10	10	10	100	
13	Dehydro-p-cymene	1	10	10	10	10	10	10	10	10	10	10	100	
14	1S,2S,5R-1,4,4-Trimethyltricyclo [6.3.1.0(2,5)]dodec-8(9)-ene	1	10	10	10	10	10	10	10	10	10	10	100	
15	Pyrrole	1	10	10	10	10	10	10	10	10	10	10	100	
16	Phenol	1	10	10	10	10	10	10	10	10	10	10	100	C,E,F
17	o-Xylene	1	10	10	10	10	10	10	10	10	10	10	100	
18	Styrene	1	10	10	10	10	10	10	10	10	10	10	100	F
19	Dodecanal	1	10	10	10	10	10	10	10	10	10	10	100	B
20	Acetone	1	10	10	10	10	10	10	10	10	10	10	100	A,F,G
21	2,4-Dimethyl-3-pentanone	1	10	10	10	10	10	10	10	10	10	10	100	

22	Hexane	1	10	10	10	10	10	10	10	10	10	10	100	
23	E or Z-1,3-Octadiene Stereoisomers1	1	10	10	10	10	10	10	10	10	10	10	100	
24	E or Z-1,3-Octadiene Stereoisomers2	1	10	10	10	10	10	10	10	10	10	10	100	
25	3-(2-propenyl)-Cyclohexene	0	10	10	10	10	10	10	10	10	10	10	100	
26	1R-α-Pinene	1	10	10	10	10	10	10	10	10	10	10	100	
27	trans-Carane	1	10	10	10	10	10	10	10	10	10	10	100	
28	unknown_37 (Retention time 37.75mins)		10	10	10	10	10	10	10	10	10	10	100	
29	Indole	1	10	10	10	10	10	10	10	10	10	10	100	F
30	2-Butyl-1-octanol	0	10	10	10	10	10	10	10	10	10	10	100	
31	Calamenene isomer 1	1	10	10	10	10	10	10	10	10	10	10	100	
32	2,3-Pentanedione	0	10	10	10	10	10	10	10	10	10	10	100	
33	Z -2-Octene	1	10	10	10	10	10	10	10	10	10	10	100	
34	p-Xylene	1	10	10	10	10	10	10	10	10	10	10	100	
35	(Z)-3-Dodecene	0	10	10	9	10	10	10	10	10	10	10	99	
36	Acetaldehyde	1	10	10	10	10	10	10	8	10	10	10	98	C, G
37	(E)-3-Dodecene	0	9	10	10	10	10	10	9	10	10	10	98	
38	Methyl isobutyl ketone	1	10	10	10	10	10	10	10	8	10	10	98	
39	2-Undecanone	1	8	10	10	10	10	10	10	10	10	10	98	B
40	Benzaldehyde	1	8	10	10	9	10	10	10	10	10	10	97	F
41	2-Nonanone	1	9	10	10	10	10	9	9	10	10	10	97	
42	(-)-Z-Carane	1	9	10	8	10	10	10	10	10	10	10	97	
43	1R,3Z,9S-2,6,10,10-Tetramethylbicycl	1	10	9	9	10	10	10	9	10	10	10	97	

	o[7.2.0]undeca-2,6-diene													
44	(-)-Menthol	1	10	10	9	10	10	10	9	10	8	10	96	
45	2,4-Octadiene stereoisomers1	1	10	10	10	10	10	10	10	8	9	10	97	
46	2,4-Octadiene stereoisomers2	1	10	10	10	10	10	10	10	8	8	10	96	
47	Toluene	1	10	10	6	10	10	10	10	10	10	10	96	F
48	2-Pentylfuran	1	10	10	10	8	10	7	10	10	10	10	95	
49	2-Pentanone	1	10	10	10	9	9	10	10	9	8	9	94	
50	2,3-Heptanedione	0	8	10	10	8	10	10	10	8	10	10	94	
51	β-Terpinene	0	10	10	9	10	10	10	8	10	7	10	94	
52	(-)-β-Bourbonene	1	10	9	9	10	10	10	10	10	6	10	94	F
53	5-Methyl-2-hexanone	1	5	10	10	10	10	10	9	9	10	10	93	
54	2,5-Dimethyl-2,4-hexadiene	0	10	10	10	8	9	10	10	7	9	10	93	
55	Acetonitrile	1	10	10	10	9	6	10	9	10	10	9	93	
56	Methylene chloride	1	10	10	10	9	6	10	9	10	10	9	93	
57	2-Butanone	1	10	9	10	7	9	10	10	10	10	7	92	A
58	(S)-Carvone	1	10	6	10	10	10	10	9	8	9	10	92	
59	1-Dodecene	0	10	10	10	10	10	10	10	9	3	10	92	
60	Humulen-(v1)	1	10	10	10	10	10	10	10	8	4	10	92	
61	unknown_38 (Retention time 39.5mins)		10	10	9	10	10	10	10	9	4	10	92	
62	unknown_39 (Retention time 39.6mins)		10	10	9	10	10	10	10	9	4	10	92	
63	unknown_40 (Retention time 39.7mins)		10	10	9	10	10	10	10	9	4	10	92	
64	2-Decanone	0	5	10	10	9	10	10	8	9	10	10	91	

65	Undecane	1	10	10	2	10	10	10	10	10	9	10	91	
66	β-Caryophyllene	1	10	10	8	10	10	10	10	8	5	10	91	B,F
67	3-Methylbutanal	1	10	10	10	10	10	9	10	4	10	7	90	
68	Isothujol	0	10	8	9	10	10	10	10	10	4	8	89	
69	Camphene	1	10	9	0	10	10	10	10	10	10	10	89	
70	Benzene	1	10	10	10	2	10	10	10	9	8	10	89	F
71	cis-3-Decene	1	10	10	10	10	10	9	10	3	6	10	88	
72	2-Methylpropanoic acid	1	10	10	10	6	10	10	10	6	8	8	88	
73	Anethole	1	10	10	9	10	9	10	9	10	1	10	88	
74	2-Propenoic acid, 2-methyl-ethenyl ester	0	10	10	10	5	9	10	10	6	8	9	87	
75	2-n-Butylfuran	0	10	10	10	7	10	10	10	9	1	10	87	
76	2-methyl-3-Hexanone	0	9	9	9	10	8	10	1	10	10	10	86	
77	Terpinolene	1	10	7	5	10	10	10	10	9	5	10	86	
78	Estragole	0	10	10	9	10	10	10	8	9	0	10	86	
79	Pentanal	1	10	10	10	2	9	6	10	8	10	10	85	
80	1-methyl-3-(2-methyl-2-propenyl)-Cyclopentane	0	10	10	0	10	10	10	6	9	10	10	85	
81	Eucalyptol	1	9	7	3	10	9	10	10	9	10	8	85	
82	β-Cubebene	0	10	6	3	10	10	10	9	9	8	9	84	
83	5-Methyl-2-heptanone	1	1	10	10	9	10	9	6	10	8	10	83	
84	Ethyl acetate	1	10	9	7	8	10	10	10	3	9	7	83	
85	2-Methyl-pentanal	0	7	10	9	5	9	9	7	7	9	10	82	
86	1-Octen-3-one	1	3	9	10	7	7	10	10	8	8	10	82	
87	Calamenene	1	10	7	9	10	10	10	9	7	1	9	82	

	isomer 2													
88	1-Tetradecene	0	10	9	8	1	10	10	9	10	5	10	82	B
89	γ- terpinene	1	10	5	0	10	7	10	10	9	10	10	81	
90	(E)-β-Bourbonene	0	10	7	5	10	10	9	9	5	6	10	81	
91	δ-Cadinene	1	10	7	4	10	7	10	10	8	7	8	81	
92	2,4-Dimethyl-2-pentene	0	10	10	10	0	10	10	10	4	8	8	80	
93	unknown_45 (Retention time 40.2 mins)		6	8	9	10	10	4	2	10	10	10	79	
94	Heptanal	1	9	10	10	1	10	3	9	9	7	10	78	
95	Octanal	1	10	9	10	1	10	1	7	10	10	10	78	
96	α-Terpinene	1	7	4	5	10	10	10	8	9	6	9	78	
97	β-Clovene	0	10	7	9	8	10	10	9	7	1	7	78	
98	4-Methylphenol	1	9	10	6	8	8	9	8	7	4	9	78	C, F
99	2-Menthene	0	10	6	4	10	9	10	8	10	0	10	77	
100	(E)-5-Undecene,	0	10	9	2	0	10	9	10	10	7	10	77	
101	Isomenthone	1	1	10	3	10	10	10	9	9	6	8	76	
102	α-Muurolene	1	10	5	4	10	10	10	10	5	3	9	76	
103	(Z)-3-Octene	1	6	10	10	10	9	9	10	0	9	2	75	
104	β-Phellandrene	1	10	5	0	10	9	10	8	9	5	8	74	
105	(E)-7-Tetradecene	0	9	6	10	9	10	10	10	1	0	9	74	
106	2-Dodecanone	0	1	8	9	9	10	8	4	4	10	10	73	B
107	isocaryophyllene	0	10	4	9	10	9	10	9	1	1	9	72	
108	p-Menthene	0	6	8	1	10	7	10	8	9	6	6	71	
109	Decanal	1	10	4	9	10	4	1	8	7	8	8	69	B
110	unknown_28 (Retention time 32.82mins)		10	8	0	10	4	10	6	10	4	7	69	
111	unknown_34 (Retention time 35.5mins)		10	5	10	8	8	10	9	0	0	8	68	

112	Cyclohexane	1	9	4	3	10	6	2	4	10	10	9	67	
113	unknown_48 (Retention time 42.55mins)		7	10	9	4	7	6	7	1	9	7	67	
114	6-Methyl-5-hepten-2-one	1	2	10	7	0	10	4	7	9	6	10	65	
115	Cyclotetradecane	0	10	6	9	4	9	9	8	0	0	10	65	
116	α-Bourbonene	0	10	3	6	10	9	8	9	1	1	7	64	
117	α-Calacorene	1	10	4	6	10	4	10	10	0	10	0	64	
118	3-Methyl-3-phenyl-azetidine	0	9	10	7	0	10	9	9	10	0	0	64	
119	Menthofurane	1	6	4	2	10	9	10	10	9	1	3	64	
120	Allo-ocimene	1	10	0	3	10	9	10	6	4	0	10	62	
121	n-Propyl acetate	1	0	7	10	9	9	3	7	3	5	9	62	
122	(Z)- 2-Octen-1-ol	0	8	7	7	5	7	2	2	7	8	8	61	
123	(+)-Ledene	1	10	1	7	10	7	9	8	9	0	0	61	
124	2-Ethyl-1-hexanol	1	10	4	10	0	6	6	7	1	6	10	60	F
125	2-Nonadecanone	0	1	9	10	3	9	7	5	1	5	10	60	
126	α-Cubebene	0	10	2	7	8	6	9	9	1	4	4	60	
127	3-Methyl-pentane	0	9	5	6	9	4	1	3	9	6	7	59	
128	nerol	0	10	0	0	10	10	10	5	9	4	0	58	
129	Ylangene	0	9	1	0	7	5	8	8	4	7	8	57	
130	α-Phellandrene	1	10	0	0	10	10	10	6	6	4	0	56	
131	thymol	1	0	5	4	10	1	9	7	10	8	1	55	
132	2-methyl-2-pentenal	0	4	5	6	10	6	8	6	2	8	0	55	
133	unknown_18 (Retention time 26.83mins)		10	0	9	10	10	10	4	1	0	0	54	
134	2-Tridecanone	0	1	7	10	6	3	7	1	0	8	10	53	B
135	2,7,7-trimethyl-Bicyclo[2.2.1]hep	0	8	0	0	10	6	10	10	7	0	0	51	

	t-2-ene												
136	Allyl isothiocyanate	1	0	9	6	0	2	9	10	5	6	4	51	F
137	dimethyldisulfide	1	7	10	1	6	8	1	1	0	9	7	50	F
138	β-Elemene	0	10	0	0	10	5	8	10	2	2	1	48	
139	Neoclovene	0	1	0	9	9	10	10	8	0	1	0	48	
140	1-Hexanol	1	2	9	10	0	4	0	0	2	9	10	46	
141	3-Methylene-1,5,5-trimethylcyclohexene	0	1	0	0	9	5	10	7	8	5	0	45	
142	unknown_15 (Retention time 25.11mins)		10	6	3	0	4	7	6	1	5	3	45	
143	unknown_43 (Retention time 39 mins)		9	2	5	7	2	10	8	0	2	0	45	
144	Heptane	1	4	6	7	0	8	9	6	1	0	2	43	
145	unknown_41 (Retention time 38.2 mins)		9	2	6	3	3	9	8	0	0	3	43	
146	1R,3Z,9s-4,11,11-Trimethyl-8-methylenebicyclo [7.2.0]undec-3-ene	0	6	1	4	7	3	10	10	0	0	0	41	
147	unknown_23 (Retention time 30.86 mins)		0	0	9	8	4	5	3	6	4	2	41	
148	Copaene	1	9	1	8	5	4	5	7	0	0	0	39	
149	Methacrolein	1	0	8	4	0	5	7	3	0	9	2	38	
150	unknown_16 (Retention time 26.1 mins)		0	1	4	10	3	10	5	1	4	0	38	
151	3,5-dimethyl-Cyclohexanol	0	8	4	3	0	0	0	2	7	6	7	37	

152	E-2-Octene	1	0	6	9	1	5	2	4	1	7	1	36	
153	R or S (-) 3,7-Dimethyl-1,6-octadiene	0	0	0	9	3	6	2	5	8	3	0	36	
154	Hexylbenzene	0	4	8	3	0	4	5	3	3	0	6	36	
155	1-Dodecanol	0	10	5	0	0	3	0	7	0	0	10	35	C,F
156	unknown_13 (Retention time 23.92 mins)		5	4	4	1	3	2	3	4	0	9	35	
157	2-Methylbutanal	1	0	3	9	3	8	0	0	1	9	1	34	
158	unknown_1 (Retention time 15.44 mins)		1	6	7	0	5	7	6	0	1	0	33	
159	3-octanone	1	0	10	0	0	0	0	4	1	6	10	31	
160	Methylcyclopentane	1	9	0	0	3	0	0	0	9	6	4	31	C
161	unknown_22 (Retention time 30.72 mins)		8	0	0	10	1	6	5	0	1	0	31	
162	unknown_29 (Retention time 32.85 mins)		0	0	0	10	2	10	7	0	2	0	31	
163	3,4-Dimethylcyclohexanol	0	8	6	1	0	4	1	2	1	2	4	29	
164	Nonane	1	0	8	2	0	4	3	1	0	10	0	28	
165	1-methyl-3-(2-methyl-1-propenyl)-Cyclopentane	0	2	0	1	8	0	10	2	0	5	0	28	
166	3-Methyl-1-butanol	1	0	8	0	0	2	0	9	0	0	8	27	
167	1-Pentanol	1	3	4	0	0	1	1	0	4	6	6	25	
168	(E)- 2-Butenal	1	0	6	3	0	6	9	0	0	1	0	25	
169	(+)-Dihydrocarvone	1	3	0	0	9	3	0	1	0	5	4	25	

170	Piperitone	1	2	1	2	3	2	4	6	0	0	5	25	
171	unknown_36 (Retention time 36.12 mins)		0	0	3	4	1	8	6	1	0	1	24	
172	unknown_12 (Retention time 20.71 mins)		1	0	3	1	4	6	0	4	4	0	23	
173	4,7-Dimethylbenzofuran	1	0	0	0	10	0	2	2	8	1	0	23	
174	unknown_4 (Retention time 16.03 mins)		2	4	2	0	2	1	1	4	0	5	21	
175	unknown_30 (Retention time 40.83 mins)		4	8	3	0	0	3	1	0	0	2	21	
176	(2α,3β,5β)-1,1,2-trimethyl-3,5-bis(1-methylethenyl)-Cyclohexane	0	1	4	6	0	4	1	3	1	0	0	20	
177	4,5,9,10-dehydro-Isolongifolene	1	6	0	1	4	0	4	4	0	0	1	20	
178	(E,E)-2,4-Nonadienal isomer??	0	0	0	0	8	0	8	3	0	0	0	19	
179	3-Methylheptane	0	7	0	0	7	0	0	0	3	1	1	19	
180	Dodecane	1	0	0	0	0	0	0	2	7	10	0	19	B
181	Δ-Cadinene	0	1	0	3	7	1	0	5	2	0	0	19	
182	(Z)- 2-Heptenal	1	0	0	0	0	0	0	0	0	8	10	18	
183	4-Heptanone	0	0	0	6	0	0	0	0	0	6	6	18	
184	(E)-4,4-Dimethyl-2-pentene	0	0	0	0	0	0	0	0	9	0	9	18	
185	(E)- 1-(methylthio)-1-propene,	1	0	3	1	0	1	5	2	0	5	1	18	

186	3-Methyl-3-buten-2-one	0	0	5	3	0	1	5	1	1	1	0	17	
187	trans-Dihydrocarvone	1	0	0	0	8	3	0	0	0	2	4	17	
188	unknown_42 (Retention time 38.7 mins)		6	1	4	0	0	1	5	0	0	0	17	
189	Allyl methylsulfide	0	0	1	0	0	5	5	2	0	4	0	17	
190	unknown_2 (Retention time 15.53 mins)		1	2	2	1	3	3	1	0	1	2	16	
191	(dimethylamino)-Acetonitrile	0	0	0	0	0	0	0	0	8	1	7	16	
192	3-Methyl-1-penten-3-ol	0	7	0	2	0	0	0	0	1	4	1	15	
193	Isoterpinolene	0	6	0	0	5	0	1	3	0	0	0	15	
194	unknown_3 (Retention time 15.69 mins)		3	4	0	1	2	1	3	0	0	1	15	
195	(E)- or (Z) 2-Nonenal	1	0	0	0	0	0	0	0	6	8	0	14	C
196	(E)- 3-Octene	0	0	0	3	6	0	0	0	0	5	0	14	
197	Tricyclene	1	1	0	0	9	3	1	0	0	0	0	14	
198	Isolimonene	1	0	0	0	10	0	1	0	0	3	0	14	
199	(E,Z)-2,4-Dodecadiene	0	5	3	0	0	1	0	1	0	0	4	14	
200	unknown_11 (Retention time 19.93 mins)		2	4	1	0	1	2	2	0	2	0	14	
201	(Z)-1-(methylthio)-1-propene,	1	0	1	0	0	0	5	2	1	5	0	14	
202	1-(3-methylbutyl)-Cyclopentene	0	0	1	0	0	0	8	3	0	1	0	13	
203	2-Propenal	1	0	5	0	1	0	5	0	0	1	0	12	

204	Propanal	1	0	0	0	10	0	0	0	0	1	1	12	
205	1-Penten-3-one	1	0	5	0	1	0	2	1	0	1	2	12	
206	(-)-β-Pinene	0	2	0	0	10	0	0	0	0	0	0	12	
207	α-Fenchene	0	0	0	0	10	0	0	0	0	2	0	12	
208	β-Myrcene	1	0	2	0	0	0	0	0	0	10	0	12	
209	2-Octanone	0	0	1	0	0	0	4	0	0	0	6	11	
210	2-Methylpentane	0	0	1	0	4	0	0	0	2	0	4	11	
211	unknown_17 (Retention time 26.25 mins)		0	0	0	9	0	0	0	0	2	0	11	
212	E,E-2,6-Dimethyl-1,3,5,7-octatetraene	0	3	0	0	5	2	0	0	0	0	0	10	
213	1,3,8-p-Menthatriene	1	1	1	0	5	0	0	0	0	1	2	10	
214	(E)-or (Z) 6-Tridecene	0	0	0	0	0	0	0	2	0	0	8	10	
215	unknown_10 (Retention time 17.83 mins)		2	3	4	0	0	0	0	0	0	1	10	
216	unknown_31 (Retention time 33.85 mins)		0	0	8	0	1	0	1	0	0	0	10	
217	unknown_32 (Retention time 34 mins)		0	0	8	0	1	1	0	0	0	0	10	
218	Isopropyl myristate	1	3	0	0	0	0	0	3	0	0	4	10	F
219	(R)-2-Butanol,	0	0	0	0	0	0	0	0	0	8	0	8	E
220	(E) 2-Methyl-2-butenal,	1	0	0	0	1	0	0	0	0	7	0	8	
221	(2-methylenebutyl)-Cyclopropane	0	0	0	4	3	0	1	0	0	0	0	8	
222	3,3,5-Trimethyl-heptane	0	0	1	2	1	1	1	2	0	0	0	8	

223	unknown_44 (Retention time 40.12 mins)		0	0	0	0	0	2	6	0	0	0	8
224	Dodecanoic acid ethyl ester	0	0	0	0	8	0	0	0	0	0	0	8
225	2,3-Octanedione	1	7	0	0	0	0	0	0	0	0	0	7
226	trans-β-Ocimene	1	0	0	0	0	0	0	6	0	1	0	7
227	1,2-dimethyl-3,5-bis(1-methylethenyl)-Cyclohexane	0	0	0	6	0	0	0	1	0	0	0	7
228	(-)-β-Cadinene	0	0	0	0	7	0	0	0	0	0	0	7
229	unknown_21 (Retention time 29.71 mins)		0	2	0	0	1	1	2	0	0	1	7
230	unknown_47 (Retention time 41.42 mins)		2	1	1	0	1	0	2	0	0	0	7
231	1-(Methylthio)-propane	1	0	2	0	0	0	1	2	0	2	0	7
232	(-)-Calamenene isomer 3	1	0	1	2	0	1	1	1	0	0	0	6
233	unknown_14 (Retention time 23.98 mins)		0	0	0	2	0	0	0	3	1	0	6
234	unknown_24 Isomer 1 (Retention time 40.83mins)		0	0	5	1	0	0	0	0	0	0	6
235	Isomenthyl acetate	0	0	6	0	0	0	0	0	0	0	0	6
236	2-Acetyl-5-methylfuran	0	0	0	0	0	0	0	0	4	2	0	6
237	Dihydrocarveol	0	0	0	0	1	0	0	1	0	1	2	5
238	2-Ethyl-2-hexenal	1	0	0	5	0	0	0	0	0	0	0	5
239	Methyl vinyl ketone	1	0	3	0	0	0	2	0	0	0	0	5

240	3-Methyleneheptane	0	0	0	5	0	0	0	0	0	0	0	5
241	3-Methyl-3-heptene	0	0	0	4	0	0	0	0	0	1	0	5
242	1-Acetyl-2-methylcyclopentene	0	0	0	5	0	0	0	0	0	0	0	5
243	2,4-Dimethyl-1-(1-methylethyl)-benzene	0	0	0	0	0	0	0	0	0	5	0	5
244	1-(1-methylethenyl)-3-(1-methylethyl)-Benzene	0	0	0	5	0	0	0	0	0	0	0	5
245	unknown_25 (Retention time 31.03mins)		0	0	0	0	0	0	0	1	0	4	5
246	n-Propyl isobutyrate	0	0	0	1	4	0	0	0	0	0	0	5
247	1-Methoxy-4-propyl-benzene	0	0	0	0	1	0	1	3	0	0	0	5
248	(E)-2-Hexenal	1	0	0	0	0	0	0	0	1	3	0	4
249	2,3-Hexanedione	0	0	1	2	0	1	0	0	0	0	0	4
250	(E)-2-Heptene	0	0	0	3	0	0	1	0	0	0	0	4
251	3-Heptene	0	0	0	4	0	0	0	0	0	0	0	4
252	3-Methyl-1-heptene	0	0	0	4	0	0	0	0	0	0	0	4
253	4-Methyl-3-heptene	0	0	0	4	0	0	0	0	0	0	0	4
254	(E)- 4-methyl-2-heptene	0	0	0	4	0	0	0	0	0	0	0	4
255	(E)-4-Octene	0	0	0	4	0	0	0	0	0	0	0	4
256	(Z)- 4-Octene	0	0	0	4	0	0	0	0	0	0	0	4
257	(3-methyl-2-butenyl)-benzene	0	0	0	4	0	0	0	0	0	0	0	4

258	Methyl propionate	0	0	1	0	0	1	0	0	0	2	0	4	
259	Dioctyl ether	0	0	0	0	0	0	1	0	0	0	3	4	B
260	Perilla aldehyde	0	0	0	0	0	0	0	0	0	0	3	3	
261	(Z)-3-Heptene	0	0	0	3	0	0	0	0	0	0	0	3	
262	Santolina triene	0	0	0	0	3	0	0	0	0	0	0	3	
263	(R)-(+)-p-Menth-3-ene	0	0	1	0	0	0	1	0	0	1	0	3	
264	1-methyl-4-(1-methyl-2-propenyl)-Benzene	0	0	0	0	0	0	0	0	0	3	0	3	
265	γ-Muurolene	0	0	0	3	0	0	0	0	0	0	0	3	
266	unknown_7 (Retention time 17.06mins)		0	0	0	0	0	0	0	2	1	0	3	
267	unknown_24 Isomer 2 (Retention time 31.12 mins)		0	0	2	1	0	0	0	0	0	0	3	
268	unknown_46 (Retention time 40.83mins)		0	1	1	0	1	0	0	0	0	0	3	
269	Isothiocyanatocyclopropane	0	0	0	0	0	0	0	0	0	3	0	3	
270	n-Propyl butyrate	0	0	0	0	3	0	0	0	0	0	0	3	
271	Acetic acid 2-ethylhexyl ester	0	0	0	3	0	0	0	0	0	0	0	3	
272	Heptylbenzene	0	0	0	0	0	0	0	0	0	0	3	3	
273	2-Propen-1-ol	0	0	0	0	0	0	0	0	0	2	0	2	
274	γ-Nonalactone	1	0	0	0	1	0	0	0	0	1	0	2	B
275	3-Penten-2-one	1	0	0	0	0	0	0	0	0	0	2	2	
276	Megastigmatrienone	0	0	0	0	0	1	0	1	0	0	0	2	
277	1,1'-	0	0	0	2	0	0	0	0	0	0	0	2	

	ethylidenebis-Cyclopentane													
278	1-methyl-4-(1-methylpropyl)-Benzene	0	0	0	2	0	0	0	0	0	0	0	2	
279	(3-methyl-2-butenyl)-Benzene	1	0	0	2	0	0	0	0	0	0	0	2	
280	Tetradecane	0	0	0	1	0	0	0	1	0	0	0	2	C, B
281	unknown_5 (Retention time 16.18mins)		0	0	0	1	0	0	0	0	1	0	2	
282	unknown_8 (Retention time 17.13mins)		0	0	0	0	0	0	0	2	0	0	2	
283	unknown_19 (Retention time 27.47mins)		0	0	0	0	0	0	2	0	0	0	2	
284	Acetic acid 1-methylethyl ester	0	0	0	0	0	1	0	0	0	1	0	2	
285	Furan, 2,4-dimethyl-	0	0	0	0	0	0	0	0	0	2	0	2	
286	(Z)- 2-Butenal,	0	0	0	0	0	0	0	0	0	1	0	1	
287	α-Methyl-cinnamaldehyde	1	0	0	0	0	0	0	0	0	1	0	1	
288	3-Phenyl- 2-propenal	1	0	0	0	0	0	0	0	0	1	0	1	
289	γ-Heptalactone	1	0	0	0	0	0	0	0	0	1	0	1	B
290	γ-Undecalactone	0	0	0	0	0	0	0	0	1	0	0	1	
291	1,3-Dihydro-2H-indol-2-one	0	0	0	0	0	1	0	0	0	0	0	1	
292	Pulegone	1	0	0	0	0	0	1	0	0	0	0	1	
293	Propane	1	0	0	0	0	1	0	0	0	0	0	1	
294	2-Methyl-heptane	1	0	0	0	0	0	0	0	1	0	0	1	
295	3-(2-Methylpropyl)-cyclohexene	0	0	0	0	0	0	1	0	0	0	0	1	

296	β-Z-Ocimene	0	0	0	1	0	0	0	0	0	0	0	1
297	4-Methyl-2-methylene-1-(1-methylethylidene)-cyclohexane	0	0	0	0	0	0	0	0	0	1	0	1
298	1,3-bis(1-methylethyl)-1,3-Cyclopentadiene	0	0	0	0	0	0	0	0	0	1	0	1
299	(E)-2-Dodecene	0	0	0	1	0	0	0	0	0	0	0	1
300	(E,Z)-2,4-Dodecadiene	0	1	0	0	0	0	0	0	0	0	0	1
301	1-(1-Methylethenyl)-4-(1-methylethyl)-benzene	0	0	0	1	0	0	0	0	0	0	0	1
302	1,8-Nonadiene, 2,7-dimethyl-5-(1-methylethenyl)-	0	0	0	1	0	0	0	0	0	0	0	1
303	Cadalene	0	0	1	0	0	0	0	0	0	0	0	1
304	1-Methyl-2-n-hexylbenzene	0	0	0	0	0	0	0	0	0	0	1	1
305	Ar-curcumene	0	0	0	0	0	0	0	0	0	1	0	1
306	unknown_6 (Retention time 16.23mins)		0	0	0	0	0	0	0	0	1	0	1
307	unknown_9 (Retention time 17.33mins)		0	0	0	0	0	0	0	0	1	0	1
308	unknown_20 (Retention time 29.57mins)		0	0	1	0	0	0	0	0	0	0	1
309	unknown_26 (Retention time 31.47mins)		0	0	0	0	0	0	0	0	1	0	1
310	unknown_27 (Retention time		0	0	0	0	0	1	0	0	0	0	1

	32.05mins)												
311	unknown_33 (Retention time 35.32mins)		0	0	1	0	0	0	0	0	0	0	1
312	unknown_35 (Retention time 35.7 mins)		0	0	1	0	0	0	0	0	0	0	1
313	Allyl mercaptan	1	0	0	0	0	0	0	0	0	1	0	1
314	Thiazole	1	0	0	0	0	0	0	0	0	1	0	1
315	Menthyl acetate	1	0	0	0	1	0	0	0	0	0	0	1
316	3-Methyl-pyridine	0	0	0	0	0	0	0	0	0	1	0	1
317	2-Methyl-pyridine	0	0	0	0	0	0	0	0	0	1	0	1

A: Kumar *et al* 2009, B: Soini *et al* 2010, C: Alagendran *et al* 2010, D: Penn *et al* 2007, E: Lochner *et al* 1986, F: Kostelc *et al* 1981, G: Larsson 1965.

Table (3): Numbers of volatile organic compounds in saliva across subjects and between samples in the longitudinal study

	Number of compounds identified													
	day 1	day 2	day 3	day 4	day 5	day 6	day 7	day 8	day 9	day 10	AVG	STDEV	Total compounds in all 10 samples	compounds in every sample
Subject_1	139	138	134	143	146	144	138	139	140	142	140	3	179	102
Subject_2	118	122	124	128	133	144	133	147	146	131	133	10	187	85
Subject_3	127	131	142	151	138	103	160	139	141	137	137	15	206	74
Subject_4	143	158	152	155	151	128	152	147	132	149	147	10	189	104
Subject_5	119	138	132	144	150	135	149	142	145	144	140	9	194	94
Subject_6	153	159	145	151	148	146	132	160	155	150	150	8	199	109
Subject_7	123	130	146	141	121	154	160	159	155	140	143	14	202	81
Subject_8	95	124	114	124	129	119	118	124	122	137	121	11	171	65
Subject_9	115	127	128	142	122	138	119	122	121	128	126	8	211	66
Subject_10	143	141	129	132	136	147	138	140	134	138	138	5	180	94
											137 (AVG), 3.6 (STDEV)		192 (AVG), 13(STDEV)	87 (AVG), 16(STDEV)

Table (4): The inter subject and overall variability in the levels of the 34 ubiquitous compounds. The overall variability is calculated from a ratio of the maximum and minimum peak area values for a given compound for all 10 subjects over the 10 day period. Individual variability is calculated from a ratio of the maximum and minimum peak area values for a given compound for a single subject over the 10 day period.

Compound Name	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Subject 8	Subject 9	Subject 10	over all variability
2,3-Butanedione	4	4	2	9	4	3	7	5	4	5	41
E or Z-1,3-octadiene Stereoisomers1	4	5	2	8	3	5	2	3	5	6	13
E or Z-1,3-octadiene Stereoisomers2	4	4	3	4	3	5	3	4	5	12	14
2,3-Pentanedione	7	15	5	12	5	7	9	5	8	9	48
Hexane	6	6	5	17	7	7	8	17	6	7	55
2-methyl-1-Propanol	5	3	2	7	5	3	2	3	3	3	11
Indole	6	3	6	26	4	4	7	3	7	17	29
Octane	126	101	18	337	89	61	20	588	18	169	860
Styrene	1	102	2	4	6	2	4	4	2	3	102
o-Xylene	1	102	2	4	6	2	4	4	2	3	102
unknown_37	6	3	6	26	4	4	7	4	7	17	41
2-butyl-1-Octanol	3	21	14	6	6	4	8	2	2	7	48
Dodecanal	6	3	6	26	4	4	7	6	7	17	70
Nonanal	16	5	3	10	5	9	6	10	5	4	56
Calamenene isomer 1	3	21	14	6	6	4	8	2	2	7	48
2-Heptanone	3	16	3	4	3	6	4	4	4	6	27
Ethanol	4	3	4	12	17	54	4	13	10	7	57
Acetone	4	4	3	3	27	9	12	12	4	6	73
Isopropyl Alcohol	3	3	8	5	108	12	11	65	36	8	253
1S,2S,5R-1,4,4-Trimethyltricyclo[6.3.1.0(2,5)]dodec-8(9)-ene	3	62	18	7	5	4	8	4	5	7	131
Hexanal	13	5	2	8	6	5	3	2	3	21	141
2,4-dimethyl-3-Pentanone	3	5	9	4	5	5	6	2	6	2	40

2-Octene Z or E	5	8	36	7	3	3	2	8	4	6	124
1-Butanol	1	2	3	5	3	2	4	2	3	2	10
Phenol	5	8	8	33	16	8	12	6	11	7	170
Pyrrole	13	5	2	8	6	5	3	2	3	21	106
p-Xylene	5	8	4	6	6	4	3	2	3	7	25
trans-Carane	7	13	8	17	12	20	48	9	62	13	736
Dehydro-p-cymene	11	7	9	6	17	6	12	36	128	7	759
1R-α-Pinene	9	21	10	7	20	9	21	11	8	6	377
1-Propanol	10	3	3	14	14	32	10	4	5	11	3751
3-(2-propenyl)-Cyclohexene	3	8	15	4	7	3	4	3	4	7	65
P-cymene	13	6	16	6	22	6	17	35	91	5	605
Limonene	12	11	37	3	33	7	27	19	101	65	1203

FIG 1_a: 2-methyl-1-propanol

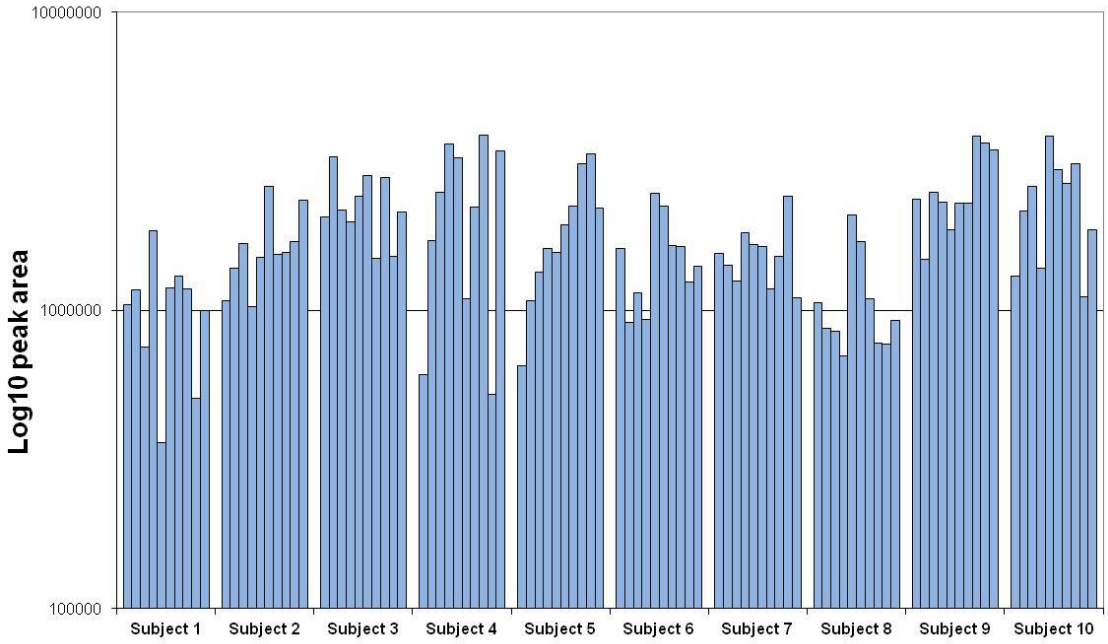

FIG 1_b: Styrene

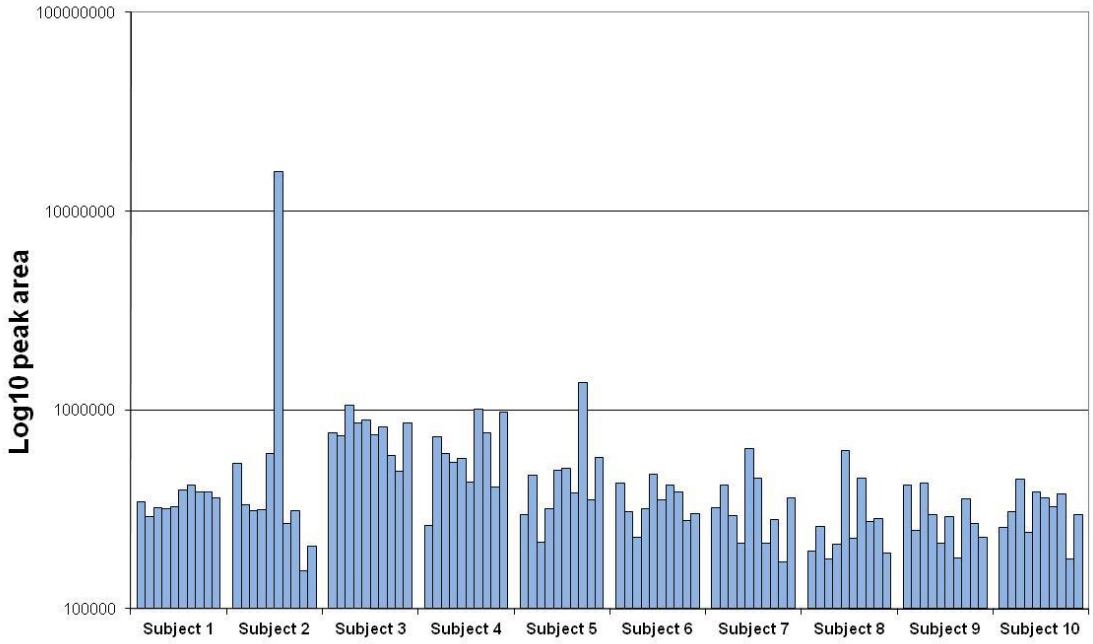

FIG 1_c: Isopropyl alcohol

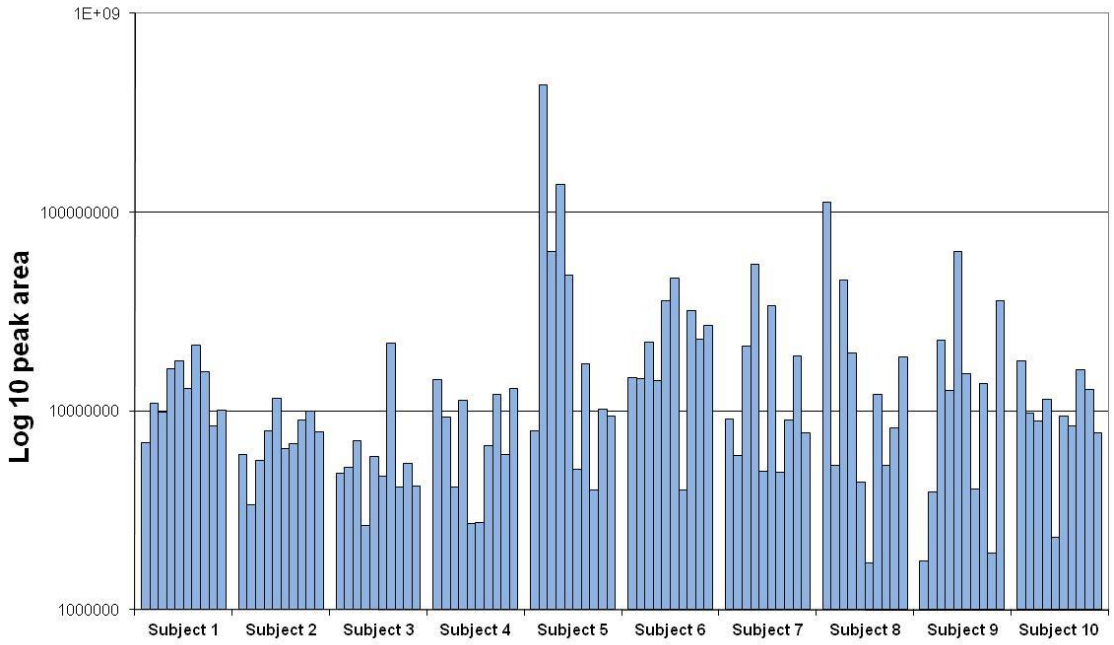

FIG 1_d: Acetone

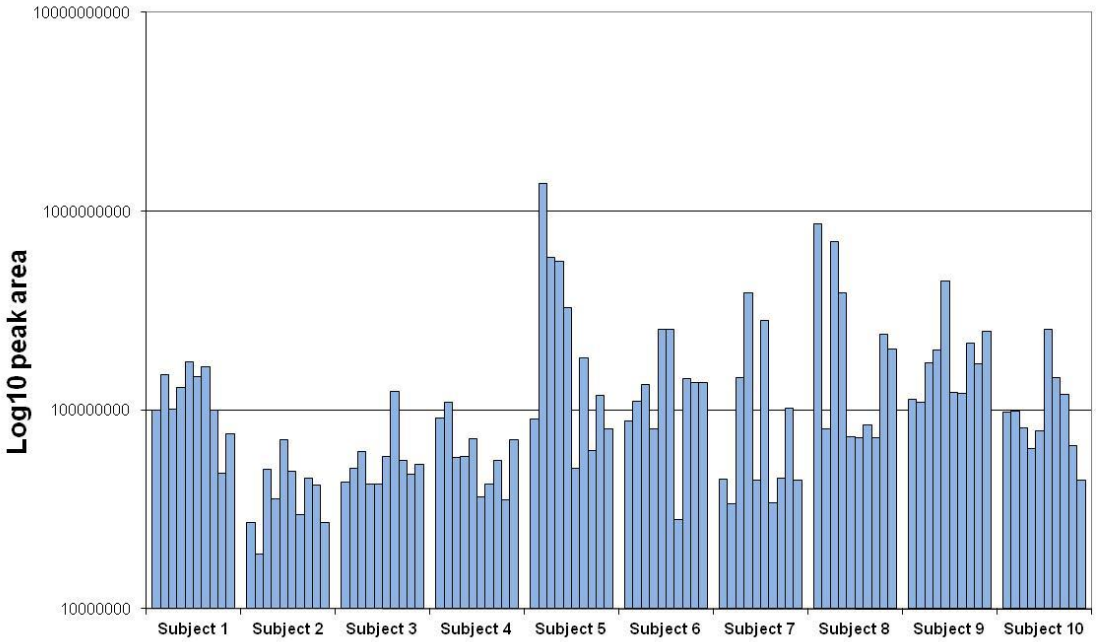

FIG 1_e: Ethanol

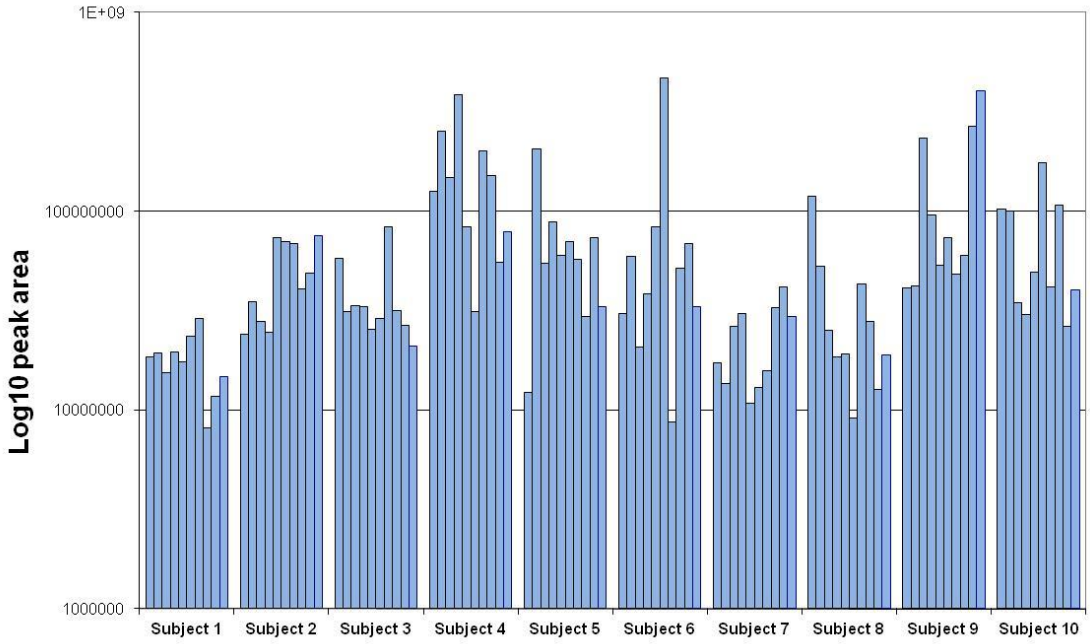

FIG 1_f: Limonene

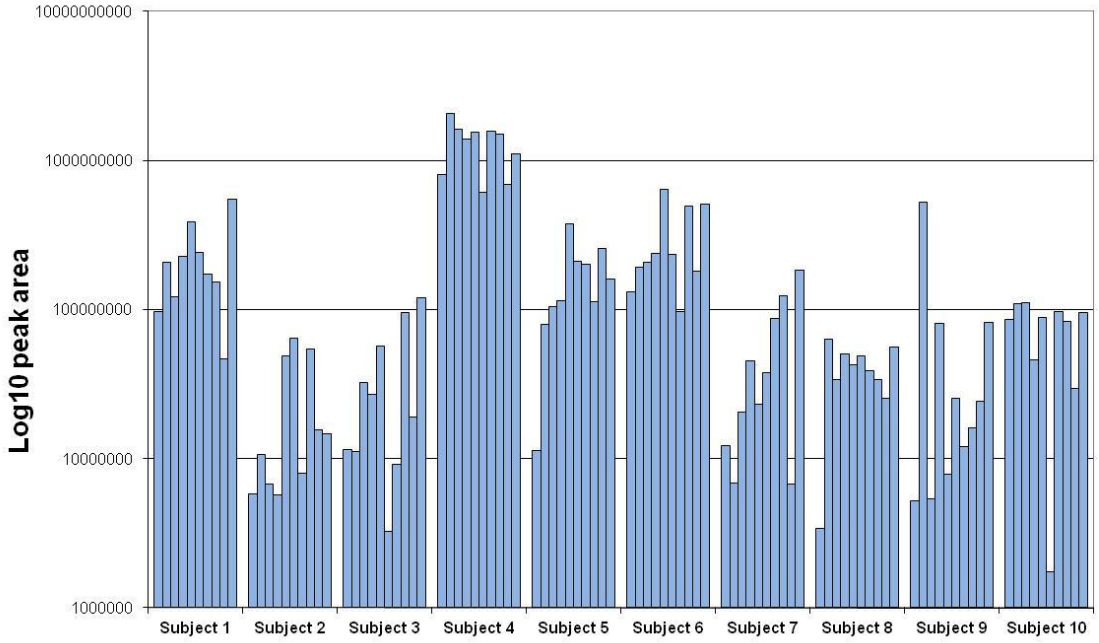

FIG 1_g: 1-Propanol

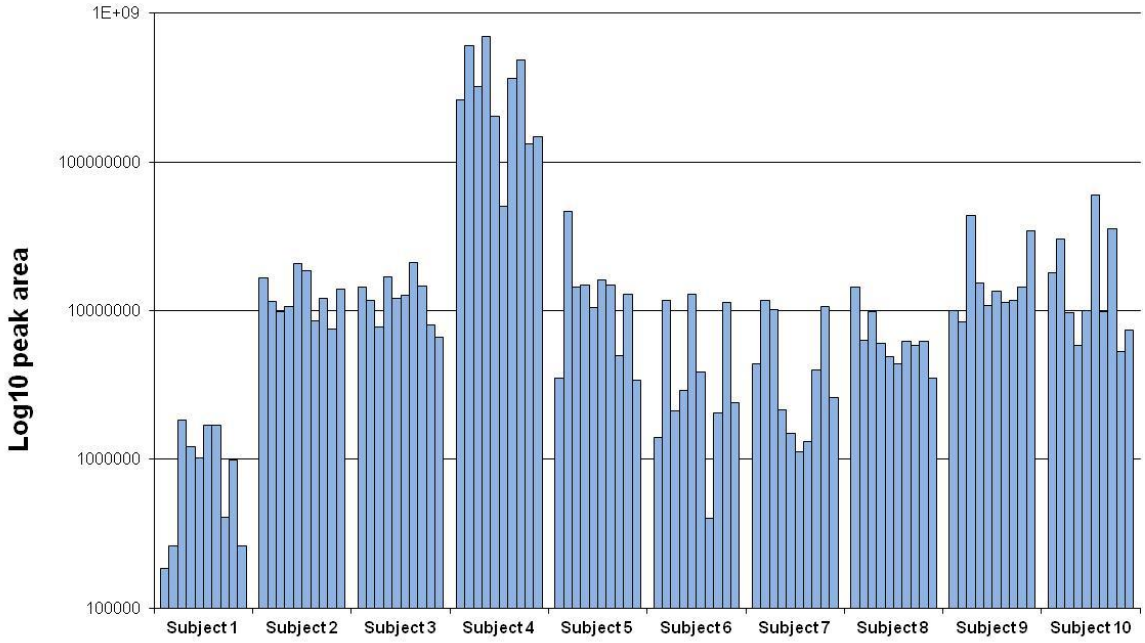

FIG 1_h: Octane

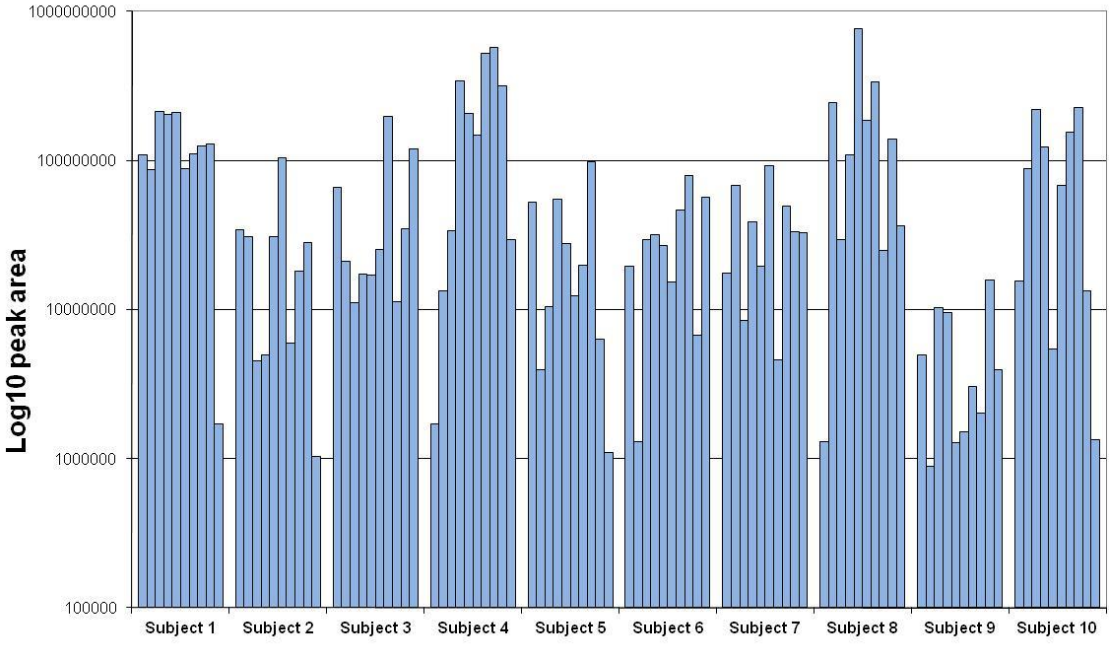

Figure (1): Showing the daya to day variation in 10 ubiquitous compounds (a: Styrene, b: Isopropyl alcohol, c: Acetone, d: Limonene, e: 1-Propanol, f: Phenol, g: Ethanol, h: Octane, i: Z -2-Octene, j: Hexanal) present in the saliva of 10 healthy individuals.

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6. References

1. Alagendran S, Archunan G, Rameshkumar K, Kadalmani B Rodriguez J A, Fernandez G, Martinez G and Guzman R G 2010 2-Nonenal-Ovulatory Specific Volatiles in Human Saliva throughout Menstrual Cycle by Gas Chromatography and Mass Spectrometry Analysis *Am. J. Biochem. Biotechnol*. **6** 187-194.

2. Barani F, Dell'Amico N, Griffone L, Santoro M and Tarabella C 2006 Determination of volatile organic compounds by headspace trap *J Chromatogr Sci*. **44** 625-30.

3. Belitz H D, Grosch W and Schieberle P 2009 Aroma compounds: Food Chemistry. Chap 5: 340-400. Springer-Verlag, Berlin Heidelberg

4. Breitmaier E, Terpenes 2006 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

5. Buszewski B, Kesy M, Ligor T and Amann A 2007 Human exhaled air analytics: biomarkers of diseases *Biomed Chromatogr*. **21** 553-66

6. Calil CM and Marcondes FK 2006 Influence of anxiety on the production of oral volatile sulfur compounds *Life Sci*. **79** 660-4

7. Calil CM, Lima PO, Bernardes CF, Groppo FC, Bado F and Marcondes FK 2008 Influence of gender and menstrual cycle on volatile sulphur compounds production *Arch Oral Biol.* **53** 1107-12

8. Chiappin S, Antonelli G, Gatti R and De Palo EF 2007 Saliva specimen: a new laboratory tool for diagnostic and basic investigation *Clin Chim Acta*. **383** 30-40

9. Cooke M, Leeves N, White C 2003 Time profile of putrescine, cadaverine, indol and skatole in human saliva. *Archives of Oral Biology* **48** 323-327

10. De Lacy Costello B, Evans P, Ewens R, Gunson H, Jones P, Ratcliffe N, and Spenser-Phillips P 2001 Gas chromatography-mass spectrometry analysis of volatiles organic compounds from potatoes tubers inoculated with Phytophthora infestans or fusarium coeruleum *Plant Pathology* **50** 489-496

11. El-Egami A A, Burham B O and El-Kamali H H 2011 Essential oil composition of the flowering aerial parts of Sudanese *Morettia phillaeana* (Del.) DC. *Current Research journal of Biological Sciences* **3** 100-103

12. Frampton MW, Pryor WA, Cueto R, Cox C, Morrow PE and Utell MJ 1999 Aldehydes (nonanal and hexanal) in rat and human bronchoalveolar lavage fluid after ozone exposure *Res Rep Health Eff Inst* **90** 1-15

13. Fuchs P, Loeseken C, Schubert J and Miekisch W 2010 Breath gas aldehydes as biomarkers of lung cancer *International Journal of Cancer*. **126** 2663-2670

14. Garner CE, Smith S, De Lacy Costello B, White P, Spencer R, Probert C S J and Ratcliffe NM 2007 Volatile organic compounds from feces and their potential for diagnosis of gastrointestinal disease. *FASEB J* 21(8):1675-1688

15. Gröschl M and Rauh M 2006 Influence of commercial collection devices for saliva on the reliability of salivary steroids analysis *Steroids* **71** 1097–1100

16. Imamura T 1982 Influences of amino acids on the phenol and indole production of salivary microorganisms *Shigaku* **70** 21-35

17. Jelen HH, Mildner S and Czaczyk K 2002 Influence of octanoic acid addition to medium on some volatile compounds and PR-toxin biosynthesis by *Penicillium roqueforti Letter Applied Microbiology*. **35** 37-41

18. Jermak S, Pranaityte B and Padarauskas A 2006 Headspace single-drop microextraction with in-drop derivatization and capillary electrophoretic determination for free cyanide analysis *Electrophoresis*. **27** 4538-4544

19. Kakoei S, Barkhori F, Mirzazadeh A, Mohammadi M, Ahmad Gholamhoseinian A 2012 Influence of menstrual cycle and salivary ß-estradiol on volatile sulfur compound *J Oral Health Oral Epidemiol* **1** 41-45

20. Kalapos M P 2003 On the mammalian acetone metabolism from chemistry to clinical implications Biochim Biophys *Acta Gen Subj* **1621** 122–39.

21. Kaufman E and Lamster IB 2002a Analysis of saliva for periodontal diagnosis-A Review *J Clin Periodontol* **27** 453-65

22. Kaufman E and Lamster IB 2002b The Diagnostic Applications of Saliva-A Review *Crit Rev Oral Biol Med* **13** 197-212

23. Kidwell DA, Holland JC and Athanaselis S 1998 Testing for drugs of abuse in saliva and sweat *J Chromatogr B Biomed Sci Appl*. **713** 111-35

24. Kostelc JG, Zelson PR, Preti G, Tonzetich J 1981 Quantitative differences in volatiles from healthy mouths and mouths with periodontitis *Clin Chem*. **27** 842-5

25. Kriek M, Martins F, Leonardi R, Fairhurst S A, Lowe D J and Roach PL 2007 Thiazole synthase from Escherichia coli: an investigation of the substrates and purified proteins required for activity in vitro *J of Bio Chem*. **282** 17413-17423

26. Kumar Gaurav A, Kumar Malik A and Matysik FM 2009 Analysis of biological samples using solid-phase microextraction *Bioanal Rev.* **1** 35-55

27. Kusano M, Mendez E and Furton KG 2011 Development of headspace SPME method for analysis of volatile organic compounds present in human biological specimens *Anal Bioanal Chem*. **400** 1817-1826

28. Lamsen E and Atsumi S 2012 Recent progress in synthetic biology for microbial production of C3–C10 alcohols *Front. Microbio*. **3** 1-13

29. Larsson BT 1965 Gas Chromatography of organic volatiles in human breath and saliva Acta *Chem Scand* **19** 159-64

30. Lawson LD and Wang ZJ 2005 Allicin and allicin-derived garlic compounds increase breath acetone through allyl methyl sulfide: use in measuring allicin bioavailability *J Agric Food Chem*. **53** 1974-83

31. Lima DP, Diniz DG, Moimaz SA, Sumida DH and Okamoto AC 2010 Saliva: reflection of the body *Int J Infect Dis* **14** 184-8

32. Lochner A, Weisner S, Zlatkis A and Middleditch BS 1986 Gas chromatographic-mass spectrometric analysis of volatile constituents in saliva *Journal of Chromatography B: Biomedical Sciences and Applications* **378** 267-282

33. Martin HJ, Riazanskaia S and Thomas CL 2012 Sampling and characterisation of volatile organic compound profiles in human saliva using a polydimethylsiloxane coupon placed within the oral cavity *Analyst*. **137** 3627-34

34. McKarns SC, Hansch C, Caldwell WS, Morgan WT, Moore SK and Doolittle DJ 1997 Correlation between hydrophobicity of short-chain aliphatic alcohols and their ability to alter plasma membrane integrity *Fundam Appl Toxicol* **36** 62-70

35. Meng Y, Hui X, Yong L, Hua H, Jie J, Yasong Z and Ting M 2009 Measuring Volatile Organic Compounds by Headspace Trap GC-MS in the Beijing Food Laboratory *American Laboratory*- online Edition. **April 2009.**

36. Mohammed R, Cambell J, Cooper-White J, Dimesky G and Punyadeera C 2012 The impact of saliva collection and processing methods on Crp, Ige, and Myoglobin i*mmunoassays Clinical and Translational **Medici**ne* **1** 19

37. Mudge S, Belanger S and Nelson A 2008. Fatty Alcohols Anthropogenic and natural occurrence in environment. Royal Society of chemistry, Cambridge, UK. pp 1-55

38. *Nagler RM, Hershkovich O, Lischins*k**y** S, Diamond E and Reznick AZ 2002 Saliva analysis in the clinical setting: revisiting an underused diagnostic tool. *J Investig Med* **50** 214-25

39. Newkome GR 1984 The Chemistry of Heterocyclic Compounds, Pyridine and Its Derivatives. John Wiley & Sons, Inc. Canada

40.

41. Olivier C, Vaughn S, Mizubuti E and Loria R 1999 Variation in allyl isothiocyanate production within Brassica species and correlation with fungal activity. *Journal of Chemical Ecology* **25** 2687-2701

42. Ott A, Germond JE and Chaintreau A 2000 Vicinal diketone formation in yogurt: (13)C precursors and effect of branched-chain amino acids. *J Agric Food Chem*. **48** 724-31

43. Penn DJ, Oberzaucher E, Grammer K, Fischer G, Soini H A, Wiesler D, Novotny MV, Dixon SJ, Xu Y and Brereton RG 2007 Individual and gender fingerprints in human body odour *J. R. Soc. Interface* **4** 331-340

44. Pfaffe T, Cooper-White J, Beyerlein P, Kostner K and Punyadeera C 2011 Diagnostic potential of saliva: current state and future applications. *Clin Chem* **57** 675-87

45. Pićurić-Jovanović K and Milovanović M 1993 Analysis of volatile compounds in almond and plum kernel oils *Journal of the American Oil Chemists Society* **70** 1101-1104

46. Røen BT, Unneberg E, Tørnes JA and Lundanes E 2010 Headspace-trap gas chromatography-mass spectrometry for determination of sulphur mustard and related compounds in soil *J Chromatogr A.* **1217** 2171-8

47. Sánchez Mdel N, García EH, Pavón JL and Cordero BM 2012 Fast analytical methodology based on mass spectrometry for the determination of volatile biomarkers in saliva *Anal Chem*. **84** 379-385

48. Schipper RG, Silletti E and Vingerhoeds MH 2007 Saliva as research material: biochemical, physicochemical and practical aspects *Arch Oral Biol* **52** 1114-1135.

49. Schulz K, Dressler J, Sohnius EM and Lachenmeier DW 2007 Determination of volatile constituents in spirits using headspace trap technology. *J Chromatogr.* **1145** 204-9

50. Shimizu S, Watanabe N, Kataoka T, Shoji T, Abe N, Morishita S, Ichimura H 2002 "Pyridine and Pyridine Derivatives" *Ullmann's Encyclopedia of Industrial Chemistry*- online Edition. June 2000.

51. Smith E A and Macfarlane G T 1997 Formation of Phenolic and Indolic Compounds by Anaerobic Bacteria in the Human Large Intestine. *Microbial Ecology* **33** 180-188

52. Soini HA, Klouckova I, Wiesler D, Oberzaucher E, Grammer K, Dixon SJ, Xu Y, Brereton RG, Penn DJ and Novotny MV 2010 Analysis of volatile organic compounds in human saliva

by a static sorptive extraction method and gas chromatography-mass spectrometry. *J Chem Ecol*. **36** 1035-1042

53. Tonzetich J and Carpenter P A W 1971 Production of volatile sulphur compounds from cysteine, cystine and methionine by human dental plaque *Archives of Oral Biology* **16** 599–607

54. Varlet V, Prost C and Serot T 2007 Volatile aldehydes in smoked fish: Analysis methods, occurence and mechanisms of formation. *Food Chemistry*. **105** 1536–1556

55. Wang V S, Lu M Y 2009 Application of solid-phase microextraction and gas chromatography–mass spectrometry for measuring chemicals in saliva of synthetic leather workers *Journal of Chromatography B* **877** 24–32

56. Yamai K, Ohkuwa T, Itoh H, Yamazaki Y and Tsuda T 2009 Influence of cycle exercise on acetone in expired air and skin gas *Redox Rep*. **14** 285-9

57. Yang CM 2002 Response of forage fiber degradation by ruminal microorganisms to branched-chain volatile fatty acids, amino acids, and dipeptides *J Dairy Sci* **85** 1183-90

58. Yang Sun S, Jiang WG and Zhao Y 2010 Characterization of the aroma-active compounds in five sweet cherry cultivars grown in Yantai (China) *Flavour and Fragrance Journal* **25** 206–213